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Compartment syndrome: pathophysiology, clinical presentations, treatment, and prevention in human and veterinary medicine

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Abstract

Objective – To review the human and veterinary literature pertaining to all forms of compartment syndrome (CS).
Data Sources – Data sources included scientific reviews and original research publications from the human and veterinary literature.
Human Data Synthesis – While CS affecting the extremities has been recognized in people for decades, other forms of CS in the abdominal and thoracic cavities are recently gaining more attention. The role of CS in critically ill people is a rapidly growing area of interest. More research on prevention and treatment of CS is being conducted in people because some studies have found mortality rates as high as 80% for those suffering from these conditions.
Veterinary Data Synthesis – While a significant amount of experimental studies of CS have been performed on small animals, there is a marked lack of primary veterinary studies. The majority of the veterinary literature includes case reports and series, and many of these studies were published over a decade ago. However, the increased recognition of CS in people has sparked an interest in veterinary critical care medicine and this has been demonstrated by the recent increased evaluation of compartment pressures in veterinary patients.
Conclusions – CS is a complex clinical condition where increased pressure within a compartment can cause significant adverse effects within the compartment as well as throughout the body. Systemic inflammatory responses and local ischemia-reperfusion elements can contribute to the detrimental effects seen in CS. This cascade of events results in increased mortality rates and contributes to the development of CS elsewhere. A better understanding of CS will help veterinarians improve patient care and outcome. Future studies on incidence, prevention, and treatment of CSs in the critical care patient are needed in veterinary medicine.

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Keywords: complication, intraorgan hypertension, monitoring, perfusion

Abbreviations

ACS	abdominal compartment syndrome
AV	arteriovenous
CS	compartment syndrome
ECS	extremity compartment syndrome
GDV	gastric dilatation volvulus
IAH	intra-abdominal hypertension
IAP	intra-abdominal pressure
NG	nasogastric
NSAID	nonsteroidal anti-inflammatory drug
PD	peritoneal dialysis
RAAS	renin angiotensin aldosterone system
ROS	reactive oxygen species
SECS	secondary extremity compartment syndrome
SIRS	systemic inflammatory response syndrome
TCS	thoracic compartment syndrome
WSACS	World Society on Abdominal Compartment Syndrome

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Introduction

Compartment syndrome (CS) is defined as the dysfunction of organs or tissues within a compartment that develops secondary to increased pressures within that compartment.[1] The increased pressure within the compartment limits the blood supply resulting in reduced or

absent perfusion to the tissues. Eventually this process can cause physiologic dysfunction in tissues, leading to irreversible damage, and in some cases death.[2] CS can affect many organ systems and is being recognized with increasing frequency in people as a compounding factor in many critically ill states. This is especially true for abdominal compartment syndrome (ACS). An increased recognition of ACS has led to early monitoring of at-risk patients, and earlier interventions for those affected.[3] However, CS have not been extensively investigated in veterinary patients. There has been some research determining normal pressure levels of various compartments in veterinary patients.[4,5] In addition, there have been some case reports and series, but CS as a whole has gone unrecognized and under-reported in critically ill veterinary patients.[6–11] The goal of this article is to review the pathophysiology underlying CS, discuss the most common clinical manifestations, and to review treatment and prevention strategies in the management of CS in people and veterinary patients.

Pathophysiology

There are 2 theorized pathways that may lead to the eventual cellular hypoxia resulting in CS. The first theory used to explain CS is known as the "arteriovenous pressure gradient theory." The second theory is known as the "ischemia-reperfusion syndrome theory." Even though these 2 theories can be considered complimentary, the "arteriovenous pressure gradient theory" is the more widely accepted theory in explaining CS because of early experiments and studies corroborating the described mechanism of this theory.[1,12,13] This theory proposes that CS develops because of increased pressure within the tissue resulting in an increase in pressure of the veins in the compartment, and that this causes a decrease in the arteriovenous (AV) pressure gradient within that compartment.[14] This decrease in pressure gradient results in decreased oxygen delivery, which then results in ischemia of the affected organs or tissues within the compartment.

The second theory of CS states that as the pressure within the individual compartment increases the interstitial fluid pressure will rise above the capillary pressure, and when this happens there is an inability to perfuse the organs or tissues within that compartment. Following a period when perfusion is impaired, the ensuing reperfusion causes a massive production of reactive oxygen species (ROS) in addition to the decreased oxygen delivery. A vicious cycle of hypoxia, anaerobic metabolism, further vasoconstriction, and continued cellular damage ensues.[15] The underlying principle of this theory of CS is that the ischemia and subsequent reperfusion is the cause of the edema and cellular damage characteristic of this condition.

An awareness of both theories can help clinicians appreciate the pathogenesis of CS. Elements of both pressure gradients as well as ischemia and reperfusion contribute to the development of CS and its systemic effects.

Primary and Secondary Forms CS

Central to CS is the dysfunction of organs or tissues within a compartment that develops secondary to increased pressures within that compartment.[1] CSs can also be further subdivided into primary or secondary CS. Primary CS occurs as a result of an injury or disease process within the affected compartment. In the human literature this is sometimes referred to as a CS that stems from the initial insult, hemorrhage, or edema.[16] Examples of primary CS include hemorrhage, significant edema due to a primary injury, obstruction of blood supply (such as thrombosis), or soft tissue or orthopedic injury to that compartment.[17–19]

Secondary CS in contrast is caused by an injury or disease outside the compartment that is affected. Instead an extra-compartment contributing factor or factors results in a CS away from the site of injury or disease. In the human literature this is often known as a CS that occurs secondary to iatrogenic factors.[16] An example is secondary extremity compartment syndrome (SECS) where the fascial planes of muscle bellies form a compartment in the limb. SECS can develop following fluid resuscitation of patients suffering from systemic inflammatory response syndrome and shock. Moreover, SECS was found to be an indicator of increased mortality in 1 human study.[20] The most common cause of secondary CS is overaggressive fluid resuscitation,[18,a] but other causes include constrictive bandaging or improper surgical placement or positioning,[19] prolonged preoperative times, or inappropriately chosen surgical approach.[21] An experimental study investigating fluid rates for intraosseous delivery of fluids in dogs found that high rates of fluid administration resulted in CS of that limb, demonstrating an iatrogenic cause of SECS in dogs.[22] Careful administration of fluid therapy could potentially lower the rates of secondary CS seen in people and veterinary patients.

Extremity or skeletal muscle CS

This occurs with marked increases in the content or reduction in the volume of a muscle compartment resulting in increased pressure within this compartment. This increased pressure results in ischemia of the affected muscle bodies and can lead to loss of muscle or limb function and eventual muscle death. Skeletal muscle CS is the most widely recognized CS entity in veterinary

medicine. It is also known as extremity compartment syndrome (ECS).

ECS can be a result of 3 factors: (1) decreased compartmental volume within the extremity compartment, (2) increased tissue or fluid volume within the compartment, or (3) externally applied pressure on the extremity.[23] The pathophysiology behind the above-mentioned changes resulting in ECS has been explained using parts of the ischemia-reperfusion model.[24] The neutrophil invasion of the area along with the associated cytokine production caused during the ischemic and reperfusion phases appear to have a significant impact on the severity of the syndrome.[25]

In people these causes of ECS are further broken down into orthopedic (ie, fracture) related, vascular related, iatrogenic, or soft tissue related.[19] The most common causes in people include fractures (with tibial fractures being the most common to result in ECS), blunt soft tissue injury to a limb, arterial injuries, venous thrombosis, burns, and prolonged limb compression.[17,19] Secondary ECS can also occur. An experimental study in dogs that induced hemorrhagic shock and then resuscitated the animals with high volumes of crystalloids resulted in a secondary increase in extremity compartment pressures.[26] Although snake bite wounds were thought to lead to an extremity CS that might require a fasciotomy in the past,[27] fasciotomy in these cases has fallen out of favor and is not recommended in cases where anti-venin is available. Fasciotomy is reserved for rare and extreme cases in which the CS has not responded to medical management with anti-venin in people, and at this time the veterinary literature is following the recommendations set forth in the human literature.[27,28]

A normal intracompartmental pressure in human muscles is 10–12 mm Hg (13.6–16.3 cm H_2O).[19] Normal values in dogs have not been specifically published but are assumed to be similar to those of people since dogs are frequently used as experimental models for human ECS.[22,24–26] The normal values in dogs typically cited in these studies is approximately 5.7 $\pm$ 5.1 mm Hg (7.8 $\pm$ 6.9 cm H_2O).[24] Normal intracompartmental pressure for cat and horse muscles have not been documented at this time.

In people, the most important clinical sign is pain of the limb, but other important clinical signs include palpable tenseness of the limb, parasthesia or paresis of the limb, or pulselessness in the limb.[17] While veterinary patients might mask pain more readily than people, pain was also the most common complaint in veterinary patients later found to be experiencing ECS secondary to a bandage injury.[29]

A definitive diagnosis of ECS is made with measurement of the intracompartmental pressure. This can be done using several devices. The first is by inserting a needle into the compartment and then using different forms of manometry to measure the pressure.[19] More recently a noninvasive measurement of intracompartmental pressure using a near-infrared spectroscopy device has been developed in people.[30] While diagnosis requires confirmation of elevated compartmental pressure, clinical signs indicative of ECS warrant intervention without confirmation of an increased pressure in human medicine. In veterinary patients this may also be a more acceptable approach due to the difficulty measurement poses. However, with a decreased ability to assess clinical signs, obtaining an actual pressure of the compartment may be advantageous.

ECS is still considered a relatively rare process in veterinary medicine. However, of all the CSs it is probably the most extensively discussed in the veterinary literature. In dogs, CS secondary to specific muscle body injuries can contribute to worsening of muscular contractures from the injury, and the infraspinatus muscle may be more susceptible than others based on a review of muscle contractures in small animals.[31] Athletic, large breed dogs that sustain injuries to their infraspinatus or supraspinatus muscles are most at risk for suffering acute CS, and early intervention is critical to help prevent the development of fibrous replacement of muscle tissue.[32] Just as in people, fractures or trauma-related injuries to the extremities has been documented as a common cause of ECS in dogs.[6–8] There is speculation that "limber tail," a term used to describe muscle damage to the tail in large breed dogs, may be a variant of an ECS,[32] and further investigation into this theory is warranted. There have also been 2 recent case reports where intramuscular neoplasia has resulted in the development of ECS in dogs.[9,10] Both dogs had intramuscular hemangiosarcoma, which has a tendency to bleed into its confined compartment and might have contributed to the development of CS.

In large animal medicine, CS has been most recognized in the form of a postanesthetic myopathy. Horses that have undergone anesthesia can develop a significant lameness postanesthesia that could be secondary to CS.[33] The AV gradient theory of CS can develop along with hypotension and poor positioning during anesthesia, and this can result in ECS in horses. The gluteal and tricep muscles appear to be the most susceptible.[33] Clinical signs in addition to the lameness postoperatively include pain, sweating, trembling, and presence of a firm, swollen muscle belly in the affected limb that may or may not have neurologic deficits.[33] Additionally, many horses seem unable to extend the digit and carpus of the affected limb.[33–35] Two experimental studies[36,37] confirmed, respectively, circulatory compromise and increased intracompartmental pressure in anesthetized horses, and that protective and padded surfaces decreased the elevation

of compartmental pressure seen in anesthetized horses. One case series[34] confirmed the presence of increased intracompartmental pressures in a horse with this suspected condition. Another case report of 2 horses[38] with this suspected condition found histopathologic lesions consistent with ischemia-reperfusion injury in the affected muscle bellies. In another case report with 2 horses suspected to be suffering from postanesthesia ECS,[35] there was improvement and recovery following fasciotomy of the affected muscle bellies.

The definitive treatment for ECS is surgical decompression, typically via fasciotomy.[15,39] Fasciotomy is recommended when pressures in the compartment rise above 30 mm Hg (40.8 cm H_2O), or within 30 mm Hg (40.8 cm H_2O) of the patient's diastolic pressure if clinical signs are also consistent with ECS.[17,40] Up to 80% of those affected may require delayed primary closure of the incision site in addition to the initial decompressive procedure.[39] Vacuum-assisted closure may speed recovery.[17] Additionally, while hyperbaric oxygen therapy is not a readily accessible treatment modality in the majority of veterinary facilities, an experimental study in which ECS was induced in dogs found that the use of hyperbaric oxygen therapy significantly improved outcome.[41]

Pain management is of the utmost importance in treatment in human medicine[17] and, aside from decompression, should also be considered a cornerstone of treatment in veterinary patients. It can also be theorized that the ischemia-reperfusion aspect of the ECS would benefit from the administration of anti-inflammatories and antioxidants.[42] A recent study found that administration of the nonsteroidal anti-inflammatory drug (NSAID) indomethacin to rats following experimentally induced ECS resulted in decreased muscle necrosis as well as increased perfusion to the compartment for those given the study drug.[43] A study of experimentally induced ECS in dogs found that treating with a cyclooxygenase inhibitor, lysine-acetyl-salicylate, resulted in decreased thromboxane levels as well as overall lower compartmental pressures.[44] These studies and our knowledge of ECS in people suggest that in addition to decompression, the use of pain management and anti-inflammatories may benefit veterinary patients with ECS. Although there has been no research into the use of antioxidants as being beneficial in ECS, it would seem in theory that their utilization might be helpful in veterinary and human patients.

Prevention of ECS may be achieved in those patients that have sustained orthopedic trauma by operating on them as soon as they are stable for anesthesia. However, it can be more difficult to have a surgeon available in smaller hospitals or after hours. Although closed fractures are generally considered a nonemergent surgical procedure, there is a decreased risk of ECS when surgery is not delayed[45] and this may be an argument for pursuing surgery as soon as possible in patients with fractures following initial stabilization. If left untreated, ECS may result in loss of the limb, in which case amputation would be needed. In severe cases that are untreated or in which treatment is delayed, complications such as systemic inflammatory response syndrome or acute kidney injury have resulted in death in people.[19]

In summary, although detection of pain in veterinary patients may be more complicated than in people, in clinical presentations where ECS might be suspected measurement of extremity compartment pressure should be considered. A clinical picture consistent with the syndrome can serve as sufficient reason for intervention. Confirmation of ECS can be achieved by measuring intracompartmental pressures, and if these measurements are consistent with elevated intracompartmental pressure, surgical intervention is warranted. Certain situations may allow clinicians to take measures to prevent the development of ECS, such as using protective padding for large animal species about to undergo anesthesia. Future treatments with antioxidants or NSAIDs may prove beneficial in patients suffering acute ECS. While some individual small animals may suffer fewer consequences from a limb amputation, limb amputation may not be an option for other large breed dogs and large animals. Therefore clinicians should be proactive in the prevention and treatmentof ECS.

Abdominal compartment syndrome

ACS is a syndrome in which increased intra-abdominal pressure (IAP) results in progressive intra-abdominal organ dysfunction as well as detrimental effects on the cardiovascular, respiratory, and CNS systems.[18] Intra-abdominal hypertension (IAH) is defined as a sustained or repeated pathological elevation in IAP $\geq$ 12 mm Hg (16.3 cm H_2O). In people the consequences of IAH include a secondary increase in intrathoracic pressure, which results in decreased left ventricular compliance and decreased ventricular filling, as well as a tertiary increase in intracranial pressure due to obstruction of cerebral venous blood outflow.[46] Additionally, people with ACS are more likely to develop kidney failure and had a much higher mortality, with 1 study having only 20% of those with ACS surviving.[47] Primary ACS has also been found to result in decreased to absent mesenteric lymph flow, which was then associated with gut wall edema and could thus further worsen IAH.[48] ACS has serious consequences and knowledge of the syndrome is very important for human and veterinary emergency clinicians and surgeons. The diagnosis of IAH and ACS is becoming much more widely reported in the human

Table 1: A summary of systemic effects caused by intra-abdominal hypertension and abdominal compartment syndrome

Organ system effected	Pathophysiology
Cardiovascular	Compression of the vena cava resulting in decreased left ventricular filling and decreased cardiac output; Decreased left ventricular compliance because of a secondary rise in intrathoracic pressures; Direct myocardial ischemia
CNS	Increased intracranial pressure secondary to a combination of decreased cardiac output and obstruction of cerebral venous outflow causing a decreased cerebral perfusion pressure
Renal	Increased pressure in the abdomen resulting in compression of the urinary collecting ducts and renal vessels, leading to oliguria
Lymphatics	Decreased to absent mesenteric lymph flow secondary to compression from the increased abdominal pressure
Pulmonary	Decreased diaphragmatic excursion secondary to compression from the abdomen's distension, resulting in decreased ventilation and hypoxia; Intra-abdominal pressure is also transmitted into the thorax through the diaphragm resulting in increased intrathoracic pressures
Gastrointestinal	Impaired wound healing; Gut wall edema secondary to increased inflammatory mediators, decreased lymphatic flow, and capillary leakage; Potential translocation of bacteria through compromised gastrointestinal tract
Hepatic	Decreased hepatic blood flow resulting in hypoxia and hepatic dysfunction; Possible acute hepatic failure

literature over the last several years, and with a better understanding of the syndrome and its consequences may help veterinary medicine follow suit.

Normal IAP in people is approximately 5–7 mm Hg (6.8–9.5 cm H_2O).[3] Two studies have determined normal values for IAP in small animals. In dogs they have been reported to be 1.5–5.1 mm Hg (2–7 cm H_2O),[4] while in cats they have been reported to be 3.8–6.5 mm Hg (5.2–8.8 cm H_2O).[5] In standing horses IAP is not expected to exceed 5.1 mm Hg (7 cm H_2O), and in recumbent horses IAP should not exceed 7.4 mm Hg (10 cm H_2O).[49]

Previously, a diagnosis of ACS in people was made with a combination of: (1) IAH with a pressure >25 mm Hg (>34 cm H_2O), (2) one of the following: oliguria, increased pulmonary pressure, hypoxia, decreased cardiac output, hypotension, or acidosis, and (3) improvement with abdominal decompression.[50] Others suggested a diagnosis when there was IAH in combination with a rigid and tense abdomen, an increased peak inspiratory pressure, renal dysfunction, and hemodynamic instability requiring catecholamines for management as the criteria for diagnosing ACS.[51] However, the recent World Society on Abdominal Compartment Syndrome (WSACS) has given a more specific definition stating that ACS is defined as a sustained IAP >20 mm Hg (>27.2 cm H_2O), with or without an abdominal perfusion pressure of <60 mm Hg that is associated with new organ dysfunction or failure.[3]

The systems known to be affected in ACS include cardiovascular, pulmonary, CNS, renal, heptic, gastrointestinal, and lymphatic. The cardiovascular changes occur because of decreased venous return due to compression on the vena cava, which results in decreased ventricular filling and a decreased cardiac output.[52] Diaphragmatic excursion is decreased, resulting in decreased functional residual capacity and impaired pulmonary function. The increased intrathoracic pressure then results in obstruction of cerebral venous outflow.[52] These changes in combination with the decreased cardiac output results in a decreased cerebral perfusion pressure.[46] Direct compression of the renal veins and urinary collecting system results in oliguria and the kidney ischemia results in stimulation of the renin angiotensin aldosterone system (RAAS), all resulting in a decreased urinary output.[52] Gastrointestinal changes include impaired wound healing and gut wall edema.[2,52] Additionally, rodent models of ACS have shown an increased incidence of bacterial translocation in rats.[53] Lastly, the hepatic system can be expected to suffer hypoxia and edema just like other organs in the abdominal cavity.[2] In a canine model of ACS, progressive increases in IAP resulted in worsening impairment of hepatic blood flow with subsequent hypoxia and dysfunction.[54] A summary of all the different systemic effects of IAH and ACS can be found in Table 1.

The confirmation of a diagnosis of ACS, requires the presence of IAH. IAP is most frequently measured via placement of a urinary catheter. When the pressure is measured with a urinary catheter in people the values obtained are generally similar, although possibly slightly lower than the actual IAP (by 1 mm Hg [1.4 cm H_2O]).[55] Measurement of IAP in people has been standardized with guidelines from the WSACS which recommends that patient's IAP be measured with a urinary catheter and measurements should be taken at end-expiration in the supine position and zeroed to the level of midaxillary.[56] Specific factors that have been found to alter IAP measurement in people include positioning, body

Table 2: Instructions for measurement of intra-abdominal pressures in small animals[5,61]

1. A urinary catheter is placed using sterile technique with sedation if required.
2. A sterile urinary collection system is then attached to the urinary catheter with two 3-way stop cocks within the system.
3. A water manometer should be placed at the level of the first upright stop cock and should also be level with the approximate center of the patient's symphysis pubis.
4. A 250 mL bag of 0.9% NaCl should be attached to the second stop cock with an attached 20–35 mL syringe.
5. Prior to any measurement the bladder should be emptied.
6. To measure the pressure one should first instill 0.5–1 mL/kg of saline into the bladder.
7. The manometer should then be filled with saline and the intra-abdominal pressure (IAP) is the difference between the zero level and the level at which the meniscus of the saline lies.
8. If serial measurements are to be performed, positioning should be consistent between measurements.

weight, application of external pressure, and phase of respiration.[3,57–60]

A similar method of abdominal pressure measurement has also been demonstrated in dogs[4,61] and cats.[5] This method entails placement of a urinary catheter, which in some patients will require sedation. Table 2 provides stepwise instructions for measuring IAP this way in small animals. Although there is no consensus on what position the patient should be lying in or when to measure IAP as is the case with people, it has been noted that in cats the pressure measured in right lateral was statistically higher than those measured in sternal recumbency.[5] In any case, if serial measurements are anticipated the clinician should ensure that the measurements are taken in the same position every time. In a recent abstract investigating IAP measurements, a commercially available IAP measuring system was used in canine patients with low intra- and interobserver variability when measuring IAP.[62] Another method of abdominal pressure measurement that appears to be accurate in dogs involves using a nasogastric (NG) catheter placed under anesthesia as this was validated in a study with experimentally induced ACS.[63] IAP can also be measured through the inferior vena cava and from placement of a catheter directly into the peritoneal cavity in people using a peritoneal dialysis (PD) catheter.[18] While 1 study indicated that subjective clinical signs such as abdominal distension visible to the clinician may be an appropriate indicator of IAH in dogs,[4] human studies have shown the contrary and physical exam findings show very poor sensitivity and accuracy for diagnosing IAH or ACS.[64]

In horses measurement of IAP has not yet been well standardized. Similar to people and small animals, a technique using urinary catheterization appears to be valid.[50,65] Other techniques using NG tubes and directly through an intraperitoneal catheter appear to lack consistency and validity when trying to determine IAP in horses.[66,67] Additionally, body weight, body position (ie, standing versus recumbent), and volume of infused fluid if using the urinary catheterization technique can all effect and significantly change IAP in horses.[66]

Table 3: Intra-abdominal Hypertension Grading System as set forth by The World Society of the Abdominal Compartment Syndrome (WSACS)[3]

Grade of intra-abdominal hypertension	Intra-abdominal pressure
Grade I	12–15 mm Hg (16.3–20.4 cm H_2O)
Grade II	16–20 mm Hg (21.8–27.2 cm H_2O)
Grade III	21–25 mm Hg (28.6–34 cm H_2O)
Grade IV	>25 mm Hg (>34 cm H_2O)

Recently guidelines on IAH have been determined in human medicine. The WSACS published a grading system for ranking severity of IAH and these can be seen in Table 3.[3] Grading can be used to guide clinician decisions for both intervention and monitoring. Patients with more severe degrees of IAH may require more aggressive forms of treatment.[3] It may be reasonable to use a similar grading system in critically ill veterinary patients as normal IAP in dogs, horses, and cats seem similar to those in people.[68] Drellich[61] did propose a grading system of IAH for dogs in a review on IAP; however, no studies were performed to support these values. Further studies are warranted to establish an ideal grading system of IAH for horses, dogs, and cats.

Although the exact causes of ACS are not always known, just as with ECS any increases in the pressure within the confined intraperitoneal space can result in ACS. Also similar to ECS, the causes of CS within the abdomen can be secondary to a decrease in compartmental volume in the abdomen, an increase in externally applied pressure on the abdomen, or most commonly an increase in tissue or fluid pressure within the compartment. Take for example the development of IAH and ACS in a case of fluid or gas distension of the abdomen. In this model of ACS development, first a primary insult occurs such as effusion secondary to hepatic failure or the development of gastric dilatation and volvulus. The excessive fluid or gas distension results in an increase in volume within the intraperitoneal space, contributing to IAH. If the underlying disease is severe enough, shock will develop. In shock states the sympathetic

nervous system is stimulated and blood is preferentially shifted toward the brain and heart due to vasoconstriction elsewhere. This results in a relative state of cellular hypoxia in the gut, triggering the release of cytokines, the production of ROS, and a decrease in the overall levels and production of ATP. Proinflammatory cytokines, ROS, and decreased ATP resulting in dysfunction of cellular pumps such as the Na-K-ATPase pump all result in cellular edema, which results in further swelling of tissues and increases IAPs.[52] If decompression is performed via drainage of ascites or trocharization or decompression of the stomach, this may result in reperfusion injury to the abdominal organs, activation of proinflammatory cytokines further increasing IAP, and resulting in IAH. Eventually this IAH may become severe enough to cause organ dysfunction, satisfying criteria for ACS.

Just as with other CSs, causes or risk factors can be primary, occurring within the abdomen or pelvic cavity, or secondary when an insult outside of the peritoneal cavity results in development of ACS.[67,69] The most common risk factors cited in human literature for IAH that may or may not lead to ACS include severe shock, sepsis, or pancreatitis.[2] Those at risk include: those receiving high volume resuscitative fluids,[18,a] any patient with acute respiratory distress syndrome (ARDS) or undergoing mechanical ventilation,[47] those who have suffered severe abdominal or pelvic injury that may or may not have undergone surgical interventions,[18,51,52] and any patient that has already experienced emergency laparotomy.[70] Other conditions in which ACS has been documented include gastrointestinal perforations, bile peritonitis, abdominal masses, and pregnant individuals.[70,71] The WSACS presented another way to think about these conditions in groups rather than as a list of diseases associated with ACS.[3] These groupings are summarized in Table 4.

Table 4: Conditions at risk for the development of IAH and subsequent ACS[3]

Condition	Example
Diminished abdominal wall compliance	Counterpressure applied by a belly wrap
	Positioning and immobility in the critically ill
Increased intra-luminal contents	Ileus
	GI obstruction
	GI paresis
Increased abdominal contents	Free gas, blood, ascites in the abdomen
	Abdominal mass
Any condition resulting in capillary leakage in association with fluid therapy	Sepsis
	Systemic inflammatory response syndrome (SIRS)
	ARDS
	Pancreatitis

The veterinary literature on ACS is very limited consisting of a few review papers as well as some case reports. The normal values for IAP in cats were recently evaluated, and better standards for measurement in cats were established in this landmark study.[5] Normal IAP values in dogs have also been documented, and in this study it was acknowledged that dogs' IAP is mildly elevated postlaparatomy.[4] It was also determined that elevated abdominal pressures were found commonly in canine patients with a disease that resulted in a visibly distended abdomen.[4] One case report described a dog with babesiosis that developed IAH and subsequent respiratory distress and renal dysfunction consistent with ACS.[11] An experimental study in cats in which chronic obstructive pancreatitis was induced found that these cats also commonly suffered from IAH.[72] Although bandaging of the abdomen is sometimes used to provide abdominal counterpressure to help slow bleeding, it has been suggested that this may be contraindicated as it may increase the risk for development of ACS.[73] However, the presence of IAH has yet to be verified as a component in animals with a hemoabdomen.

In large animal medicine the presence of ACS has yet to be definitely diagnosed. However, a case report did acknowledge the presence of IAH in 2 critically ill horses.[74] Common clinical situations where large animal clinicians suspect ACS may play a role in equine medicine include colitis, hydrops, colonic displacement, uroperitoneum, pregnancy, and lymphatic obstruction.[49,74] Additionally, the use of abdominal insufflation with CO_2 in horses undergoing laproscopy appeared to have significant negative cardiopulmonary affects in dorsally recumbent horses, suggesting a component of ACS.[75] Further studies into this found that these same negative effects were not seen when the insufflation occurred in standing horses.[76,77]

In summary, while there have been limited reports of IAH and ACS, the veterinary literature has acknowledged that IAH and ACS have many negative systemic effects. A better understanding of the condition as well as implementing IAP monitoring will most likely lead to better management in critically ill veterinary patients.[65]

As with ECS, the treatment for ACS is decompression, and this is definitively accomplished via a decompressive laparatomy and potentially management with an open abdomen postoperatively in certain human cases.[78] Following decompression patients with ACS have an increase in cardiac index, tidal volume, oxygen delivery, and urine output, while heart rate, central venous pressure (CVPs), positive inspiratory pressure (PIP), and lactate all decrease to more acceptable levels.[51,70] Additionally, a recent prospective study looking at intervention with an open abdomen to decrease the development of ACS in people found that these interventions

significantly improved survival rates from 50% to 72%.[79] There were also trends toward decreased ICU stay and days on the mechanical ventilator for patients managed with an open abdomen, but these findings were not statistically significant.[79] Although management of an open abdomen is more easily achieved in people, it should be noted that ACS is the only situation in which postoperative open abdomen management is the definitive recommendation.[78] Open peritoneal drainage following surgery has been successfully used in veterinary patients with abdominal sepsis[80,81] but has fallen out of favor in this patient population with the development of closed suction drains, except for cases with overt gross contamination that cannot be lavaged or debrided clean.[82] However, in cases at risk for or with confirmed IAH and ACS it may be reasonable to consider using open peritoneal drainage postlaparotomy.

As ACS becomes more widely recognized, the focus will hopefully shift to prevention. The consequences of surgery may worsen the patient's condition since the surgical insult can further exacerbate the inflammatory response, and this also advocates for earlier monitoring and prevention. Aggressive monitoring in those at risk may help identify IAH before ACS and organ dysfunction develops. Depending on the underlying cause of the IAH, some nonsurgical interventions that might help reduce IAP prior to ACS include gastric decompression, rectal decompression with enemas, changing body position, diuretics to remove excess fluid and edema from tissues, paracentesis to remove excess fluid from the abdominal cavity, hemodialysis, and neuromuscular blockade.[18] The WSACS also set up specific medical management recommendations for those with known IAH in an effort to lower the IAP and prevent the development of ACS that would require surgical intervention.[56] Their recommendations found that sedation, analgesia, or neuromuscular blockade can all improve abdominal wall compliance and therefore lower IAP.[18] To help with emptying luminal contents within the gastrointestinal tract, the use of prokinetics should be instituted and emptying of the stomach via an NG tube or instituting enemas to empty the colon should be considered.[18] Overzealous fluid therapy should be avoided and the use of hypertonic fluids that will lower the overall fluid volume administered is recommended.[56] Another study found that the use of lower volume colloid fluid therapy as opposed to high volume crystalloid therapy was beneficial in keeping IAP within normal limits while those receiving the crystalloids did go on to develop IAH.[83] If oliguria or anuria has already developed, then the use of diuretics or dialysis should be considered.[56] Attempts at draining specific areas found to have gas or fluid taking up abdominal space can be made, but if the medical management strategies are not working the clinician should not hesitate to pursue surgical decompression.[3,56] Additionally, when surgery is deemed necessary for treatment of ACS or when a patient must undergo abdominal surgery but there is a risk of postoperative development of ACS, use of epidural analgesia in people has been found to significantly decrease IAP in postoperative abdominal surgery patients.[84] These preventative and treatment measures have not been investigated in veterinary medicine as of yet. However, all of these recommendations from human medicine might be helpful for the veterinary clinician when ACS is suspected, or when a patient is deemed at risk for development of ACS. If ACS is confirmed or if medical measures to decrease IAH fail, surgical decompression may be necessary in veterinary patients.

In people with IAH that went on to develop ACS, survival has been documented to be as low as 20%.[47] Obviously the detrimental consequences of ACS including cardiovascular compromise, renal compromise, decreased pulmonary function, and potential CNS damage all make it a very dangerous condition that should be monitored for, prevented if possible, and rapidly treated with surgical decompression when it does develop. Based on the large amount of human literature on the subject and the smaller amount of veterinary literature, more aggressive monitoring of our veterinary patients for IAH and ACS, instituting preventative measures when possible, and treating once a diagnosis of ACS is confirmed should be recommended. Monitoring IAP via placement with a urinary catheter might be more difficult in some small animal patients, mainly female cats and dogs where urinary catheter placement might require anesthesia. Also, consistent measurement protocols for measuring IAP in horses have not been established, leaving another hurdle for large animal clinicians to overcome. Hopefully this will not stop the veterinary clinician from attempting monitoring for IAH in those at risk. Additional monitoring with measurements of pulmonary function and cardiac output in conjunction with IAP measurements help physicians confirm a diagnosis of ACS, but these measurements are also not always as closely or easily monitored in veterinary patients. This makes the diagnosis of both IAH and ACS more difficult in both small and large animals.

Certain preventative measures as recommended from the human literature might be easier to implement than others with veterinary patients. More frequent administration of epidurals in those undergoing surgery deemed to be at risk for postoperative IAH may increase the prevention of ACS in veterinary patients. NG tube placement for emptying of luminal contents and enemas to empty colonic contents are easily achievable, but other preventative measures like dialysis or neuromuscular

blockades might prove to be difficult for most veterinary practices.

When ACS is strongly suspected or confirmed in veterinary patients, surgical decompression should be recommended. Following surgical decompression people are typically managed with an open abdomen, and the decision to manage our veterinary patients in this manner should be made on a case-by-case basis. People managed with an open abdomen postoperatively have good success rates[78] and this might be achievable in veterinary patients as well.[80,81] Lastly, the element of financial capability is often a large component to decision making in veterinary patients, and because of this the recommendation of surgical decompression for treatment of ACS might not be as economically feasible in veterinary medicine as it is with people.

Thoracic compartment syndrome

Thoracic compartment syndrome (TCS) is the dysfunction of organs and tissues within the thoracic cavity secondary to an increased intrathoracic pressure. In people it has also been referred to as a "tight mediastinum."[1] TCS is the rarest form of CS and is just now gaining more recognition in human medicine. The most common situation in which TCS is encountered is following open heart or intrathoracic surgery. Human cardiologists have acknowledged this phenomenon for the last couple of decades and have found that after intrathoracic surgeries a significant amount of edema and inflammation secondary to tissue manipulation at surgery results in a significant increase in intrathoracic pressure following sternal closure.[85]

Clinical signs are vague and include increasing airway pressure, decreasing cardiac output, worsening acidosis, and hemodynamic instability.[21,86] TCS is the 1 CS where diagnosis does not require measurement of pressures but the syndrome is suspected based on worsening clinical signs and then confirmed via response to therapy (ie, decompressive thoracotomy).

Risk factors for TCS include overaggressive resuscitative fluid therapy during a prolonged preoperative period, significant chest trauma resulting in tissue edema, significant intrathoracic bleeding, and patients undergoing open heart surgery.[21,87] The most documented and recognized cause of TCS is myocardial and pulmonary edema secondary to manipulation in open heart surgery.[85] The development of edema from manipulation and secondary to concurrent fluid therapy can cause TCS to develop as soon as the chest is closed. Other documented cases include significant thoracic trauma, which might result in significant intrathoracic hemorrhage,[21,87] or complications from routine intrathoracic surgery.[86] It has been hypothesized that components of ischemia-reperfusion syndrome may contribute to the postoperative development of TCS,[21] but in any case where edema or hemorrhage is involved, direct compression on the lungs or heart can contribute to impaired functionality.

At this time no documented cases of TCS have been reported in the veterinary literature. It is not known whether the condition does not occur or is just not recognized in veterinary patients. In theory, situations where it may be encountered in veterinary medicine include postoperative intrathoracic surgery or in patients with extensive thoracic trauma.

As with all other CSs, the treatment of TCS entails decompression via opening the chest surgically, and delayed sternal closure following decompression is advocated.[1] The sternum exerts a significant amount of pressure on the thoracic cavity and the heart specifically, and opening the sternum is similar to relieving pressure exerted on the heart in pericardial disease.[88] Although infection and subsequent sepsis seem like significant complications associated with delayed sternal closure, in one study of 150 children undergoing delayed sternal closure none died from sepsis and only 15 developed minor skin infections at the site of surgery.[85]

In theory, chest tubes would eliminate the possibility of TCS developing, but they were present in several documented human cases where the syndrome developed.[86,87] Therefore, chest tubes are not preventative of TCS.[86,87] As with other CSs, aggressive pain management might help prevent development of TCS. There is still some controversy on the ideal approach to thoracotomy (sternal versus lateral) and whether or not 1 approach is more painful than the other. A human study of 815 thoracotomies found that those undergoing median sternotomy had a shorter postoperative hospital stay as opposed to those undergoing lateral thoracotomies.[89] Additionally, a preliminary experimental study in dogs found that the intercostal approach may be more painful because of secondary nerve entrapment depending on the closure technique used.[90] This suggests that using the median sternotomy approach may be advantageous to the patient, and with regards to TCS it might enable the surgeon to quickly reopen the area causing the increase in intrathoracic pressure to relieve the consequences of TCS.

In summary, TCS is one of the CSs that relies exclusively on the clinician for identification, so an awareness of the syndrome is very important. Although situations where TCS may arise are very rare in veterinary medicine, an awareness of the condition may help clinicians make appropriate surgical recommendations in situations where patients are decompensating. One of the more commonly encountered situations where awareness of TCS would be very beneficial in small animal patients is after sustaining thoracic trauma. In cases of significant hemothorax and pulmonary contusions the

use of low volume resuscitation with hypertonic saline also helps decrease the risk of TCS[87] and should be recommended for veterinary patients with this sort of trauma. Additionally, in patients with significant thoracic disease that have an acute or unexplained decompensation, the development of TCS should be considered and potential thoracotomy should be discussed. As thoracic surgery becomes more common in small animal medicine, knowledge of this condition will hopefully help clinicians develop plans for intervention when postoperative patients decline. In veterinary patients where TCS appears to be developing, management with delayed sternal closure may be possible with significant sedation and ventilation for a limited period of time.

Conclusions

All forms of CS deserve more attention in veterinary medicine as they are all associated with various complications and significant morbidity and mortality. An understanding of both the AV pressure gradient theory as well as the ischemia-reperfusion phenomenon will help clinicians understand the repercussions of CS. The growing abundance of human literature may help guide veterinarians in managing all forms of CS. As the individual CSs discussed in this review article are more readily recognized, the clinician is urged to look at the impact of any individual CS from a more global perspective. Rather than viewing the problem as a single compartmental issue, the clinician should recognize the systemic effects that occur throughout the body in any CS. As it is being increasingly acknowledged in human medicine, considering these syndromes as a "polycompartment" syndrome will help the clinician understand how important and detrimental their effects can be on the entire body.[91]

Footnote

[a] Daugherty EL, Liang H, Taichman D, et al. Abdominal compartment syndrome is common in the medical ICU patients receiving large volume resuscitation. Abstract. Crit Care Med 2006; 32(12S): A83.

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State-of-the-Art-Reviews

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Hyperbaric oxygen therapy. Part 1: history and principles

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Abstract

Objective – Review the historical development and physiologic principles of hyperbaric oxygen therapy (HBOT) based on human and veterinary experimental literature and current equipment in use.

Data Sources – Review of basic physiologic concepts. Data from human and veterinary journals were reviewed through Pubmed and Veterinary Information Network database searches as well as reference searches on several articles covering hyperbaric therapy in clinically applicable situations.

Human Data Synthesis – HBOT has been gaining acceptance as an adjunctive treatment in human medicine. The understanding of the physiology and application of hyperbaric therapy is increasing through ongoing research and greater access to hyperbaric equipment.

Veterinary Data Synthesis – Several animal models have been utilized to examine the effects of HBOT. Most models utilize dogs and rats but pigs, cats, and other species have been studied.

Conclusions – Hyperbaric therapy utilizes several physiologic principles of how gases respond under pressure and more specifically of how oxygen responds under pressure. The increase in concentration of oxygen in solution, based on its solubility under pressure, increases the diffusion gradient for its delivery deeper into tissues, which is the premise of HBOT. Ultimately the increases in dissolved oxygen generated by hyperbaric therapy have several physiologic effects that can alter tissue responses to disease and injury. As this technology becomes more available to clinical practice, HBOT should be considered as a therapeutic option.

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Keywords: HBOT, hyperbaric chambers, oxygen concentration, pressure

Introduction

Hyperbaric therapy, although a relatively old therapy, is increasing in use as a treatment option in both human and veterinary medicine as more chambers become available and knowledge of its benefits increase. It has several applications in emergency conditions such as carbon monoxide poisoning, envenomation from spider and snake bites, compartment syndrome, and central nervous system injury, as well as in more chronic disease states such as delayed wound healing. The aim of this article is to review the history, physiology, and equipment available for hyperbaric therapy. A companion article will review the human and veterinary literature examining the indications, contraindications, animal research literature, and current clinical usage of hyperbaric therapy.

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History

In 1662, a British physician, Henshaw, first utilized compressed air for hyperbaric therapy. An English scientist named John Priestly first discovered oxygen in 1775, which ultimately would have a profound effect on hyperbaric medicine. Unfortunately, Lavoisier and Seguin reported ill-defined toxic effects of concentrated oxygen in 1789, thereby increasing the hesitation to use hyperbaric oxygen therapy (HBOT).[1] In 1878, Paul Bert documented more clearly the toxic effects of oxygen on the central nervous system that were manifested as seizures.[1] In spite of the prevailing idea that excess oxygen was toxic, Arntzenius did a review in 1887 noting up to 300 references in the literature to hyperbaric therapy indicating that early interest in hyperbaric medicine was blooming.[1] During this time, several historic hyperbaric chambers were built, including the first

hyperbaric chamber constructed in North America, built in 1860 in Oshawa, ON, Canada, followed closely in 1861 by the first chamber in the United States that was built in New York. Cunningham in Kansas City, KS built the most well known and utilized hyperbaric chamber in the United States in 1921, which became the only operational chamber in the world by 1925. In 1928, he built the largest chamber in the world in Cleveland, OH that measured 64 feet in diameter, was 5-stories tall and had 12 bedrooms on each floor.[1]

References on the use of hyperbaric oxygen in animals can be traced back to 1887 when Valenzuela studied the effects of HBOT on rabbits demonstrating a decrease in febrile events and increased survival after they had been injected with the *putrid contents* from a dead rabbit.[2] In 1937, Behnke and Shaw first used hyperbaric oxygen successfully for the treatment of decompression sickness. Since that time, HBOT has been utilized in the treatment of numerous medical conditions[3] including carbon monoxide poisoning, infections, and trauma, and research continues to prove its effectiveness in several others such as wound healing in diabetics.[4,5]

Gas Laws

The principles of HBOT are based on how gases of different solubilities, most importantly oxygen, behave under changing pressures and volumes, within tissues and fluid described by Henry's, Fick's, and Boyle's Laws of gas behavior. Henry's Law describes how the pressure of gas affects its concentration within a tissue or fluid. Henry's Law states that the concentration of a dissolved gas ($Conc_{gas}$) equals the pressure (P) times the solubility coefficient (Sol) of that gas.

Henry's Law

$$Conc_{gas} = P(Sol)$$

The 3 main gases of concern in HBOT are oxygen, carbon dioxide, and nitrogen. The solubility coefficients of these gases at normal body temperature are as follows:

Oxygen	0.024
Carbon dioxide	0.57
Nitrogen	0.012[6]

From this, it is evident that carbon dioxide is 24 times more soluble than oxygen and 48 times more soluble than nitrogen and that oxygen is twice as soluble as nitrogen. The delivery of a gas to the tissues is dependent, not only on the concentration of a gas in solution, but also on its diffusion into the tissue, both of which can be affected by pressure. Where Henry's Law determines the concentration of a gas within a tissue or fluid, Fick's Law describes the rate of diffusion of a gas through tissues or fluids.

Fick's Law states that the gas flow (volume of gas per unit time [Vgas]) through a tissue or membrane is equal to the area (A) divided by the thickness (T) multiplied by the diffusion constant (D) times the difference in partial pressures (P_1–P_2) of the gas across the tissue or membrane. The partial pressure of a gas is calculated by multiplying the pressure of the mixture of gases times the percentage of that mixture that is a particular gas.

Fick's Law

$$V_{gas} = \frac{A}{T} D(P_1 - P_2)$$

As the area and thickness of a tissue or membrane is usually unmeasurable, the equation is often reduced to gas flow is equal to the diffusion constant times the change in partial pressure.

Fick's Law

$$V_{gas} = D\,(P_1 - P_2)$$

The diffusion constant is proportional to the solubility of the gas (Sol) divided by the square root of the molecular weight (MW) of the gas.

Diffusion constant

$$D = \frac{Sol}{\sqrt{MW}}$$

Carbon dioxide is slightly heavier than oxygen in molecular weight, but as mentioned above, the solubility coefficient of carbon dioxide is 24 times greater. This means that carbon dioxide will diffuse through tissue 22 times faster than oxygen over the same distance and under the same pressure. If there is an increase in the partial pressure difference (driving pressure) between 2 tissue areas, not only the rate of diffusion, but the distance the gas diffuses into the tissue will increase. The driving pressure of a gas can be increased by either increasing the fraction of an inspired gas or the atmospheric pressure. In this way, inspiring oxygen enriched air under hyperbaric conditions, the pressure gradient of oxygen is increased allowing the oxygen to diffuse further into tissues especially those that may be increased in thickness secondary to inflammation or those that have decreased blood flow. Another method of increasing the relative oxygen concentration is through vasoconstriction of well-oxygenated tissues so that the body can divert oxygen to less well-oxygenated tissues.

Finally, Boyle's Law relates to how volumes of gas behave under pressure. Boyle's Law states that with increasing pressure (P) the volume (V) of a gas decreases proportionately.

Boyle's Law

$$P_1V_1 = P_2V_2$$

This means a volume of gas will be halved when the pressure is doubled and conversely that the volume of a gas will double when the pressure is halved. This becomes important when gases are trapped in various cavities during compression and decompression of the patient and is the main cause of barotrauma. When the patient is compressed if there is air trapped within a body cavity the volume will contract and may alleviate some clinical conditions. Alternatively, when the patient is decompressed the trapped gas will expand and may cause complications.

Physiology

Oxygen is needed to provide energy and support cellular respiration. Decreased delivery of oxygen can affect cell survival. Injury or disease decreases the body's ability to transport oxygen to the tissues, increases the tissue demands for oxygen, and may increase the distance that the oxygen must travel from the capillary to reach the cell. Conditions such as hemolytic anemia, toxin exposure, and hemorrhage can affect the body's ability to transport oxygen. Infections and tissue healing can increase the demands for oxygen. Edema, decreased perfusion and microthrombosis can affect the distance that oxygen must travel from the patent capillaries to the cells.

There are several pressure gradients that exist to help bring oxygen to the cells as well as to transport byproducts, most notably carbon dioxide, away from the cells. In an ideal system the gradients for oxygen, called the oxygen cascade, start with ambient air that has a PO_2 of 160 mm Hg at sea level. The air then enters the respiratory tract and becomes diluted by water vapor to yield an alveolar PO_2 of 104 mm Hg. The mixed venous blood coming into the lung has a PO_2 of approximately 40 mm Hg so oxygen diffuses down the pressure gradient from the alveoli into the blood reaching an arteriolar PO_2 of 95 mm Hg. The arteriolar PO_2 is a reflection of oxygen bound to hemoglobin for transport as well as oxygen that is dissolved in the plasma phase of the blood. In the capillaries, the oxygen again flows down the pressure gradient to 40 mm Hg in the interstitium. From here, the oxygen diffuses into the cells, which have a PO_2 of 3–40 mm Hg but average about 3 mm Hg. The cells require an intracellular PO_2 of 1–3 mm Hg to fully support metabolic processes[6] (Figure 1). The absolute values in any given area in the pulmonary or circulatory system depend on many variables including, but not limited to, barometric pressure, inspired oxygen concentration, ventilation, and oxygen delivery and uptake to name of few. However, the general principle still holds that a pressure gradient is required to allow oxygen diffusion.

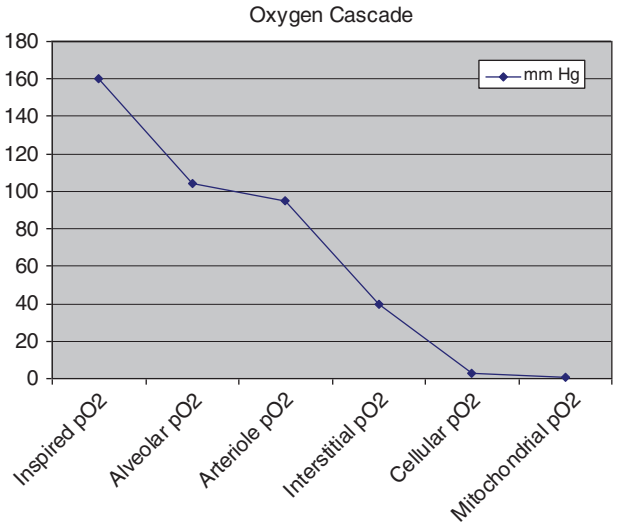

Figure 1: The diffusion gradients along the oxygen supply chain to the tissues of a normal body at sea level. Modified with permission from Nunn's Applied Respiratory Physiology[11] (Elsevier Publishing Inc.).

Oxygen is far more soluble in lipid than in water so the diffusion of oxygen in tissues becomes limited by its rate of diffusion through the fluid portions of the system (ie, plasma, interstitial fluids, and cytoplasm). Under normal atmospheric pressure, there is a limit to the amount of oxygen that can be carried in blood, which is quantified by the equation for arterial oxygen content (CaO_2). The CaO_2 equals 1.34 times the hemoglobin concentration (Hgb) times the arterial saturation of oxygen (SaO_2) plus 0.003 times the arterial partial pressure of oxygen (PaO_2).

Arterial oxygen content

$$CaO_2 = (1.34 \times Hgb \times SaO_2) + (0.003 \times PaO_2)$$

Because the hemoglobin is 97% saturated in the normal body at sea level, limited improvement in oxygen delivery to tissues can be achieved by increasing hemoglobin saturation. However, the concentration of dissolved oxygen in the plasma can be influenced greatly with hyperbaric therapy. Based on Henry's Law, increased pressure will cause more gas to go into solution, and therefore more oxygen will be transported in the plasma. Increasing the concentration of a gas within a fluid increases its partial pressure within the fluid. The increased partial pressure increases the driving force for diffusion and thereby increases its diffusion distance as defined by Fick's Law. Additionally, it is the oxygen dissolved in plasma that is most bioavailable to the tissues. By increasing the PaO_2 in arterial blood, more oxygen can be delivered deeper into the tissues. Increasing the pressure from 1 atmosphere absolute (ATA) to 2–2.5 ATA, which is the typical work-

ing pressure with hyperbaric therapy, the oxygen dissolved in plasma increases approximately 3-fold if the patient is breathing room air. If the inhaled oxygen concentration is increased to 100% under pressure, the plasma oxygen concentration increases by almost 17-fold. In theory, with 100% oxygen at 2.5 ATA, enough oxygen can be dissolved in plasma to meet the normal requirements of the body at rest without the need for hemoglobin.

The delivery of oxygen is also affected by perfusion and by variable degrees of vasodilation and vasoconstriction within different tissues. Typically, arterioles and venules vasoconstrict at high oxygen tensions ($PO_2 > 500$ mm Hg) and primarily is thought to be related to a decrease in the availability of endogenous nitric oxide. This is a protective mechanism in response to hyperoxia to protect tissues from increased oxidative damage. Despite the decrease in blood flow, overall tissue oxygenation remains normal because of the increased PO_2. In ischemic and postischemic tissue, these vasoconstrictive mechanisms are impaired, thus, allowing for improved oxygen delivery. Additionally, carbon dioxide build up in these areas contributes to vasodilation and is a more potent vasodilator than the oxygen is a vasoconstrictor. This vasodilation and enhanced oxygenation of injured tissues helps to preserve the ATP levels and to inhibit swelling and edema formation by maintaining energy-dependent cellular functions.

Although carbon dioxide removal from the tissue is impaired by the saturation of the hemoglobin with oxygen, it has minimal effects on the venous PCO_2, with increases as little as 5 mm Hg. As the hemoglobin transport system is responsible for only 20% of CO_2 removal from tissues, the bicarbonate system and increased plasma carrying capacity limit the overall impact of decreased hemoglobin removal thereby limiting any further rise in venous or tissue PCO_2 levels.[7]

In addition to its effects on cellular function, HBOT affects the immune system. Oxygen has an antimicrobial effect especially in anaerobic infections. The oxygen-derived free radicals that are formed in the reperfusion state have bactericidal affects. Likewise, HBOT stimulates phagocytosis within affected tissues. HBOT also has been shown to have beneficial effects on fibroblast activity,[8] angiogenesis,[9] and modulation of neutrophil activity,[10] which will be discussed further in a companion article reviewing the experimental literature.

Equipment

There are currently 3 types of chambers in use for delivering hyperbaric therapy. In human medicine, the most common chambers are high-pressure multiplace and monoplace chambers with low-pressure monoplace chambers being relatively new. A multiplace chamber is designed to be compressed with air and accommodate several patients who are individually breathing oxygen usually through a mask or hood. The multiplace chambers allow for a higher volume patient load as well as being adaptable for more critically ill patients by allowing an attendant to be present in the chamber during therapy to address any complications that may arise. Monoplace chambers are designed for a single person and the compressed gas is oxygen, negating the use of a mask or hood. These are more frequently used in smaller facilities allowing for more individualized therapy and are often found in chronic wound treatment centers. Most of these chambers are designed to operate at high pressure usually in the 2–2.5 ATA range. Newer low-pressure collapsible monoplace chambers are available that operate in the 1.2–1.3 ATA pressure range. These chambers are currently used most frequently for in-home use, and by plastic surgeons to improve postoperative recovery. They may be more attractive in some situations due to their portability, lower cost, and increased availability. Because these low-pressure chambers are relatively new, differences in therapeutic benefits have not been researched; however, based on the principles of hyperbaric therapy, tissue oxygen delivery will be improved even though it will not be to the same degree as with the high-pressure units.

Conclusions

Henry's, Fick's, and Boyle's Laws of how gases behave under pressure are crucial in the understanding of how hyperbaric therapy exerts its physiologic effects. These physiologic effects include enhanced, or improved oxygen delivery to cells, antimicrobial effects, stimulation of phagocytosis, stimulation of fibroblasts, modulation of neutrophils, and angiogenesis. Hyperbaric therapy has been in existence for over 350 years suggesting it has stood the test of time and is deserving of consideration as a therapeutic modality.

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Hyperbaric oxygen therapy. Part 2: application in disease

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Abstract

Objective – Review the mechanisms of action and clinical application of hyperbaric oxygen therapy (HBOT) based on human and veterinary clinical and experimental literature.
Data Sources – Pubmed and Veterinary Information Network databases were searched for human and veterinary journal articles on hyperbaric therapy in clinically applicable situations. Historical reference searches on several articles in addition to basic physiologic concepts were also reviewed.
Human Data Synthesis – HBOT has gained acceptance as an adjunctive treatment in clinical conditions other than diving-related injuries, such as select problem wounds and central nervous system diseases, in human medicine. Access to hyperbaric therapy has increased and ongoing research has furthered understanding of the mechanisms and potential therapeutic uses of HBOT.
Veterinary Data Synthesis – Several animal models have been utilized to examine the effects of HBOT; primarily rodents (mice, rats) and rabbits but also dogs, cats, and pigs. Data related to animal model research as it pertains to clinical application of HBOT is reviewed.
Conclusions – There is a substantial body of literature that has examined the adverse and beneficial effects of HBOT in animal models. As technology becomes more readily available to clinical practice and more clinical trials are performed to define its effectiveness, HBOT may be considered as an additional therapeutic option in many conditions including select problem wounds, spinal cord injury, and cerebral ischemic injury. Understanding the mechanisms by which HBOT exerts its effects will help guide research and use of the modality in clinical patients.

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Keywords: angiogenesis, HBOT, nitric oxide, reperfusion injury, sepsis, wound healing

Clinical Application

Historically, citations can be found in the literature regarding the use of hyperbaric oxygen therapy (HBOT) in several conditions in animals (primarily rats and dogs) dating back to the 1950s and 1960s. Cited conditions include bowel obstruction,[1] myocardial ischemia,[2,3] anaphylactic and hypovolemic shock,[4–7] severe anemia,[8] and anaerobic infections.[9] The more recent literature discusses uses in immunomodulation,[10–14] wound healing,[15–18] central nervous system injury and disease,[19–24] and sepsis.[25,26] A companion article reviewed how gases behave under pressure to understand how HBOT exerts some of its physiologic effects.[27] Briefly, the volume of a gas decreases as pressure increases (Boyle's law); the solubility of a gas is proportional to the pressure of the gas in equilibrium with the liquid (Henry's law); and diffusion radius increases as the concentration gradient increases (Fick's law). These principles determine the rate and distance of diffusion of gases within body tissues and fluids. Gas volume and solubility changes created by changes in pressure are the main causes of adverse effects associated with hyperbaric therapy. The aim of this article is to review the mechanisms of action of HBOT and some of the conditions where it might be useful in a clinical setting as well as indications and contraindications for its use.

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Mechanisms of Action

In a hyperbaric oxygen environment, the effects of pressure, and changes in solubility and diffusion characteristics of gases lead to several of the physiologic effects seen with this therapy. The physiologic effects of HBOT include intravascular and tissue gas bubble reduction, improved oxygenation, vasoconstriction, increased antimicrobial activity, modulation of inflammation and immune function and angiogenesis. The following is a

discussion of each of these effects and potential applications in the treatment of disease or injury.

Gas bubble reduction

The use of HBOT to reduce gas bubbles in the circulation and tissues is 1 of the oldest applications of this therapy. HBOT is widely accepted for the treatment of air or gas embolism[28,29] because increased pressure decreases the volume of the gas in addition to increasing the solubility of gases such as nitrogen and carbon dioxide, which aids in resorption and elimination of the air or gas embolism. Gas emboli can be seen as a complication after various surgical and diagnostic procedures, such as neurosurgery or angiography,[30] as well as being the cause of decompression sickness in divers,[31] commonly called *the bends*. In a prospective experimental pig model, HBOT decreased the deleterious effects of cerebral air embolism on intracranial pressure (ICP) and brain metabolism.[31] A retrospective human clinical study identified HBOT, with or without conventional nasogastric or intestinal tube decompression, to be beneficial in ameliorating symptoms of gas accumulation in bowel loops caused by postoperative paralytic ileus.[32]

Improved oxygenation

Blood and tissue oxygen tensions were documented to remain elevated for over an hour following a single HBOT treatment in an experimental rat wound model.[15] Improved oxygenation is the mechanism by which HBOT has its primary beneficial effect in conditions such as carbon monoxide (CO) toxicity and select problem wounds. HBOT is widely accepted for the clinical treatment of CO toxicity. In a prospective human experimental study it was shown to enhance CO's dissociation from hemoglobin.[33] In spite of resolution of carboxyhemoglobinemia, delayed injuries to the cerebral cortex, such as cognitive sequelae, can occur. In experimental rat models, these delayed injuries are associated with impaired energy production[34] and prolonged mitochondrial oxidative stress.[35] Two randomized controlled double-blinded clinical trials in humans yielded conflicting results when comparing HBOT with normobaric oxygen therapy for treatment of adverse cognitive sequelae after CO toxicity. One trial showed no benefit or worse outcome with HBOT[36] while a more recent study demonstrated less frequent adverse cognitive sequelae in the HBOT group.[37]

The hyperoxic effects of HBOT are used to enhance healing in hypoxic wounds associated with arterial insufficiency and diabetes in humans.[28,29] A prospective clinical study in human diabetics showed improved spontaneous healing in chronic nonhealing wounds with HBOT through improvement of wound oxygen tension.[38] In cell culture models, the proposed mechanism for the improved wound healing is maintenance of a critical level of hyperoxia required for fibroblast stimulation[39] and increased production of growth factors, thereby enhancing fibroblast proliferation.[39,40] Fibroblasts are important for the generation of collagen and ultimately to wound healing and strengthening.[41–44] In a laboratory rat tubed pedicle flap model, the percentage of flap survival was improved with HBOT by sparing the margins of the flap where blood supply was most compromised[45]; however, an experimental model of uncompromised fresh and granulating wounds in horses showed HBOT impaired healing of full thickness skin grafts,[18] suggesting variable effects of HBOT in hypoxic versus normal wounds.

Vasoconstriction

In experimental rat models, normal vasculature constricts in response to hyperoxia,[46,47] while tissue oxygenation is maintained because of the increased arterial dissolved oxygen content. Experimentally in animals, vasoconstriction decreases tissue exposure to reactive oxygen species (ROS) such as superoxide that are generated under hyperbaric conditions.[19] Vasoconstriction also helps reduce tissue edema formation.[48] Nitric oxide (NO) is an important smooth muscle relaxant that leads to vasodilation. In ischemic tissue in experimental rat models, increased NO activity can inhibit progressive arteriolar vasoconstriction,[46] which could explain differences in healthy and ischemic tissue reactions to HBOT.[46,47–51] In contrast, the ability of HBOT to decrease inducible nitric oxide synthase (iNOS) expression in several rat lipopolysaccharide-induced septic shock models, reduces NO production and helps maintain vascular responsiveness[52,53] as evidenced by minimal changes in mean arterial pressure. Maintenance of vascular responsiveness has been correlated to decreased mortality in some studies.[54,55]

Decreased vasogenic edema has been demonstrated in animal models of cerebral ischemic events[19,56] and traumatic brain injuries.[57] In addition, experimental rodent models have demonstrated that hyperoxic vasoconstriction decreases brain blood flow[47] and blood brain barrier permeability,[19,56] thereby decreasing ICP[19] and improving ICP dynamics.[20] Correction of blood brain barrier permeability changes and ICP dynamics decrease mortality and neurologic deficits.[19,20]

Antimicrobial activity

HBOT enhances endogenous antimicrobial activity in several ways. HBOT helps restore tissue oxygen tension, which is required for leukocytes to function normally with regards to oxidative killing mechanisms.[58] In addition, increased tissue oxygen levels have direct

bacteriostatic or bactericidal effects on various infectious organisms.[59] These effects were reported in a combined experimental guinea pig model and clinical human case series of clostridial myositis where HBOT improved outcome.[9] Rat models have also demonstrated the antibacterial effects of HBOT in aerobic bacterial infections.[60,61] HBOT was shown in an experimental human clinical study to stimulate phagocytic activity of neutrophils in diabetic patients with infected foot wounds in conjunction with standard therapy including intensive insulin therapy, antimicrobials, and surgical debridement.[62]

Finally, HBOT has been shown to have synergistic effects with antimicrobials. In an experimental rat model of *Escherichia coli*-induced sepsis, HBOT was found to be a useful adjuvant to antimicrobial therapy in eliminating histopathologic injury and biochemical derangements of the liver. Changes in ROS bactericidal effects and increased antioxidant levels were the proposed mechanisms for the improvements seen.[25] Other rat models have shown decreased *Staphylococcus aureus*-induced osteomyelitis when HBOT is used in combination with antimicrobials administered either locally[63] or systemically.[60]

Inflammation and immune modulation

HBOT has been shown to modulate neutrophil and macrophage function, which explains many of its effects during reperfusion injury, inflammation, and immune-mediated disease. In an experimental model of CO poisoning, HBOT inhibited the formation of xanthine oxidase, which thereby decreased lipid peroxidation in the brain during ischemia/reperfusion.[64–66] Increases in NO generation with HBOT have been associated with decreased neutrophil adhesion and sequestration, both from functional inhibition of the neutrophil β2 integrin,[66,67] and through downregulation of endothelial intracellular adhesion molecule-1 expression.[68] In several laboratory models, decreased neutrophil adhesion reduces ROS formation, especially during the reperfusion phase,[46,64,66,68] which decreases production of inflammatory mediators. Laboratory rat models have demonstrated the ability of HBOT to decrease neutrophil sequestration in various tissues such as the lung,[69–73] brain,[74] and intestinal mucosa[75] in response to inflammation.

In a rat stroke model, HBOT decreased cerebral neutrophil accumulation, which in turn decreased infarct volume and reperfusion injury in the ipsilateral hemisphere and improved neurologic outcome.[74] HBOT-induced decreases in microgliosis (proinflammatory) and increases in astrocytosis (neuroprotective) explain some of the improvements in infarct volume.[76] By decreasing neutrophil adhesion to the endothelium, neutrophil infiltration into tissues is limited. Decreased neutrophil infiltration is associated with improved neurologic outcomes in several experimental rat models including permanent ischemic[77,78] and transient ischemic events.[79]Alterations in neutrophil migration and ROS activity during HBOT have also been proposed as explanations for improved neurologic outcomes and attenuated neuronal injury in a postresuscitation cardiac arrest model in dogs.[80]

In addition to its anti-inflammatory effects, HBOT has other immunomodulatory actions. HBOT attenuates disease severity in experimental models of autoimmune disease,[11] and improves graft tolerance through major histocompatability complex protein changes.[12] In a cecal ligation and puncture-induced sepsis model in mice, HBOT showed protective effects that appeared to be linked to enhanced interleukin 10 (IL-10) expression by macrophages.[10] HBOT also decreases the serum concentration of tumor necrosis factor-α (TNF-α) as shown in reperfusion[71] and heatstroke[13] rat models.

Angiogenesis

Experimental animal models have demonstrated HBOT creates the necessary oxygen gradients between the blood and injured tissues to promote angiogenesis[81] and increased blood flow (on the order of 20% increase in mean perfusion).[16,82] Oxygen gradients have been shown to be mandatory in angiogenesis during wound healing through regulation of macrophage-derived growth factors, specifically vascular endothelial growth factor.[16,83,84] Neovascularization is important in helping fight infection as well as in the later phases of wound repair as it facilitates the migration of fibroblasts and epithelial cells that continue the healing process. Improved angiogenesis with HBOT has been associated with improvements in healing in such diverse experimental animal models as burns,[85] cartilage and skin grafts,[17,86] dermal wounds,[82,85,87] and bone healing.[88]

Indications in human medicine

In human medicine the accepted indications for the use of HBOT vary between organizations as well as countries. Few randomized-controlled clinical trials exist on the use of HBOT. Many of the widely accepted uses in human medicine are based on experimental animal models and clinical case experience.[28] The Undersea and Hyperbaric Medical Society performs an evidence-based medicine review of the available literature and publishes a list of indications that is referenced by Medicare and other third party carriers in reimbursement determinations in the United States.[28] HBOT is currently covered by Medicare in the United States for reimbursement in the treatment of several acute conditions including decompression sickness, CO toxicity,

Clostridial myonecrosis and crush injuries, in addition to chronic conditions including refractory osteomyelitis, radionecrosis, and select diabetic wounds. A complete listing of approved indications from Medicare and the Undersea and Hyperbaric Medical Society are provided in Tables 1 and 2.[28,29] A list of conditions not covered for reimbursement by Medicare or considered nonapproved/research conditions is provided in Table 3 for comparison.

Clinical investigational use in veterinary medicine

There are no prospective randomized controlled studies on the indications for HBOT in veterinary medicine. The investigational uses of HBOT in veterinary patients are often based on accepted human indications and are similar to those (approved and nonapproved) reported in human medicine. In small animal medicine, clinical investigational cases include: brain and spinal cord injury, postoperative patients with intervertebral disc herniation, pancreatitis, peritonitis, pyothorax (especially Nocardiosis and Actinomycosis), postcardiopulmonary cerebral resuscitation neurologic impairment, severe soft tissue inflammation, aortic embolization, and post-traumatic and reperfusion myocardial injury.[89] Additional references also include treatment for skin flaps,[90] refractory osteomyelitis, clostridial infections, acute traumatic ischemias,[91] and rattlesnake envenomations.[a] In equine medicine, proposed applications include: laminitis, osteomyelitis, desmitis/tendonitis, postsurgical wounds, slow-healing wounds, thermal burns, smoke inhalation, rhabdomyolysis, head trauma, peripheral nerve trauma, anaerobic infections, lymphangitis, intestinal surgeries, envenomations (spider and rattlesnake),[92,93] internal abscesses, dummy foals,[94] and infertility.[95] Another proposed use for HBOT is to enhance recovery from athletic performance in horses.[93]

Table 1: This table presents a list of indications for hyperbaric oxygen therapy covered for reimbursement by Medicare[29]

Acute conditions	Chronic conditions
Acute carbon monoxide intoxication	Actinomycosis (only when refractory)
Acute peripheral arterial insufficiency	Preparation and preservation of compromised skin grafts
Acute traumatic peripheral ischemia	Diabetic wounds meeting select criteria
Crush injuries and suturing of severed limbs	Chronic refractory osteomyelitis
Cyanide poisoning	
Decompression sickness	
Gas embolism	
Gas gangrene	
Osteoradionecrosis	
Progressive necrotizing infections (necrotizing fasciitis)	

Table 2: This table presents a list of accepted indications for hyperbaric oxygen therapy from the Undersea and Hyperbaric Medical Society[28]

Acute conditions	Chronic conditions
Air or gas embolism	Enhancement of healing in selected problem wounds
Carbon monoxide poisoning with or without cyanide poisoning	Osteomyelitis (refractory)
Clostridial myositis and myonecrosis (gas gangrene)	Skin grafts and flaps (compromised)
Crush injury, compartment syndrome, and other acute traumatic ischemias	
Decompression sickness	
Delayed radiation injury (soft tissue and bony necrosis)	
Exceptional blood loss (anemia)	
Intracranial abscess	
Necrotizing soft tissue infections	
Thermal burns	

Table 3: This table presents a list of nonapproved/research conditions for hyperbaric oxygen therapy from Medicare, and the Undersea, and Hyperbaric Medical Society[28,29]

Nonapproved/Research conditions
Acute cerebral edema
Acute frostbite
Acute or chronic cerebral vascular insufficiency
Acute thermal and chemical pulmonary damage (smoke inhalation with pulmonary insufficiency)
Aerobic septicemia
Anaerobic septicemia and infection other than clostridial
Arthritic diseases
Brown recluse spider bites
Cancer
Cardiogenic shock
Chronic peripheral vascular insufficiency
Cognitive performance, psychology, and chronic neurology (including senility and cerebral palsy)
Cutaneous, decubitus, and stasis ulcers
Headache
Hearing loss
Hepatic necrosis
Multiple sclerosis
Myocardial infarction
Nonvascular causes of chronic brain syndrome (including Alzheimer's disease)
Organ storage
Organ transplantation
Pulmonary emphysema
Sickle cell anemia
Sports and athletic performance
Tetanus

Contraindications in Human Medicine

In human medicine, pneumothorax is the only absolute contraindication for HBOT. This contraindication can be overcome by inserting a thoracostomy tube with a Heimlich valve attached during the HBOT treatment. Relative contraindications include emphysema with bulla formation, asymptomatic pulmonary lesions, history of thoracic or ear surgery, uncontrolled high fever, pregnancy, claustrophobia, and upper respiratory infection.[96] Many of these contraindications are related to the known adverse effects of HBOT, such as barotrauma that can be exacerbated by upper respiratory infection and emphysema, or seizures for which an uncontrolled high fever can be a predisposing factor. Adverse effects from some of these conditions can often be mitigated with medications such as anxiolytics or decongestants administered before HBOT.

Complications

Complications with HBOT are related to the toxic effects of oxygen, including myopia and cataracts,[97–99] barotrauma, decompression sickness, oxidative stress associated with lipid peroxidation as well as the generation of ROS, oxygen-induced seizures, and pulmonary oxygen toxicity.[100,101]

Barotrauma is related to Boyle's law and the pressure trauma caused by volume changes of gases in closed spaces.[96] Sinus and middle ear pain are potential problems during compression and decompression as the gas in these enclosed rigid structures contracts or expands causing pressure differentials across the structure and is the most common form of barotrauma. Pulmonary barotrauma occurs mainly during decompression as gas in the lungs expands. If the lung becomes overdistended it can rupture, which can lead to air embolism, mediastinal emphysema (which can cause cardiovascular compromise), or tension pneumothorax. Barotrauma, especially pulmonary barotrauma, is more problematic in monoplace chambers (single occupant) where the clinician cannot get to the patient until the chamber is decompressed, which may take several minutes even in an emergency. Barotrauma effects are difficult to diagnose in veterinary patients but common sense suggests they should be considered. The patient should be monitored for any sign of anxiety or discomfort during compression or decompression such as head shaking or pawing at the head. If such signs are noted the clinician should evaluate the situation and appropriate steps such as slowing compression, immediate decompression, or premedication before further therapy should be taken.

Decompression sickness, related more to Henry's law, occurs when gas bubbles (primarily nitrogen) form in tissues as solubility is reduced on decompression. Decompression sickness is less common than barotrauma in HBOT and can be treated through recompression of the patient and slowing of the decompression cycle.

The oxidative effects are well correlated with increasing pressure[102]; however, in several experimental rodent studies HBOT has been demonstrated to decrease overall oxidative stress[103–105] by increasing ROS scavengers[105–108] and anti-inflammatory mediators.[10,53,75]

HBOT can cause oxygen-induced seizures,[109,110] as NO effects can increase excitatory CNS activity.[111] The incidence of oxygen-induced seizures in humans has been shown to be $\leq$0.03%,[112,113] and an existing seizure condition unrelated to oxygen therapy is not considered a contraindication to HBOT.[114] There are species differences in susceptibility to oxygen toxicity, based on basal metabolic oxygen consumption[82,115]; therefore, dogs may be more sensitive to oxygen-induced seizures than humans.[89]

Pulmonary oxygen toxicity is another concern with HBOT. Pulmonary oxygen toxicity is dependent on the concentration and duration of exposure to high oxygen concentrations[101] in addition to species and individual variability.[116,117] Patients receiving supplemental oxygen therapy may be predisposed to pulmonary oxygen toxicity with HBOT.[96,100] Many patients who may benefit from HBOT also have conditions that require supplemental oxygen therapy. Strategies such as using the lowest inspired oxygen concentrations possible between sessions[101] and using moderate pressure (1.5–2 ATA) short duration (45 min to 1 h) therapy sessions may help reduce the risk of pulmonary oxygen toxicity in these patients.[118]

Because many of these complications are associated with the increased formation of ROS that overwhelm the intracellular and extracellular antioxidant defense systems, maintaining proper nutritional support and dietary antioxidants such as vitamin E may help to decrease sensitivity to oxidative stress caused by HBOT.[119]

Practical considerations in veterinary medicine

Veterinary patients are reported to accept treatment well. Most will fall asleep during a session but some patients do require an anxiolytic before placement in the chamber. Because sparks from static electricity can cause fires in the 100% oxygen environment in some chambers used in veterinary medicine, metal collars should be removed and preferably metal skin staples be covered. Cotton has less risk of developing static compared with other materials and should be considered

when selecting towels or bandaging materials for use with HBOT patients. The chamber should be grounded and the use of static ground limb straps should be considered.[89,118]

Monoplace chambers are most commonly used in veterinary medicine, which presents challenges for patient access and monitoring such as vital signs, ECG, or perfusion parameters should problems arise. Although HBOT chambers can be rapidly decompressed it still takes several minutes for this to occur, depending on where the patient is in their treatment cycle, and concerns for barotrauma and decompression sickness increase. Some chambers are equipped with integrated monitors or pass through ports to allow monitoring, IV therapy, or mechanical ventilation during HBOT, which mitigates some of these concerns. Additionally, personnel should be properly trained in patient monitoring, chamber safety, and operations.[120]

Conclusion

HBOT in veterinary medicine is in its infancy and current prospective clinical research is lacking. Understanding the physiology and mechanisms of action of HBOT will help guide case selection and clinical research in HBOT and help establish indicated veterinary uses as well as standardized treatment protocols. Areas of interest may include smoke inhalation, wound healing, and air embolism but also more challenging areas such as postcardiac arrest resuscitation, spinal cord injury, systemic inflammatory response syndrome, and sepsis.

Footnote

[a] Edwards ML, unpublished data.

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The association of physical examination abnormalities and carboxyhemoglobin concentrations in 21 dogs trapped in a kennel fire

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Abstract

Objective – To evaluate physical examination findings and their association with carboxyhemoglobin (COHb) concentrations in 21 dogs that were exposed to smoke during a kennel fire.
Series Summary – Twenty-one dogs were exposed to a kennel fire. Physical exam findings, presenting, and posttherapy COHb concentrations as well as therapeutic interventions were evaluated. COHb concentrations upon presentation were increased in all smoke inhalation exposed dogs. These dogs were compared to a small set of clinically normal staff-owned dogs who were not exposed to fire. Physical parameters significantly associated with higher COHb concentrations included lower body temperature, increased respiratory effort, abnormal respiratory auscultation, altered neurologic status, and length of hospital stay. Oxygen therapy resulted in a more rapid decline in COHb concentrations although 5 dogs still had mildly increased COHb concentrations 24-hour postadmission.
Unique Information Provided – This study describes the relationship of admitting clinical findings of dogs exposed to a kennel fire with their initial blood COHb concentrations. It also describes the resolution of increased COHb concentrations with use of oxygen therapy and hospitalization. Additionally, COHb concentrations for a control group of dogs was evaluated and compared to the dogs exposed to smoke inhalation.

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Keywords: carbon monoxide, respiratory distress, smoke inhalation

Introduction

Smoke inhalation in small animals and people is most commonly associated with structural fires, although exposure to sources of pure carbon monoxide (CO) has been described.[1–5] Case studies of CO toxicity have been uncommonly reported in the veterinary literature, possibly because of a high preadmission mortality.[6] Additionally, measurement of carboxyhemoglobin (COHb) is not readily available making verification of CO toxicity difficult.

Abbreviations

BAER	brainstem auditory evoked response
CO	carbon monoxide
COHb	carboxyhemoglobin
MRI	magnetic resonance imaging
HBO	hyperbaric oxygen
V/Q	ventilation-perfusion

Smoke inhalation and accompanying CO toxicity have many mechanisms for their complex pathophysiology. Most importantly, COHb displaces oxygen from oxyhemoglobin and results in cellular hypoxia. This occurs by CO binding to hemoglobin with more than 200 times the affinity than that of oxygen to hemoglobin as well as shifting the oxygen–hemoglobin dissociation curve to the left thus resulting in tissue hypoxia.[7] Also, CO

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binds to substances such as cytochromes, myoglobin, and guanylyl cyclase.[8] This protein binding may lead to the generation of oxygen-free radicals through disruption of oxidative metabolism and subsequent hypoxia.[8] Also, stimulation of guanylyl cyclase can result in cerebral vasodilation and loss of consciousness.[8] Carbon monoxide toxicity also increases nitric oxide production and leads to free radical formation, leukocyte adhesion, and activation in brain microvasculature, which may be a mechanism for delayed neurological sequelae.[8] Other proposed mechanisms of CO toxicity include glutamate-mediated neuronal injury, involvement of cytochrome P-450 and apoptosis.[8]

The high heat of smoke inhalation can cause thermal damage that is most often limited to the upper airways. The lower airways, however, are not spared from pulmonary irritants. Irritants cause chemical and mechanical acidic and alkaline mucosal burns and reflex bronchoconstriction can occur.[a] The combination of these factors can lead to a severe ventilation-perfusion (V/Q) mismatch and subsequent worsening of hypoxia.

The clinical signs associated with smoke inhalation are highly variable. This variability is attributable to many factors including the intensity of the heat and flames and the duration of exposure to the fire, smoke, and noxious gases. Systems commonly affected include the respiratory, neurologic, ophthalmic, and dermatologic.[8]

COHb concentrations are routinely performed on suspected CO toxicity human patients.[8] Human nonsmokers are expected to have a normal baseline level between 1% and 3%. Low COHb levels (<20%) are typically associated with mild symptoms and concentrations greater than 60–70% are usually rapidly fatal.[8] The relationship between intermediate COHb levels (20–60%) with clinical signs and outcomes in humans is debatable.[9]

Though smoke inhalation has been described in small animals, very little information is provided in the veterinary literature documenting CO toxicity in these patients or the relationship of COHb to physical examination findings. One of the challenges in characterizing this relationship in veterinary medicine is the lack of a normal reference interval for COHb values. The primary goal of this study is to characterize the COHb concentration in a group of dogs that were exposed to a structural fire and to relate the concentration of COHb with clinical parameters, to describe the use of oxygen therapy in these patients and document the duration of hospitalization in these dogs. A group of clinically normal dogs served as controls to enable comparisons between groups.

Materials and Methods

Study dogs

Complete medical records of dogs that were presented to the emergency service of our hospital after being trapped in a kennel building fire and had COHb concentrations measured were included in analysis. Dogs were excluded from analysis if the medical record was incomplete or pretreatment COHb concentrations were not obtained.

Clinically normal dogs

Carboxyhemoglobin levels were measured in 6 staff-owned, clinically normal dogs that were boarding in the hospital, and these served as a control group. These dogs were from nonsmoking households and they had no exposure to smoke inhalation. They had physical exams performed at time 0 and 12 hours. Venous blood samples (whole blood, sodium citrate tubes) were obtained at time 0, placed on ice until transport to a local human hospital and analysis was performed within 1–2 hours by a standard cooximeter.[b]

Clinical parameters evaluated

Description of the fire scene, signalment, initial body temperature, pulse rate, respiratory rate and effort, pulmonary auscultation (eg, increased bronchovesicular sounds, crackles, wheezes), neurologic abnormalities (eg, presence of altered mentation, disorientation, depression, obtunded state), use of supplemental oxygen and outcome were evaluated.

Blood sampling and handling

Venous blood samples for blood COHb determination were obtained from the smoke inhalation dogs at the time of arrival to the hospital and 24 hours after therapy was initiated. The samples were handled and analyzed in a similar manner as the staff-owned dog samples described above.

Description of fire scene

The fire was located in the mechanical and kitchen end of the building; the dogs were exposed primarily to smoke combustion products while they were in their adjoining kennels. It is suspected that they were exposed to the heat and smoke for approximately 30–60 minutes before the arrival of first responders to the scene. Three dogs were found deceased at the scene. Twenty-two dogs were transported by first responders to the hospital. At the time of presentation, no prior medical history for any

of the dogs was known. One dog was excluded from analysis due to lack of pretreatment COHb levels.

Statistical analysis

Continuous variables were evaluated for normality using the Shapiro–Wilks test. Nearly all of the continuous variables were not normally distributed, so median and range were used as descriptive statistics for consistency. The Mann–Whitney test was used to compare continuous variables between groups while the Wilcoxon signed rank tests were used to compare baseline COHb concentrations with 24-hour levels. Finally, the Spearman rank correlation was used to assess association between 2 continuous variables. For all statistical comparisons, a P value less than 0.05 was considered significant. A commercial statistical software program was used for all analyses.[c]

Results

Signalment

The study population of smoke inhalation dogs comprised 21 dogs, of which 13 were spayed females and 8 were male dogs (7 neutered, 1 intact). Median age was 19 months (range 19 months–14 years). Median weight was 17.2 kg (range 5.5–33 kg). The following breeds were represented: mixed breed ($n = 8$), Labrador retriever ($n = 2$), Springer Spaniel ($n = 2$), Lhaso Apso ($n = 2$), and 1 each of American bull terrier, Chihuahua, beagle, Tibetan terrier, Cairn terrier, Jack Russell terrier, and Australian cattle dog. Age or weight was not significantly associated with admission COHb concentrations.

Staff dogs included 2 spayed females and 4 male dogs (3 intact, 1 neutered). The following breeds were represented: Schanauzer ($n = 2$), and 1 each of the following breeds: Labrador, Queensland Blue Heeler, mixed breed.

Carboxyhemoglobin concentrations at admission

The median COHb concentrations dogs suffering from smoke inhalation was 24.2% (range 8.8–37%) and this was significantly higher than normal controls dogs 6.1% (5.6–6.4%) ($P < 0.001$).

Carboxyhemoglobin and physical parameters

Relationships between presenting physical examination findings with initial COHb levels are summarized in Table 1. Rectal temperature ranged from 32.2°C [90°F] to 39.8°C [103.7°F] (median 38.2°C [100.8°F]). Pulse rate (median 120/min, range 100–180/min) and respiratory rate (median 30/min, range 16–70/min) were not significantly correlated with initial COHb concentrations ($P = 0.06$, $P = 0.3206$, respectively).

Table 1: Relationship between carboxyhemoglobin level (%) in dogs with smoke inhalation with the absence or presence of various clinical signs. Abnormal respiratory auscultation included increased bronchovescicular sounds, crackles, and wheezes. Altered mental status was defined as disorientation, depression, or obtundation

Clinical parameter	Absent	Present	*P*-value
Hypothermia (<37.8°C [100°F])	14.8 (8.8–30.9)	33.0 (24.2–37.0)	0.0005
Respiratory effort	12.4 (8.8–29.5)	31.0 (21.7–37.0)	0.0011
Abnormal respiratory auscultation	11.5 (8.8–26.7)	31.0 (21.7–37.0)	0.0004
Altered mentation/ataxia	18.3 (8.8–37.0)	32.7 (24.2–34.5)	0.0132

Twelve dogs were eupneic at the time of hospital presentation while 9 dogs had some degree of tachypnea and increased inspiratory effort. The median COHb concentration was 31% (range 21.7–37.0%) in dogs with increased respiratory effort and this was significantly higher compared to eupneic dogs (median 12.4%, range 8.8–29.5%) ($P = 0.0011$). However, respiratory rate was not correlated with COHb concentrations ($P = 0.3206$).

Serial COHb concentrations

The median blood COHb concentration 24-hour posttherapy ($n = 21$, median 6%, range 3.4–7.5%) was significantly lower than the median at presentation in all smoke inhalation patients ($P < 0.001$). Six dogs still had COHb concentrations exceeding that the highest control dog blood COHb concentration 24 hours after presentation, although the concentrations were only mildly increased above the control group (range 6.5–7.5%). The median 24-hour posttherapy blood COHb change for all smoke inhalation dogs was 18.4% (range 2.8–31.1%). Fourteen of 21 dogs were treated with supplemental oxygen via either nasopharyngeal oxygen cannula or oxygen cage. Rates of administration were 50–100 mL/kg/min for nasopharyngeal administration and 100–150 mL/kg/min for cage administration. The decision to use supplemental oxygen was based on presenting clinical signs and discretion of admitting veterinarian as COHb levels were not immediately available. Dogs that received oxygen therapy had significantly higher presenting COHb concentrations (median 28.4%, range 14.8–37%) ($P = 0.003$). Additionally, the median absolute change in COHb concentrations was also significantly greater in dogs that received oxygen therapy (21.9%, range 8.8–31.1%) compared to dogs that did not (5.5%, range 2.8–7.6%) ($P = 0.003$). Since initial COHb levels were lower in dogs who did not receive

oxygen, to determine if a true difference existed, the percent change was also evaluated. The change in COHb was significantly greater in dogs that received oxygen therapy (78%, range 59–84%) compared with dogs that did not (48%, range 32–68%) ($P < 0.001$). Five of the 14 dogs (36%) that received supplemental oxygen still had blood carboxyhemoglobin concentrations above those of the control dogs and this was in contrast to 1 of the 7 dogs (14%) that did not receive oxygen supplementation. However, this was not statistically different ($P = 0.613$).

Progress of clinical signs and duration of hospitalization

Five dogs had worsening of clinical signs (3 respiratory, 2 neurologic). Three dogs developed bronchopneumonia based on clinical signs (eg, coughing, increased bronchovesicular sounds, fever), radiographic findings ($n = 3$), and results of a bronchoalveolar lavage ($n = 1$). Pneumonia resolved radiographically and clinically within 10 weeks of exposure in 2 dogs. A third dog with pneumonia was euthanized on day 6 (presenting COHb was 32.7%).

Two dogs with initial neurological abnormalities deteriorated further after admission. One dog (COHb concentration of 32.7% at presentation) that was initially obtunded had a seizure shortly after presentation and remained obtunded until euthanasia on day 6. The other dog (COHb concentration of 31% at presentation) presented alert but with extensor rigidity; became ataxic, disoriented, blind, deaf, and started head pressing on day 3. Magnetic resonance imaging (MRI) findings on day 9 were within normal limits with no evidence of basal ganglia damage, and no evidence compatible with anoxic changes. This dog was still deaf and blind but mentally appropriate at the time of discharge on day 28. The owner reported aggressive behavior 2 months later but this and all of the other neurologic abnormalities resolved at 6-month postexposure including blindness and deafness, although a brainstem auditory evoked response (BAER) test was not performed.

Duration of hospitalization ranged from 2 to 28 days (median 3.0 days). One dog was hospitalized for 13 days and boarded for another 15 days due to slowly improving neurological signs and the owner's preference. Dogs with a longer duration of hospitalization had a significantly higher presenting COHb concentration ($P = 0.017$).

One of the 21 dogs presented did not survive to discharge. A relationship between mortality and COHb levels could not be determined as there was only one nonsurvivor in this population of dogs.

Discussion

This study describes the relationship of admitting clinical findings of dogs exposed to a kennel fire with their initial blood COHb concentrations. It also details the progression of COHb concentrations with use of oxygen therapy and duration of hospitalization. In people, the presence and severity of symptoms associated with CO toxicity are extremely diverse and there is debate if there is a true relationship between the severity of symptoms and COHb levels.[8–10] Clinical findings, such as temperature, respiratory, and neurological signs, and their association with varying COHb levels has only been briefly discussed in 2 veterinary studies.[1,15] In the current study, we identified significant relationships between some presenting clinical signs and COHb levels.

Although, there is debate about whether there is a true relationship between temperature and COHb levels in human patients, a rapid return to euthermia via active heating measures improves survival following acute, severe, CO poisoning.[12] In the present study, dogs with a body temperature less than 37.7°C (100°F) had significantly higher COHb concentrations. A previous report of dogs with smoke exposure found a relatively wide range of rectal temperatures on admission (34.6–39.5°C [94.6–103.2°F]) but COHb concentrations were not reported.[11] Association with body temperature and survival could not be determined in the current study, although it is interesting to note that the single fatality had presented with marked hypothermia (<32.2°C [<90°F]).

Respiratory rate was not correlated with COHb concentrations in the dogs reported in this kennel fire. This appears to agree with a previous veterinary report of 2 cats and 4 dogs.[1] Another large veterinary study showed no difference in respiratory rate between uncomplicated and complicated smoke inhalation dogs, although COHb levels were not available.[11] The lack of correlation between respiratory rate and COHb levels may be due to the fact that ventilation, which is regulated by sensory cells in the carotid body, may not be stimulated until acidosis develops. The carotid body is believed to respond to PaO_2 but since this is not reduced in CO poisoning there is little stimulus to increase ventilation.[16] Blood gas evaluation was not performed in the current study and therefore we cannot evaluate whether blood pH impacted respiratory drive. Additionally, changes in mechanical properties of the airways as well as ventilation responses to airway irritation may also influence changes in ventilation. Although the relationship between COHb concentration and respiratory physical parameters is controversial in people, some studies do suggest that they may be related.[9,12] In one study, tachypnea was noted in people with COHb concentrations between 40% and 50% and respiratory failure was seen at COHb of 60–70%.[9]

Dogs in this study with an increased respiratory effort as well as abnormal auscultation findings had significantly greater COHb concentration compared to dogs with normal effort and breath sounds, respectively. One possible reason for this is that CO competitively and reversibly binds with hemoglobin at the oxygen-binding sites leading to decreased oxygen saturation as well as shifting the oxygen-hemoglobin dissociation curve resulting in decreased tissue off-loading of oxygen. Other possible reasons, unrelated to CO toxicity, include bronchoconstriction secondary to thermal damage from the heat of the fire and changes related to V/Q mismatch.

The pathology of CO neurotoxicity is complex and not fully understood and there is debate in people if severity of neurological signs and COHb concentrations are related.[8,12] Experimental studies have shown that hypoxemia is not the only mechanism for neurotoxicity as hypoxemia can be independent of COHb concentration.[15] Other possible contributing factors include systemic hypotension with subsequent decreased cerebral blood flow and cellular asphyxia that leads to lipid peroxidation by free radicals.[15] A recent report of 3 Chihuahuas exposed to CO evaluated clinical and neuropathological findings, and findings were consistent with that found in people with acute toxicity.[15,16] Histopathologic lesions of the gray matter areas and neuronal sites involved the caudate nucleus, globus pallidus, and substantia nigra (all part of the basal ganglia). In people, MRI lesions of the globus pallius are often considered pathognomonic for CO-induced brain damage.[8,15] Only a single dog in this case series had an MRI performed and no abnormalities were noted.

Dogs with altered mental status at the time of presentation in this case series had a significant increased presenting COHb concentration compared to normal dogs. A study in children with CO toxicity showed a significant correlation between increased COHb levels on presentation and abnormal presenting neurological signs.[18] Carboxyhemoglobin levels of 30–40% in people can lead to CNS demyelination and neurological dysfunction, but as stated above, it is debatable if COHb levels correlate well with the severity of acute neurological signs.[13,17]

All 5 dogs that demonstrated neurological abnormalities had presenting COHb concentrations greater than 24%. Both acute and delayed neurological dysfunction was noted in one dog in this study (presenting COHb level 31%). A delayed neuropsychiatric syndrome (Parkinsons-like) is described in 10–30% of human CO toxicity survivors and can occur between 3 and 240 days after exposure.[15] A similar syndrome has been described in dogs where acute, delayed neurological dysfunction occurred in 46% (5 of 11 dogs) of dogs 3–6 days after presentation.[a] One dog had clinical signs of deafness, although no BAER testing was performed. Deafness has been reported in both the human and veterinary literature as an acute sequelae to CO toxicity.[1,8,13]

In human and veterinary medicine, high-flow oxygen therapy is the standard of care to treat hypoxia induced by CO poisoning and hasten elimination of CO from the body.[8] Since oxygen supplementation shortens the half-life of COHb, it was not surprising that administration of supplemental oxygen to dogs in this study resulted in a more rapid decline of COHb levels than dogs that did not receive supplemental oxygen. Due to the lack of bedside co-oximetry in many veterinary hospitals, the use of supplemental oxygen is often based on clinical signs and clinician preference, as was the case in the current study.

Hyperbaric oxygen (HBO) therapy has been proposed as a treatment modality for many conditions in human and veterinary medicine.[20–22] Although not used in this patient population, HBO therapy is an important and controversial topic in the treatment of COHb toxicity and warrants a brief review. HBO therapy is the delivery of 100% oxygen within a pressurized chamber that results in an increase in the dissolved oxygen content in blood. In people, this leads to a reduction in the half-life of COHb from 240 to 320 minutes on room air (ie, 21% oxygen), 40–80 minutes with 100% oxygen to approximately 20 minutes at 100% hyperbaric oxygen.[8] Other theorized benefits of HBO include maintenance of vascular responsiveness due to HBO's ability to decrease nitric oxide synthesis, enhanced antimicrobial activity, and modulation of inflammatory mediators.[20] These benefits make HBO an attractive therapeutic option for CO toxicity, but in human trials, there is much controversy surrounding its utility.[8] The lack of a definitive answer stems from significant variations in study designs, delivery of oxygen protocols, outcomes measured, and patient population.[8] One recent study evaluated the use of HBO therapy for the treatment of cognitive sequelae (eg, problems with memory, attention, or concentration) in people. Investigators found that multiple doses of HBO reduced the frequency of cognitive sequelae by 46%.[8] Other studies have shown that HBO therapy might worsen outcome.[19] A recent Cochrane review evaluated 7 randomized controlled trials for use of HBO in CO toxicity.[22] Their conclusion was that there is insufficient evidence to support the use of HBO for treatment of patients with CO poisoning. The use of HBO is not without its limitations. Accessibility poses a distinct difficulty in veterinary and human medicine.[20–22] Other disadvantages and risks associated with HBO therapy in people include barotrauma to the ears and sinuses, oxygen toxicity, seizures, pulmonary edema, and hemorrhage, decompression sickness including pneumothorax and nitrogen

emboli.[19] To the authors' knowledge, there are no veterinary studies that have evaluated the use of HBO for CO toxicity at this time.

In people, there is debate whether presenting COHb levels correlate with the duration of hospital stay.[9] In the present study, higher pretreatment COHb levels were positively correlated with a longer duration of hospitalization but a cause and effect relationship cannot be concluded.

Mortality is a difficult parameter to relate with COHb levels in veterinary medicine. This is mainly due to the fact that there are only a small number of retrospective studies on CO toxicity in the literature.[1–3,11,14,15,a] However, even in human medicine with numerous large prospective and retrospective studies, there is debate whether increasing COHb levels influences mortality. In our group of dogs, the presenting COHb concentration was 32.7% in the one patient that was euthanized. There were 3 patients who survived who had higher COHb levels on presentation (33.2–37%).

Limitations of the current study include the small sample size and the fact that all cases stemmed from same event. Moreover, in this patient population then was only one nonsurvivor and thus risks for mortality cannot be assessed. Although COHb is the main cause of acute death from smoke inhalation in people, smoke inhalation has a variety of sequelae, many of which were not evaluated in this group of patients. Some of these sequelae include production of hydrogen cyanide, direct thermal injury, and irritation to the lower airways from irritant gases and particles. No dog in the present study had a presenting COHb >34.5%. In people, this level of COHb (ie, 30–40%) is associated with severe clinical signs.[10] Because COHb concentrations were measured at the time of presentation to the hospital and not at the scene, actual COHb concentrations may initially have been even higher in these dogs. The possible change in COHb levels from time of exposure to time of presentation may have affected our ability to detect correlations between presenting signs and COHb levels.

One final limitation was the lack of a validated reference interval for COHb in clinically normal dogs. In the current study, clinical cases were compared to a group of control dogs. Previous veterinary studies have extrapolated reference ranges from human values.[1] We had 6 clinically normal boarding staff animals available at the time that dogs described in this study were presented and they served as a control group. It is interesting to note that the COHb range for this group of healthy dogs (range 5.6–6.4%, median 6.1%) is slightly higher than the range reported for nonsmoking normal humans (1–3%). A possible explanation for this is the small sample size in our control population as the development of reference intervals usually requires much larger number of subjects. A consideration was also made that lab error was the reason for this difference, but these COHb determination were performed at a human hospital with a validated standard co-oximeter that is calibrated daily. This difference in reference intervals, if true, does argue for the development of a proper reference interval for COHb levels in dogs, as the use of human values may be inappropriate.

Conclusion

In veterinary patients, COHb levels have not been previously correlated to the severity of clinical signs. The findings in this study suggest that increased COHb levels are associated with multiple clinical signs such as altered mental status, abnormal respiratory sounds, increased respiratory effort, and hypothermia. Dogs with higher COHb concentrations also had a significantly longer duration of hospitalization. It is not surprising that the oxygen supplementation lowered COHb levels more rapidly and should be considered for all patients exposed to CO. The determination whether COHb levels consistently correlate with severity of signs require further evaluation.

Footnotes

[a] Jackson CB, Drobatz KJ. Neurologic dysfunction associated with smoke exposure in dogs. J Vet Emerg Crit Care 2002;12(13):193[Abstract].
[b] ABL800 Radiometer America Inc, West Lake, OH, USA.
[c] Stata 11.0 for Windows, Stata Corporation, College Station, TX.

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Mechanisms of injury and emergency care of acute spinal cord injury in dogs and cats

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Abstract

Objectives – To review the literature in regards to the pathophysiology of acute spinal cord injury, and to describe current concepts in regards to patient assessment, diagnostic, and therapeutic measures with a special emphasis on emergency and critical care considerations.
Etiology – Acute spinal cord injury occurs in 2 phases. The primary injury occurs at the time of initial injury and may include intervertebral disk herniation, vertebral fracture or luxation, penetrating injury, and vascular anomalies such as fibrocartilaginous embolic myelopathy. Secondary injury occurs following primary injury and is multifactorial encompassing numerous biochemical and vascular events that result in progression of injury.
Diagnosis – The diagnosis is based on history and physical examination findings. A neurologic examination should be performed following initial patient assessment and stabilization. Further diagnostics to characterize acute spinal injury include radiographs and advanced imaging modalities such as myelography, computed tomography, or magnetic resonance imaging.
Therapy – Initial treatment should focus on addressing the patient's cardiovascular and respiratory system. Supportive measures to support systemic perfusion are vital to minimizing secondary injury. Specific therapy toward minimizing secondary injury in veterinary medicine remains controversial, especially in regards to the utilization of methylprednisolone. Other therapies are either in need of additional research or have failed to document clinical difference.
Prognosis – The prognosis for acute spinal injury is varied and is dependent upon the presence of concurrent trauma, location, and type of primary injury sustained, and extent of neurologic impairment at the time of initial presentation. The etiology of the underlying trauma is of great importance in determining prognosis and outcome. Loss of deep pain is generally accepted as a poor prognostic indicator; however, even these patients can recover depending on their response to treatment.

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Keywords: hypertonic saline, methylprednisolone, neurologic trauma, polyethylene glycol, vertebral trauma

Abbreviations

AMPA	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl) propanoic acid
ANNPE	Acute non-compressive nucleus pulposus extrusion
ATA	atmosphere absolute
CT	computed tomography
FAST	focused assessment sonogram for trauma
MBP	myelin basic-protein
MMP-9	matrix metalloproteinase-9
MRI	magnetic resonance imaging
NASCIS	National Acute Spinal Cord Injury Study
NMDA	*N*-Methyl-D-aspartate
OSF	oscillating field therapy
PN	parenteral nutrition
PPI	proton pump inhibitor
ROS	reactive oxygen species
TSH	thyroid-stimulating hormone
TRH	thyrotrophin-releasing hormone

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Introduction

Acute spinal cord injuries are common in domestic species and the consequences can be devastating. Spinal cord injuries can result in complete or incomplete damage and compromise the major functions of the spinal cord (eg, proprioception, motor, nocioception, reflexes). Unlike in human medicine where heroic measures are often undertaken to manage spinal cord injuries, a large percentage of injured animals are euthanized. Despite

the surgical and medical options that are currently available to treat acute spinal cord injury, many cases of acute spinal cord result in poor outcome. Research in the field of acute spinal cord injury is ongoing and potentially valuable new therapies are being devised with the hope of improving long-term outcome. Understanding the pathophysiology of acute spinal cord injury is necessary to understand the therapeutic options (eg, surgical versus medical) that are currently present and those that are being investigated.

Primary Injury

The primary injury is the event that causes the initial spinal cord trauma and is considered irreversible. Numerous classification schemes of acute spinal cord injury exist in the literature. For the purposes of this review, the 2 broad categories of primary injury that will be discussed are traumatic versus nontraumatic. The principle mechanical forces that are involved in acute traumatic injury are concussion, compression, shear, laceration, distraction, and contusion. Concussion injury refers to when the spinal cord undergoes injury from external forces[1] that can cause rapid acceleration and deceleration of the parenchyma. Compression injury refers to an injury that causes an abnormal amount of pressure on the spinal cord parenchyma.[1] Shearing forces cause injury in which the parallel plane of the spinal cord is shifted in a direction perpendicular to itself.[2] Laceration injury causes tearing of neuronal and axonal tissue within the spinal cord parenchyma.[3] Distraction refers to forcible stretching of the spinal cord parenchyma in the axial plane.[4] Contusion injury refers to spinal cord parenchymal hemorrhage due to damage to the spinal cord vasculature. The most common injuries that cause acute spinal cord injury from the mechanical forces listed above are discussed below.

Intervertebral Disk Herniation

Acute intervertebral disk herniation is a common acute spinal cord injury seen primarily in canine patients (but reported also in cats) with chondrodystrophic breeds predisposed to this condition.[1,5] The anatomy of the intervertebral disk is composed of 3 parts: the outer fibrocartilage annulus fibrosus, an inner nucleus pulposus, and the cartilaginous endplate.[2] Spinal cord injury due to intervertebral disk herniation can be due to either disk extrusion or disk protrusion; the Hansen scheme has been used to describe these lesions in veterinary medicine. Rupture of the annulus fibrosus and subsequent extrusion of the degenerated nucleus pulposus into the vertebral canal is referred commonly in the literature as a Hansen Type I herniation.[2,5,6] Herniation involving dorsal protrusion of the annulus fibrosus that causes a progressive compression is referred in the literature as a Hansen Type II herniation.[2,6] Acute noncompressive nucleus pulposus extrusion[7] (ANNPE) has also been discussed in the literature and are high-velocity extrusions that cause contusion of the spinal cord without any obvious persistent compression,[6] these acute extrusions have been described as Hansen Type III disk injuries.[6,7] A definitive diagnosis for these injuries is only possible through postmortem examination with visual and histological confirmation of extruded nucleus pulposus, ruptured annulus fibrosus, and spinal cord contusion.[7] Characteristic magnetic resonance imaging (MRI) features include an area of focal hyperintensity overlying an intervertebral disk on T2-weighted images, narrowed intervertebral space, reduction in volume and signal intensity of the nucleus pulposus on T2-weighted images, and extruded material within the epidural space dorsal to an affected disk site.[7] Patients that have undergone surgery with presumed type III injuries are reported to have edema and bruising of the spinal cord with minimal evidence of extruded disk material in the extradural space;[6] it should be noted, however, that ANNPE is considered a nonsurgical disease. Type III disk injuries are associated with severe external trauma such as vehicular accidents.

Vertebral Injuries

The three-compartment model[1,5] has been utilized to describe the anatomy of the vertebrae with the concept that disruptions in any 2 of the 3 compartments will result in instability. The dorsal compartment is composed of the articular process, dorsal laminae, pedicles, and the spinous process. The middle compartment is composed of the dorsal longitudinal ligament and the dorsal portion of both the vertebral body and the annulus fibrosus. The ventral compartment is composed of the ventral longitudinal ligament, nucleus pulposus, and the remaining portions of both the annulus fibrosus and vertebral body. Injuries involving the vertebrae include fractures, dislocations, and subluxations.[3,5,7] Vertebral dislocation occurs when a vertebral body is displaced from its normal cranial/caudal synovial joint articulation with its adjacent vertebrae.[5,7,8] Vertebral subluxation occurs when there is partial vertebral displacement from its normal cranial/caudal synovial joint articulation with its adjacent vertebrae.[7–9]

Penetrating Injuries

Penetrating injuries to the spinal cord occur when a projectile penetrates or lacerates the spinal cord. Gunshot or stab wounds are common examples of penetrating

injury.[3,4] Penetration of the spinal cord can also occur as a result of vertebral fractures.

Nontraumatic Injuries

Nontraumatic acute spinal cord injury can be caused by acute disruption to the vascular supply to a section of the spinal cord, the most common being a fibrocartilage emboli. The pathophysiology of fibrocartilaginous emboli remains controversial and several hypotheses have been proposed to its etiology recently discussed in the literature.[10] These include direct penetration of nucleus pulposus fragments into the spinal cord or its vascular system, chronic inflammatory neovascularization of the degenerated intervertebral disk, embroyonic remnant vessels within the nucleus pulposus, and entrance of fibrocartilage into the spinal cord vasculature, and mechanical herniation of nucleus pulposus into the vertebral bone marrow sinusoidal venous channels with retrograde entrance into the basivertebral vein and intervertebral vein plexuses. The formation of these emboli is more common in adult large, breed dogs but have also been observed in small breeds and in puppies as young as 3 months of age.[11] Fibrocartilage emboli in cats are uncommon but have been reported.[12] The ischemic injury associated with fibrocartilage emboli affects both gray and white matter. Because neurons are more susceptible to ischemic injury than white matter, clinical signs of gray matter disease may be observed before white matter signs.[4] The clinical signs will reflect the location of the lesion with any segment of the spinal cord being susceptible. However, a higher incidence in the caudal lumbar area has been discussed in the literature.[13] In addition to fibrocartilage emboli, other pathologic states associated with hypercoagulability such as sepsis and endocarditis could cause spinal cord emboli and acute ischemic injury.[13]

Secondary Injury

Secondary injury to the spinal cord refers to the molecular and biochemical events that occur following the primary injury. While the goal of surgery, if indicated, is to relieve the physical effects of the primary injury such as compression from an intervertebral disk herniation, the goal of medical therapy is to limit the damage that occurs from secondary injury. The interrelated vascular and biochemical changes associated with secondary injury have been discussed extensively in the literature[3–5,14,15] and it should be noted that these mechanisms as well as a number of experimental therapies discussed in this review have been derived largely from rodent models. Secondary mechanisms of injury may also vary with the nature of the primary injury involved. An overview of the mechanisms involved in secondary injury is presented in Figure 1. A brief description of these mechanisms include:

Vascular damage and loss of autoregulation[3–5,14,15]
Under normal circumstances, the spinal cord's autoregulatory system serves to maintain perfusion in the presence of alterations in systemic blood pressure. Traumatic damage to the spinal cord parenchyma and its microvasculature causes loss of its autoregulatory function and compromises the ability of the spinal cord to maintain its own blood flow leading to ischemic injury. Maintaining systemic blood pressure and oxygen delivery to the tissue is paramount toward preventing secondary injury. Loss of autoregulation exacerbates ischemic injury that occurs due to the primary injury-induced damage to the local vasculature. Recent evidence has also shown that damaged capillary endothelial cells will upregulate a gene called Trpm4 following primary injury.[15] This gene is normally expressed in low levels in the central nervous system (CNS) but following injury, upregulation occurs leading to molecular changes including ion entry, oncotic swelling, and neuronal cell death.

Excessive release of the excitatory neurotransmitters aspartate[3,5] and glutamate[3–5,14,15]
Glutamate and aspartate are 2 of the principal excitatory neurotransmitters of the CNS and excessive release occurs after neuronal injury due to leakage from damaged neurons and depolarization-induced release.[3] Adequate ATP supplies are necessary for the normal astrocyte-mediated reuptake of these neurotransmitters from the extracellular compartment. Hypoxia of the spinal cord tissues results in decreased production of ATP resulting in accumulation within the extracellular compartment. Glutamate is known to be directly excitotoxic to the spinal cord and also causes receptor-mediated activation of *N*-Methyl-D-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA)-kainate receptors, resulting in further neuronal injury by exacerbation of ischemia.[4] Increases in glutamate concentration also result in generation of reactive oxygen species (ROS) that may lead to neuronal cell death.[4] Activation of glutamate receptors results in excess sodium entry into neuronal cells. Low ATP concentrations from local tissue hypoxia decrease the ability of the Na+-K+ ATPase to pump sodium out of the cell, resulting in sodium accumulation and cytotoxic edema. Increases in glutamate concentrations were observed in the CSF in dogs experiencing both acute and chronic thoracolumbar disk herniations.[16]

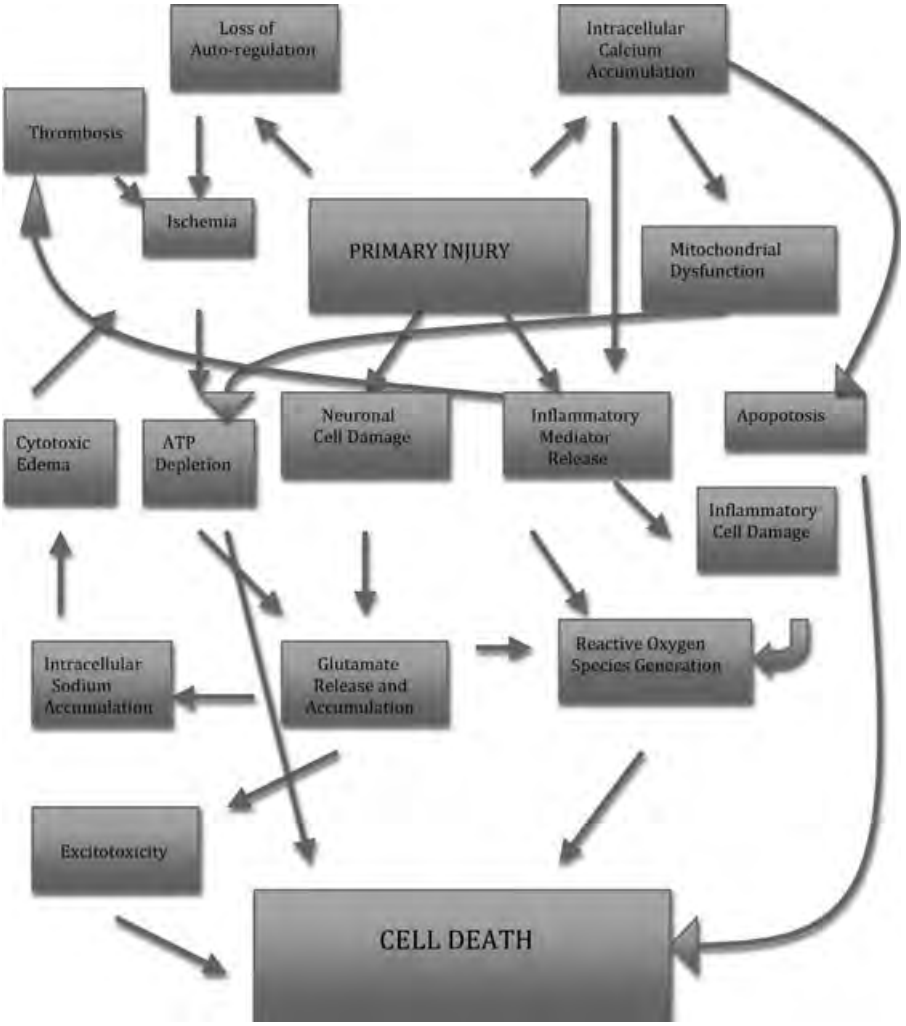

Figure 1: The interrelated vascular and biomechanical changes associated with secondary injury following primary injury in acute spinal cord injury are depicted here; the secondary mechanisms involved have been the targets of therapeutic interventions to improve patient outcome.

Intracellular neuronal calcium accumulation[3–5,14,15]
Increases in the amount of intracellular sodium cause activation of the Na^{+}-Ca^{2+} exchanger, resulting in an increase in neuronal intracellular calcium. Other mechanisms of cytosolic calcium accumulation include calcium-induced calcium release from intracellular organelles along with impaired calcium extrusion by ATP-dependent cell membrane pumps due to ATP depletion. ATP depletion occurs as a result of local ischemia, calcium-mediated inhibition of normal mitochondrial function, and through calcium binding of phosphorous.[3,15] The accumulation of intracellular calcium leads to formation of oxygen-free radical species and lipid peroxidative damage. In addition, intracellular calcium accumulation is a major factor in apoptosis and cell death. Calcium-mediated activation of phospholipase A2 also results in induction of the arachidonic acid cascade, leading to the production of inflammatory mediators and further perpetuation of cellular injury.

Cellular damage from the production of ROS[3–5,14,15]
Damage caused by ROS is a major factor in the secondary injury that occurs in acute spinal cord injury. A number of factors cause increases in ROS including ischemia-reperfusion injury, increased intracellular calcium, glutamate accumulation, and the presence of iron and copper complexes found in petechial hemorrhages. ROS cause damage to the lipid-rich membranes of the CNS resulting in glial, neuronal, and endothelial damage through lipid peroxidation.[3,15] ROS are also involved in oxidative damage to protein and nucleic acids as well as inhibition of mitochondrial respiration[15] that can worsen ischemia.

The inflammatory response to acute spinal cord injury[3–5,14,15]

The immunologic response that occurs in acute spinal cord is biphasic.[4,15] The first phase involves recruiting neutrophils to the site of injury as a direct result of ischemia-reperfusion injury and inflammatory mediator generation. The process of neutrophil infiltration to the site is complex and a full discussion of the mechanisms behind neutrophil adhesion, rolling, diapedesis, and chemotaxis is beyond the scope of this manuscript. Neutrophil activity causes further free radical production, direct parenchymal damage by proteolytic enzyme release, and by ischemic damage through capillary plugging. Following this initial phase, a delayed secondary phase caused by macrophage recruitment and migration occurs. These inflammatory cells are responsible for phagocytosis of damaged tissue. The association between inflammation and thrombosis is well recognized and local thrombosis can further exacerbate ischemic injury.[5]

Recent data in the veterinary literature has also identified increased concentrations of specific proteins involved in exacerbation of secondary injury in dogs. Myelin basic-protein (MBP), a protein known to be increased in disease processes that result in demyelinating injury, has been demonstrated to be increased in dogs with intervertebral disk herniations.[17] Increases of greater than 3 nM/mL in the CSF of affected dogs has been suggested as a poor prognostic indicator of functional recovery following injury.[17] In addition, increases in matrix metalloproteinase-9 (MMP-9) activity[18] were found in the CSF of dogs with intervertebral disk herniations. Matrix metalloproteinases are involved in both secondary spinal cord injury and repair mechanisms; MMP-9 activity has been associated with disruption of the spinal cord blood barrier following acute injury.[18]

Initial Stabilization

Any animal that presents with traumatic acute spinal cord injury should have any immediate life-threatening issues addressed prior to specifically addressing the spinal cord injury itself. The basic tenants of the ABCs (airway, breathing, circulation) should always be first priority. Close attention is made toward maintaining systemic blood pressure and oxygen delivery in an effort to reduce ischemia and progression of secondary injury in acute spinal cord injury. Shock is defined as inadequate tissue oxygen delivery[19] and is initially addressed with appropriate intravenous fluid therapy. Although 90 mL/kg for dogs and 60 mL/kg for cats is commonly stated in the veterinary literature for crystalloid volumes in shock, these volumes represent the entire blood volume for each respective species and the risk of volume overload exists with administration of this large volume. Resuscitative fluid volumes are titrated to individual patient needs and regular re-assessment for improvement and resolution of shock should be closely monitored. Rapid expansion of the intravascular compartment may also be achieved with the administration of hypertonic crystalloids (eg, 7.2% hypertonic saline at a dose 2–4 mL/kg) or synthetic colloid therapy (eg, hydroxyethyl starch, dextran) in 5 mL/kg volumes. Crystalloid fluids should always accompany hypertonic or colloidal therapy to prevent depletion of the interstitial and intracellular fluid compartments. Hypertonic saline has been shown to have specific beneficial effects of reducing spinal cord swelling, edema, and hemorrhage in rats with experimental acute spinal cord injury.[20] Patients experiencing blood loss from acute trauma may require transfusion to maintain oxygen delivery with packed red blood cells at a volume of 10 mL/kg or whole blood at a volume of 20 mL/kg. Volume resuscitated patients that remain persistently hypotensive due to inappropriate vasodilation[5] may require vasopressor therapy with dopamine and norepinephrine. Hypotensive patients with echocardiographic changes associated with depressed cardiac contractility may benefit from positive inotropic therapy with dobutamine or dopamine.

Patients sustaining traumatic injury should receive supplemental oxygen via cage, mask, or nasal catheter; particularly those with concurrent pulmonary disease due to acute trauma and should be closely monitored through pulse oximetry. Oximetry values greater than 90% (corresponding to a $PaO_2 < 60$ mm Hg) indicate severe hypoxemia. Patients with cervical injuries may experience ventilatory failure secondary to paralysis or paresis of respiratory musculature.[21] Ventilation may be further impaired if concurrent pulmonary parenchymal injury is present. Mechanical ventilation is indicated in patients with severe hypoxemia ($PaO_2 < 60$ mm Hg) despite therapy, severe hypoventilation with corresponding $PaCO_2 > 60$ mm Hg, and whose ability to ventilate is impaired due to respiratory muscle failure or fatigue.[22] Patients requiring long-term mechanical ventilation and aggressive management had a favorable outcome (64%) in 1 retrospective study.[21]

Patients must be immobilized in lateral recumbency when vertebral fractures are suspected or where the structural integrity of the vertebral column is in question. Placing these patients on a backboard and taping them to it minimizes movement and prevents worsening of the primary injury. Because pain can have a number of detrimental physiologic effects in trauma patients, analgesic drugs should be provided to all patients with acute spinal injury.

Neurologic Examination

Once the patient is hemodynamically stable, a neurologic examination is performed to determine the severity of the injury and the spinal cord segment involved. Excessive motion should be minimized in patients suspected of having spinal fractures or other injuries in which movement may cause further injury. In these patients, a complete neurologic assessment may not be possible and an abbreviated examination to determine spinal cord function should be performed. The neurologic examination should assess the animal's mentation, cranial nerve function, gait (if the patient is still ambulatory), postural reactions, spinal reflexes, and the ability to detect noxious stimuli in all 4 limbs. Loss of spinal cord function will typically develop in the following sequence (1) loss of proprioception (2) loss of voluntary motor function (3) loss of superficial nocioception (4) loss of deep nocioception.[1,2,6] Gentle examination of the vertebral column should be performed to determine spinal malalignment, instability, or discomfort in a particular region of the vertebral column. Evaluation of spinal reflexes is important for localization. Thoracic limb reflexes to assess include withdrawal, biceps, triceps, extensor carpi radialis, and cutaneous trunci. Pelvic limb reflexes to assess include patella, withdrawal, gastrocnemius, and perineal. Patients lacking superficial nocioception should be evaluated for the presence or absence of deep nocioception. Deep nocioception is typically evaluated by applying a noxious stimuli (ie, pressure via a hemostat a digit) to evaluate for a cerebral response.[23]

Table 1: The Modified Frankel Scale is a derivation from the human system that is utilized in various modifications to assess the extent of myelopathies. The table shown is a modification for thoracolumbar spinal cord disease.[2]

Grade 0	Paraplegia with absent deep nocioception
Grade 1	Paraplegia with absent superficial nocioception
Grade 2	Paraplegia with intact nocioception
Grade 3b	Nonambulatory paraparesis; inability to bear weight on the pelvic limbs without support
Grade 3a	Nonambulatory paraparesis; ability to bear weight on the pelvic limbs without support
Grade 4	Ambulatory paraparesis
Grade 5	Normal gait with paraspinal paresthesia

There are currently 3 validated scoring systems to assess the neurologic status of patients experiencing spinal cord injury. These include the Modified Frankel Score (Table 1), the 14-Point Motor Score (Table 2), and the Texas Spinal Cord Injury Score (Table 3). The examining clinician should evaluate each patient under the guidelines of a validated scoring system to assess initial patient evaluation and clinical progress. The usage of a validated scoring system also provides a means of communicating patient assessment for referral should it be deemed necessary by the examining clinician. The Modified Frankel Score[2] is a derivation from the human system that has been utilized in veterinary medicine in various modifications to evaluate all 4 limbs or for the assessment of pelvic limb function from thoracolumbar injury.[2] The 14-Point Motor Score is a system that was developed as means of

Table 2: The 14-Point Motor Scale[25] is a system developed for scoring and monitoring pelvic limb function as a result of thoracolumbar injury. The system describes 5 stages of recovery with each stage subdivided on the basis of recovery patterns described.

STAGE 1
- 0-No pelvic limb movement and absent deep nocioception
- 1-No pelvic limb movement with deep nocioception
- 2-No pelvic limb movement but voluntary tail movement

STAGE 2
- 3-Minimal nonweightbearing protraction of the pelvic limb (movement of 1 joint)
- 4-Nonweightbearing protraction of the pelvic limb with >1 joint involved <50% of the time
- 5-Nonweightbearing protraction of the pelvic limb with >1 joint involved >50% of the time

STAGE 3
- 6-Weightbearing protraction of the pelvic limb <10% of the time
- 7-Weightbearing protraction of the pelvic limb 10–50% of the time
- 8-Weightbearing protraction of the pelvic limb >50% of the time

STAGE 4
- 9-Weightbearing protraction 100% of the time with reduced strength of pelvic limb. Mistakes >90% of the time (crossing of pelvic limbs, scuffing foot on protraction, standing on dorsum, etc.)
- 10-Weightbearing protraction of pelvic limbs 100% of the time with reduced strength. Mistakes 50–90% of the time
- 11-Weightbearing protraction of pelvic limb 100% of the time with reduced strength. Mistakes <50% of the time

STAGE 5
- 12-Ataxic pelvic limb gait with normal strength, but mistakes >50% of the time (lack of coordination with thoracic limb, crossing of pelvic limbs, bunny hopping, scuffing foot on protraction)
- 13-Ataxic pelvic limb gait with normal strength, but mistakes made <50% of time
- 14-Normal pelvic limb gait

Table 3: The Texas Spinal Cord Injury Score for dogs[24] is a recently developed system that individually evaluates each limb for gait, proprioceptive positioning (knuckling), and nociception. The system was designed to reflect the typical sequence of functional loss and recovery after spinal cord injury in the progressive categories evaluated

Gait
0-No voluntary movement seen when supported
1-Intact limb protraction with no ground clearance (ability to lift the limb off the ground while it is being protracted)
2-Intact limb protraction with inconsistent ground clearance
3-Intact limb protraction with inconsistent ground clearance
4-Ambulatory, consistent ground clearance with moderate paresis-ataxia
5-Ambulatory, consistent ground clearance with mild paresis-ataxia
6-Normal gait
Proprioceptive positioning
0-Absent response
1-Delayed response
2-Normal response
Nocioception
0-No deep nocioception
1-Intact deep nocioception, no superficial nocioception
2-Nocioception present

scoring and monitoring pelvic limb function as a result of thoracolumbar injury;[24,25] it does not assess postural reactions, limb asymmetry, or assesses the thoracic limbs.[24] The most recently developed of the 3 systems is the Texas Spinal Cord Injury Score[24] and is a system that evaluates each limb individually for 3 components: gait, proprioceptive positioning, and nocioception.[24]

The examining clinician should be cognizant for the presence of spinal shock, which occurs due to interruption of ascending and descending nerve fibers resulting in flaccid paralysis of the limbs below the site of injury;[21] T3–L3 injuries that result in spinal shock will result in flaccid paralysis of the pelvic limbs. Differentiation of T3–L3 injuries with spinal shock from patients with L4–S3 injuries may be difficult during initial examination. The flaccid paralysis occurs due to disruption of input from upper motor neurons to downstream lower motor neurons.[26] This typically lasts for 12 hours or less[1] but may be apparent for 12–48 hours.[5] Spinal shock may lead to incorrect lesion localization on neurologic assessment and may alter diagnostic investigations.[26] Schiff-Sherrington syndrome, characterized by extensor rigidity of the thoracic limbs with normal postural reactions and reflexes, is due to a lesion in the thoracolumbar spine that causes loss of inhibition of the extensor motor neurons in the cervical intumescence.[6] Pelvic limb reflexes in patients experiencing Schiff-Sherrington syndrome can vary from normal to increased;[2] however, patients experiencing concurrent spinal shock may have decreased reflexes.[6] The presence of Schiff-Sherrington syndrome indicates severe injury but does not indicate that the patient cannot recover.

Prevention of Secondary Injury

One of the responsibilities of the attending veterinarian treating acute spinal cord injury patients is the provision of neuroprotection,[27] the goals of which are to attenuate the pathophysiological processes triggered after acute injury and minimize secondary damage.[27] As ischemic injury can be exacerbated by hypotension and hypoxemia, it is absolutely imperative to maintain systemic blood pressure and tissue oxygen delivery. Research is ongoing in both human and veterinary medicine to re-evaluate old recommendations and to develop new therapies to improve the outcome of these patients. Secondary injury in spinal cord patients begins immediately after the onset of the primary impact injury and thus, therapeutic measures to prevent the effects of secondary injury should be implemented as soon as possible.

Controversial use of methylprednisolone

Despite the clinical research conducted over the past 2 decades, corticosteroids remain controversial in acute spinal injury in both human and veterinary medicine. The principal benefits of corticosteroids in acute spinal injury are the free-radical scavenging properties, anti-inflammatory effects, and preservation of spinal cord blood flow.[5,28] Due to its relative deficiency of endogenous antioxidants and abundance of cell membranes, the CNS is highly susceptible to free radical damage. Corticosteroids prevent the formation of inflammatory mediators by inhibiting the arachidonic acid cascade via inhibition of the enzyme Phospholipase A_2. Methylprednisolone has been the most extensively studied corticosteroid in acute spinal injury. Clinical research has demonstrated that the principal benefit of methylprednisolone is related to its free-radical scavenging effects.[28,29] These effects were demonstrated to be absent with both prednisone and dexamethasone. However, in comparison with other steroids, methylprednisolone does not appear to be effective in decreasing the products of Phospholipase A_2.[30] The National Acute Spinal Cord Injury Study (NASCIS) was a series of prospective human clinical trials conducted in the 1990s that evaluated methylprednisolone in acute spinal trauma.[31–36] Based on the results of a post-hoc analysis of the second clinical trial, methylprednisolone was suggested to have beneficial effects if administered within 8 hours of the initial injury. The data in this study revealed that patients treated within 8 hours had improved motor and sensory scores up to a year following presentation. These beneficial effects were also observed

in the third clinical trial. However, the degree of functional improvements observed in these patients in the post-hoc analysis was considered minimal.[23,36,37] In addition, these clinical trials also demonstrated that administrating methylprednisolone after 8 hours resulted in worse outcomes with complications such as pneumonia and increased risk of sepsis. Other detrimental effects associated with high-dose glucocorticoids include gastrointestinal ulceration, immunosuppression with a predisposition for wound infection or other secondary infections, hyperglycemia, acute adrenal insufficiency,[38] increased number of hospital days, as well as impaired wound healing. Data to support using methylprednisolone in veterinary medicine is lacking. Limited studies failed to show documented clinical improvements with high-dose methylprednisolone,[39] and is not considered general standard of care. If therapy is elected, the initial dose is 30 mg/kg followed by repeated boluses of 15 mg/kg at 2 hours and 6 hours, then every 8 hours up to 48 hours after trauma.[28] The protocol used during the human NASCIS trial involving a continuous rate infusion (CRI) protocol of an initial bolus of 30 mg/kg followed by 5.4 mg/kg/h for 23 hours and a similar CRI protocol has also been used in veterinary medicine. It remains a controversial topic in veterinary and human medicine.

Due to the controversial nature of high-dose methylprednisolone, numerous studies have been performed in both human and veterinary medicine in an effort to develop therapies that may be effective in treating acute spinal cord injury. A discussion of a number of these therapies follows.

Polyethylene Glycol

Polyethylene glycol is a hydrophilic polymer compound that is capable of repairing damaged cell membranes. The mechanism by which polyethylene glycol acts is through sealing breaks along neuronal cell membranes that occur as a result of mechanical damage.[40,41] It is theorized that exposing neurons to this sealing property spares much of the damaging effects of secondary injury including ion channel abnormalities, exposure to inflammatory mediators, and exposure to ROS.[41] A preliminary study utilizing polyethylene glycol was recently published revealing that the intravenous injection of polyethylene glycol in dogs was safe[2,40] and improvements in spinal cord function were observed in treated dogs experiencing intervertebral disk herniation as compared to historical controls.[40] However, the study was not placebo controlled and further study via a blinded, controlled study is necessary prior to its recommendation as an effective treatment.

Therapeutic Hypothermia

Therapeutic hypothermia has been discussed in the human literature as an adjunctive therapy in a number of clinical situations including acute neurologic injury, cardiac arrest, liver failure, and multisystem trauma. In regards to its application in acute neurologic injury, greater emphasis has been placed in the literature on its beneficial effects for brain pathology. However, interest in its clinical application for acute spinal injury is increasing. Experimental models of acute spinal injury in rodents have shown that hypothermia resulted in improved motor function and reduced histologic parenchymal damage,[42,43] presumably through its ability to delay the onset of ischemic damage following acute injury.[42] Although the benefits of hypothermia in rodent models have been demonstrated, controlled clinical studies evaluating its use in both human and veterinary medicine for acute spinal injury are lacking. The potential harmful effects of hypothermia have been well discussed and include bradycardia, hypotension, cardiac arrhythmias, tissue hypoxia due to shifting of the oxygen–dissociation curve to the left, impairing of the immunologic system, and coagulation abnormalities.[44,45] Thus, although therapeutic hypothermia is garnering more attention in the ICU setting, its use at this time cannot be advocated until further study is undertaken.

Oscillating Field Therapy

Oscillating field therapy (OSF) involves applying alternating electrical currents. Controlled clinical trials in both dogs and people have shown promise as a therapy in acute spinal injury.[46,47] Applying an electrical field across a site of spinal injury is thought to promote axonal growth via tropic and trophic effects on injured spinal cord axons.[46] A placebo-controlled study was conducted in canine patients with intervertebral disk injury and revealed that patients undergoing OSF experienced clinical improvements in superficial and deep pain perception, although statistical significance was not achieved in regards to improvement in ambulation and proprioception.[47] A phase I clinical trial was conducted in a total of 10 human patients and also revealed clinical improvements in sensory and motor function utilizing OSF.[46] Although OSF appears to be a promising treatment modality in acute spinal injury, further research is needed.

Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy is becoming more available in veterinary facilities as an adjunctive therapy for a number of conditions including acute spinal cord injury. The principle of hyperbaric therapy involves in-

creasing the atmospheric pressure from 1 atmosphere absolute (ATA) to 2–2.5 ATA, thereby increasing the oxygen dissolved in plasma 3-fold while a patient is breathing room air (FiO_2 of 0.21).[48] Maintaining oxygen delivery is critical to preventing secondary injury from acute spinal trauma, and dissolved oxygen can diffuse into damaged tissues that may not possess adequate circulation.[49] Complications of hyperbaric oxygen therapy are related to oxygen toxicity and include barotrauma, oxygen-induced seizures, and the potential for generation of ROS.[50] Adequate published data involving hyperbaric oxygen therapy are currently lacking in veterinary medicine.

Antioxidant Therapy

Vitamin E is a natural antioxidant that plays a role in limiting the damage that occurs from free radical damage. It inhibits lipid peroxidation of the lipophilic interior by becoming oxidized to a free radical, and is converted back to itself by a process involving ascorbic acid.[51] A 5-day pretreatment course in cats with vitamin E and selenium, another endogenous antioxidant, showed improved neurologic outcome and improved spinal cord blood flow.[52] Raffinee, a mixture of antioxidants derived from natural products such as soybeans and rice germ, was studied in a clinical trial in 10 patients in the subacute phase of spinal injury.[53] This compound was administered orally and was determined to be both safe and associated with improvements in motor and sensory recovery reported over a 6-week period.[53] No control groups were utilized in the clinical trial and further research is necessary to determine its true efficacy. Melatonin is an endogenous hormone well known for its role in maintaining circadian rhythm. It also has potent antioxidant and immunomodulatory effects and has been shown in rodent models of spinal injury to reduce ischemic injury.[54] Resveratrol is a natural antioxidant polyphenol compound that is found in red wine. It has demonstrated in rodent models of spinal injury to result in improved histopathologic changes as compared to control groups.[55,56] N-acetylcysteine is a natural endogenous antioxidant that has been used in veterinary medicine for various purposes such as acetaminophen toxicity and as a mucolytic. Although it had been shown to be effective in improving neurologic function in experimental studies, a controlled clinical trial of N-acetylcysteine performed in dogs experiencing acute intervertebral disk disease failed to result in improvement of neurologic recovery;[57] however, the sample size used in the study was relatively small ($n = 70$). Although the benefits of antioxidants have been shown in experimental models, they require a prolonged time to achieve therapeutic concentrations in the CNS,[58] thus limiting their utility in treating spinal cord injury in the acute phase.[58,59]

Calcium-Channel Antagonists

Due to the role of calcium in the pathogenesis of secondary injury, calcium-channel antagonists have been evaluated as a potential therapeutic option. In experimental rodent studies, the calcium-channel antagonist nimodipine was shown to improve posttrauma spinal cord blood flow.[60] Experimental studies involving cats that were pretreated with calcium-channel blockers diltiazem and nifedipine approximately 30 m prior to injury showed improved spinal cord blood flow.[61] These studies however did not evaluate the functional recovery of the animals. In a separate study involving cats, intravenous nimodipine administration resulted in systemic hypotension although functional recovery was not affected.[62] A prospective, randomized clinical trial performed in people comparing methylprednisolone and nimodipine failed to document any clinical difference in people treated with nimodipine as compared to patients treated with methylprednisolone.[63] Clinical experience utilizing calcium-channel antagonists are limited and the potential detrimental side effects involving cardiac muscle and vascular tone must be considered as potential complications with these therapies.

21-Aminosteroids

21-Aminosteroids received much attention in the 1990s as a potential alternative to methylprednisolone in treating acute spinal cord injury. 21-Aminosteroids (also known as lazaroids) are potent inhibitors of iron-dependent lipid peroxidation and their primary advantage over corticosteroids is their lack of both glucocorticoid and mineralocorticoid side effects.[3] Prior to the Phase III clinical trials that were performed as part of the NACSIS III study in the late 1990s, tirilazad mesylate (a 21-aminosteroid) was experimentally administered to cats and revealed reduced spinal cord ischemia.[64] During the Phase III trials in people, tirilazad as a therapeutic agent in acute spinal cord injury was compared to methylprednisolone administered for 24 hours and for 48 hours postinjury. The results of the study revealed that tirilizad was as efficacious at improving functional recovery as the 24 hours methylprednisolone group. However, when compared to the 48-hour methylprednisolone group, tirilizad failed to improve functional recovery as well as methylprednisolone.[35,36] Results of this study along with lack of evidence of improvement in dogs with lazaroids[65] have precluded its usage in veterinary medicine.

Opiate Receptor Antagonists

Based upon the premise that endogenous opioid receptor activation results in a decrease in spinal cord blood flow following acute injury,[4,66] experimental and clinical trials using naloxone, an opiate-receptor antagonist, have been attempted. Naloxone is commonly used in clinical practice to reverse the effects of administered opioids, particularly when adverse effects such as bradycardia and hypotension are observed. Experiments performed on cats showed naloxone produced better functional recovery as compared to cats that received saline.[66] Similar results were found using naloxone in experimental studies in rats where it was again found that spinal cord blood flow and functional recovery were improved.[67] However, during the second NASCIS, the effects of methylprednisolone and naloxone were compared in humans with acute injury. The results of the study revealed that naloxone did not improve functional recovery following acute injury while methylprednisolone did improve functional recovery when administered to human patients within the first 8 hours.[33,34] Because naloxone does not appear to be effective and decreases analgesia of exogenous opioids, it is not recommended in acute spinal cord injury.

Thyrotrophin-Releasing Hormone (TRH)

TRH is the endogenous hormone secreted by the hypothalamus that signals the pituitary gland to secrete thyroid-stimulating hormone (TSH). In experimental studies performed in both cats and rats, TRH analogs were shown to improve spinal cord blood flow and subsequent function following spinal cord injury.[68–70] The proposed mechanism of how TRH improves spinal cord blood flow includes antagonism of endogenous opioid receptors and inhibiting the effects of inflammatory mediators and cytokines such as leukotrienes and platelet activating factor.[3] A clinical trial utilizing TRH was performed in humans involving 20 acute spinal cord patients receiving either TRH infusions or a placebo. The results of the study revealed that TRH did not improve spinal cord function in patients with complete injuries, but in patients who experienced incomplete injuries, there were improvements in spinal cord function.[71] A Phase I clinical trial in dogs was recently conducted in which 9 dogs experiencing acute intervertebral disk herniation were administered the TRH derivative, 1-ARA-35B and compared to 6 control dogs.[72] Due to the small sample size, no definitive conclusions in regards to the changes in functional outcome were determined.

Minocycline

Minocycline is a lipophilic, second-generation tetracycline derivative that has been investigated in multiple experimental studies in acute spinal injury due to its ability to cross the blood–brain barrier and exert anti-inflammatory and neuroprotective effects.[73–75] The mechanisms of action include inhibition of caspase-1 and caspase-3 involved in interleukin-1 generation and in the induction of apoptosis,[75] inhibition of nitric oxide synthetase and production of nitric oxide,[73,75] prevention of injury due to glutamate excitotoxicty,[23,75] and attenuation of microglia activation.[73] Experimental rodent models have shown that minocycline reduces the extent of gross lesions in the spinal cord with histologic sparing of both neuronal cells and white matter tracts.[73–75] No clinical trials to date have been performed and further research into its clinical application is necessary.

4-Aminopyridine

4-Aminopyridine is a potassium-channel blocker that has been shown in experimental studies to be able to reverse conduction block due to spinal cord injury.[76] A Phase I clinical trial was conducted in 39 dogs with a majority of the patient group (77%) experiencing intervertebral disk herniation and the remaining patient population consisting of patients experiencing mechanical injury such as vehicular trauma. The results of the Phase I study revealed significant improvement in pelvic limb postural reactions (46%), improved nociception to noxious stimuli (25%), and improved cutaneous trunci reflexes (23%) in the patient population tested.[76] The effects of 4-aminopyridine were short-lived in these patients with reversal observed within 3 hours of administration. Adverse side effects included seizure, hyperthermia, and anxiety. The exact mechanism of action of 4-aminopyridine has not been fully determined; however, a combination of potassium-channel blockade in demyelinated segments and enhancement of neurotransmitter release may be involved.[76] Additional research is necessary to evaluate its overall potential.

Intraspinal Olfactory Glial Cell Transplantation

Experimental studies in acute spinal injury in rodents have shown improved functional recovery following olfactory glial cell transplantation and a Phase I study in dogs was recently published involving 9 dogs to evaluate its utility in patients experiencing thoracolumbar spinal injury.[77] Five of these patients experienced vertebral subluxations or fractures due to vehicular trauma and 4 experienced intervertebral disk herniation; 8 of the patients were nonambulatory and had absent nociception of the pelvic limbs. Cells were surgically obtained from the nerve fiber layer of the right olfactory bulb and placed into cell culture prior to surgical implantation via dorsal laminectomy. Seven of the 8 patients that had

absent motor function had functional improvements and 1 of the 8 patients that had absent nocioception regained limited pelvic limb and tail sensation.[77] Although the sample size involved in this clinical trial was small, the significant functional improvements warrant further research in both canine and human spinal cord injury.

Imaging Studies

Survey spinal radiographs should be obtained following patient stabilization as part of the initial evaluation for acute spinal cord injury. Careful consideration should be made to avoid excessive patient manipulation and subsequent exacerbation of patient injury. Immobilization of patients on a backboard aids in preventing excessive motion and ideally, a backboard that is radiolucent should be utilized to avoid movement during radiography. Orthogonal radiographs should try to be obtained if possible and horizontal-beam radiography can be utilized to obtain ventrodorsal views in patients where spinal instability is suspected. The entire spinal column should be imaged as fractures and luxations have been noted in multiple sites in approximately 20% of patients experiencing spinal trauma.[1] Changes associated with fractures, luxations, or intervertebral disk herniation should be evaluated for on-survey radiographs. Radiographic findings associated with intervertebral disk herniation include narrowing or wedging of the disk space, calcified material within the disk space, and narrowing of intervertebral foramen.[78] The advantages of radiography include the fact that it is inexpensive and does not require general anesthesia. The disadvantage of radiography is its limited diagnostic accuracy in comparison to other imaging modalities. For intervertebral disk herniations, spinal radiographs were 51–61% accurate in terms of correctly diagnosing lesion location.[78] Radiographic sensitivity for spinal fractures and luxations was determined to be 72% and 77.5%, respectively, in another study.[79] Thus, plain radiographs should be interpreted with caution whenever an acute traumatic lesion is not apparent. Concurrent radiographs of the thorax and abdomen should also be taken to rule out concurrent thoracic and abdominal injuries from acute trauma. Abdominal focused assessment sonogram for trauma (FAST) examination should also be performed on all acute trauma patients,[80] particularly if serosal detail appears decreased on survey abdominal radiographs.

Prior to the availability of MRI and CT in clinical practice, myelography was the primary diagnostic modality utilized to diagnose spinal cord compression due to intervertebral disk herniation. Myelography involves injecting contrast media into the subarachnoid space[81] and observing for radiographic attenuation of the ventral and dorsal contrast columns at sites of compression. The diagnostic correlation between myelographic and surgical findings in correcting identifying the location of a disk has been reported to be 85–98%.[82–84] The advantage of myelography is that it is relatively inexpensive when compared to MRI or CT, and that special equipment is not needed. The disadvantages of myelography are the need for general anesthesia and the potential for complications associated with contrast media injection such as seizures,[81,85] contrast-induced nephropathy,[86] and cardiac asystole.[87] In addition, myelography is operator-dependent and artifacts resulting from accidental injection outside the subarachnoid space (epidural, central canal, intraparenchymal) can occur[82,88] as well as the potential for further spinal cord injury from direct parechymal injection. While myelography can be an effective modality for diagnosing diseases associated with spinal cord compression, its ability to diagnose intraparechymal spinal cord disease is limited. In patients with fibrocartilaginous embolic myelopathy, myelography may be normal or may show an intramedullary pattern that is suggestive of spinal cord swelling.[10] However, other disease processes such as focal myelitis, intramedullary neoplasia, intraparenchymal hemorrhage, and ANNPE may also show an intramedullary pattern and myelography cannot be used to distinguish between these disease processes.[10] In contrast to 3-dimensional modalities such as computed tomography and MRI; its ability as a 2-dimensional study must be recognized as a limiting factor that may complicate surgical intervention.

Due to increasing availability over the past decade, CT and MRI are more frequently used in evaluating spinal cord injuries. CT is considered a superior diagnostic modality for imaging bone and is an ideal modality for evaluating the extent of vertebral fractures and to observe for bone fragments resulting from vertebral fracture into the vertebral canal.[1,89] In comparison to both MRI and radiography, CT provides superior spatial resolution and while both CT and MRI are able to provide transverse images of the spinal column, the newer CT machines can provide thinner slice images at a much faster rate and some patients may even be able to undergo CT with sedation due to this rapidity[89] (which can also reduce client expense when CT is utilized versus MRI). CT may also be used in conjunction with contrast myelography,[83,90] which can further characterize the etiology of cord compression in comparison to normal myelography alone. In a recent study evaluating various CT modalities and myelography in the diagnosis of acute myelopathy in 46 dogs; CT myelography was determined to be superior to normal myelography, normal CT, and angiographic CT for lesion characterization, localization, and laterization of all etiologies diagnosed.[90]

CT myelography identified intervertebral disk herniations in 8% of patients that normal myelography determined to be normal in this study.[90] In comparison to MRI, however, CT is limited in its ability to diagnose noncompressive lesions such as fibrocartilaginous emboli, myelitis, and syringomyelia; MRI remains the imaging modality of choice for diagnosing these diseases.

MRI is considered a superior diagnostic modality for imaging soft-tissue structures (eg, spinal cord, nerve roots, and intervertebral discs) and is the imaging modality of choice for evaluating parenchymal injuries. In patients suspected of having fibrocartilage emboli, MRI is the imaging modality of choice. The characteristic focal, relatively sharply demarcated intramedullary appearance on T2-weighted MRI images has been recently discussed in the literature[91,92] as means of diagnosing ischemic myelopathy with the intensity of the changes associated with a worse prognosis in affected patients. Hyperintensity on T2-weighted MRI is associated with spinal cord edema, necrosis, and gliosis.[24] Other advantages of MRI include the ability to view images in multiple planes[89] (in contrast to CT which allows only visualization in the transverse plane), higher quality on reformatted images, and the avoidance of complications associated with injectable hyperosmotic contrast media.[83,89] Disadvantages of MRI include the higher cost factor, reduced availability, and the need for general anesthesia.

Poststabilization Management

Following initial assessment and stabilization, the clinical decision must be made in regards to managing the patient medically or surgically. Conservative management may include restricted exercise and cage rest for a period of 6–8 weeks. Patients with intervertebral disk herniation with mild neurologic dysfunction or pain may be treated medically, however moderate-to-severe ataxia and paresis are indications for decompressive surgery.[13] Nondisplaced spinal fractures and mild subluxations may be managed through immobilization with external coaptation. Splints utilizing fiberglass cast material, metallic rods, or other sturdy material can be used.[5,8] Bandaging material should be used to secure the splint with the extent of the bandage cranial and caudal to the fracture site. Complications associated with external coaptation include bandage loosening, skin abrasions, sores, pain, and increased difficulty with bowel and bladder management.[8] Vertebral column instability, spinal cord compression, and worsening neurologic signs despite conservative management are indications for surgical management.

The poststabilization period in surgically or medically managed patients is critical toward influencing the outcome of each patient. Intravenous fluid support to maintain hydration and normotension is absolutely imperative during this time period. The length of supportive care may be lengthy and well beyond the period of hospitalization. Proper communication to the owner of the time and effort that may be involved to achieve a favorable outcome is essential. The goals of patient care in the postacute phase involve not only continuation of supportive measures for the spinal injury but also to prevent the onset of concurrent diseases that may develop. The supportive measures needed to manage surgical and nonsurgical patients are similar and include the basic following premises.

Pain Management

Continuing pain management is vital in the poststabilization phase as uncontrolled pain can lead to patient stress and a number of detrimental complications. The physiologic responses to pain include increases in endogenous cortisol concentrations, upregulation of catecholamine release, and inflammatory mediator production.[93,94] The pathophysiologic responses are multisystemic and include cardiovascular changes (eg, tachycardia, hypertension, increased cardiac workload, and oxygen consumption), gastrointestinal complications (eg, vomiting, nausea, ileus), respiratory complications (eg, ventilation/perfusion mismatch, predisposition to infection), and immunologic impairment.[95] Clinical signs of pain include anxiety, vocalization, reluctance to move or lay down, anorexia, aggression, panting, tachycardia, elevated body temperature, dilated pupils, and lethargy.[93,95] Recognition of these signs is important and pharmacologic therapy should be implemented in these patients. Opioids are the most commonly used agents in veterinary medicine to provide analgesia and are appropriate for controlling pain in both surgical and nonsurgical patients. Commonly used opioids include the pure muagonists such as hydromorphone, morphine, and fentanyl as well as the agonist–antagonist combinations such as buprenorphine and butorphanol. Side effects of opioid administration include dysphoria, gastrointestinal ileus, respiratory depression, bradycardia, and hypotension.[96] Administration of the opioid-antagonist naloxone may be indicated if these adverse effects are observed. NSAID therapy may also be used as an analgesic agent but should be avoided in patients with previous corticosteroid administration and in patients already experiencing gastrointestinal symptoms. Other analgesic agents that may be considered include the alpha-2 agonists (ie, medetomidine),[97] NMDA-antagonists (ie, ketamine),[23,97]and sodium-channel blockers (ie, lidocaine).[23,95] Electroacupuncture has also been

described as a potential adjunctive therapy in reducing pain,[98] improving motor and sensory function, and bladder control in dogs with intervertebral disk herniation.[99] Additional research is necessary, however, to evaluate its effectiveness as an adjunctive therapy.

Gastrointestinal Complications

Gastrointestinal mucosal complications in acute spinal cord trauma are multifactorial in nature and include concurrent trauma, hypovolemia, hypotension, critical illness, and corticosteroid administration.[100,101] Spinal cord injury may also result in imbalances of parasympathetic and sympathetic tone and paralytic vagotonia, resulting in an increase in gastric acid secretion and bile reflux.[101] In addition, the stress due to pain and neurosurgery may increase endogenous cortisol production and catecholamine release, increasing the risk of gastrointestinal complications. The reported rate of gastrointestinal hemorrhage in patients undergoing neurosurgical procedures is 14.8–20%.[100,102] Administration of corticosteroids further enhances the risk of gastrointestinal complications by suppressing arachidonic acid metabolism and decreasing prostaglandin secretion. Prostaglandins maintain the gastrointestinal mucosa by increasing mucus and bicarbonate secretion.[100] Corticosteroids also increase gastric acid secretion by increasing circulating concentrations of gastrin and pepsin, decrease gastrointestinal cell renewal, and promote bacterial colonization of peptic ulcers.[101] The incidence of gastrointestinal hemorrhage in patients receiving both methylprednisolone and undergoing spinal surgery is 90% according to 1 study.[102] Thus, prophylactic therapy with gastroprotectants should be considered in patients experiencing spinal trauma and especially with those receiving corticosteroid therapy. Options to consider include H_2-receptor antagonists, proton-pump inhibitors, prostaglandin analogs, and sucralfate. Data to support the beneficial usage of any of these drugs in spinal injuries in veterinary medicine are lacking.[101,102]

H_2-receptor antagonists are histamine analogs that competitively and irreversibly bind to H_2 receptors on gastric parietal cells.[103] The available H_2-receptor antagonists include famotidine, ranitidine, cimetidine, and nizatidine. Cimetidine and ranitidine inhibit the hepatic cytochrome P-450 system, which may prolong the clearance of drugs that are metabolized by this system. Evaluation of cimetidine in patients undergoing spinal surgery failed to show any prophylactic benefit in its usage in reducing gastrointestinal hemorrhage.[102] Nizatadine and ranitidine also have anticholinesterase activity, which may be beneficial in patients that have concurrent abnormal gastric motility;[103] nizatadine does not inhibit the cytochrome P-450 system. Famotidine is the most potent of the H_2 receptor antagonists, has a longer duration of action than cimetidine, nizatadine, and ranitidine, does not interfere with the cytochrome P-450 system, and has been shown to be able to effectively increase intragastric pH in dogs.[104]

Proton pump inhibitors (PPI) are substituted benzimidazoles that are metabolized, following diffusion as a weak base into the acidic compartment of the gastric parietal cell, to its active form that covalently binds to the hydrogen ion-potassium ion ATPase enzyme to block its activity.[103] This pump is responsible for the final step in gastric acid secretion and does so by exchanging cytosolic hydrogen ions for luminal potassium ions; blocking this pump results in irreversible inhibition of gastric acid secretion.[101,103] The PPIs used in veterinary include omeprazole, panteprotazole, and lansoprazole; all are available in oral form but panteprotazole and lasoprazole are also available in intravenous formulations. PPIs are most effective when the hydrogen ion-potassium ion ATPase pumps are maximally present on the apical membrane. In people, only 10% of these pumps are present in the fasting state in contrast to the postprandial state when a large reserve of these pumps is recruited to the apical membrane.[103] Therefore, recommendations for administration of PPIs are for 1 hour prior to a meal to ensure that peak serum concentration coincides with the maximal activity of the hydrogen ion-potassium ion ATPase. PPIs are widely used in people for their efficacy and were determined to be more effective than H_2-receptor antagonists in reducing intragastric pH.[105] Although this was true when omeprazole was compared with cimetidine in dogs, there was no appreciable difference when omeprazole was compared with famotidine.[104] However, the same study did show that twice daily therapy with omperazole was more effective than famotidine at achieving therapeutic efficacy for healing gastric-related disease utilizing human criteria.[104] Omeprazole failed to prevent gastric mucosal lesions in dogs with acute disk herniation treated surgically with corticosteroid administration.[101]

Misoprostol is an orally administered synthetic analog of prostaglandin E_1 that inhibits gastric acid production and has cytoprotective effects by increasing mucosal blood flow, mucus production, and bicarbonate secretion.[101,103] Although misoprostol has been shown to be effective at preventing gastrointestinal complications in patients treated with nonsteroidal anti-inflammatory drugs,[101,106] the same protective effect was not observed in patients treated with methylprednisolone.[107] Studies involving misoprostol in patients undergoing both corticosteroid therapy and surgery for acute intervertebral disc extrusions failed to demonstrate that misoprostol prevents gastrointestinal hemorrhage.[101,102]

Sucralfate is a sulfated disaccharide-aluminum hydroxide complex that dissociates following oral administration into sucrose octasulfate and aluminum hydroxide within the acidic environment of the stomach. The sucrose octasulfate serves to form a negatively charged paste-like complex that binds to the positive charges in the base of ulcers. This complex protects the ulcer from further damage by inhibiting back diffusion of hydrogen ions, inactivating pepsin, and through absorbing gastric-damaging bile acids from the duodenum.[103] Sucralfate also stimulates prostaglandin production, which is cytoprotective by increasing mucosal blood flow and increasing bicarbonate and mucus production. Sucralfate was shown to have no effect in preventing gastrointestinal hemorrhage in dogs experiencing spinal surgery and corticosteroid administration.[102]

Although the incidence of gastrointestinal complications is high in patients with acute spinal injury, evidence-based data to support the efficacy of any of these commonly used gastrointestinal prophylaxis measures have not been documented. It should be noted that the studies performed evaluating the efficacy of these drugs occurred in patients treated with corticosteroids along with concurrent surgery for spinal trauma. To the authors' knowledge, no veterinary studies evaluating the efficacy of these medications in patients with spinal injury alone have been performed.

Nutritional Support

Nutritional support is a vital aspect in the recovery of all critically ill patients. Critically ill patients undergo metabolic alterations mediated by catecholamines, cortisol, and inflammatory mediators such as interleukin-1 and tumor-necrosis factor-alpha that place them in a catabolic state.[108] Spinal cord injury has been shown in people to result in obligatory negative nitrogen balances following acute trauma.[109,110] Although the same finding has not been verified in veterinary medicine, it is known that critically ill and injured animals are at risk of malnutrition and its complications.[111] These include loss of lean body mass and impaired wound healing, immune function, and intermediary drug metabolism.[108,111] Enteral nutrition is the preferred method for providing nutritional requirements as enteral nutrition preserves gastrointestinal permeability and function, decreases bacterial translocation, improves immune function, attenuates the release of inflammatory mediators, improves clinical outcome and in people, reduces the length of hospital stay.[109,112] Patients that are unable or unwilling to take oral feedings may have nasoenteric feeding tubes placed under sedation. Radiographic confirmation of placement should be performed. Nasoesophageal tubes may be utilized but nasogastric feeding tubes are advantageous as they allow for assessment of gastric residual volumes prior to a feeding. Patients that are unable to tolerate enteral feeding due to intractable vomiting and nausea or are unable to guard their airways are at subsequent risk for aspiration pneumonia and are candidates for parenteral nutrition (PN). PN may be administered as either centrally or peripherally. Administration of PN requires the use of a dedicated venous catheter or venous port on multilumen catheters, trained nursing staff capable of monitoring the catheter and the patient, and the ability to formulate and compound solutions based on the individual requirements of the patient in a sterile setting.[113]

Bladder Management

Urinary bladder dysfunction is common in patients experiencing acute spinal injuries and proper management is necessary to prevent complications. The hypogastric, pelvic, and pudendal nerves innervate the urinary bladder[114] and the type of bladder dysfunction present is dependent upon the location and severity of the lesion. Spinal cord lesions up through the 7th lumbar segment may result in upper motor neuron bladder dysfunction.[115] Patients experiencing upper motor neuron bladder dysfunction have increased urethral sphincter tone resulting in a large, firm bladder that is difficult or impossible to express.[114,115] Upper motor neuron bladder dysfunction may result in overflow incontinence as intraluminal pressures overwhelm the internal and external urethral sphincter.[2,114] Reflex dyssynergia in male dogs may also be observed with upper motor neuron bladder dysfunction and is characterized by normal initiation of micturition followed by interruption of the urine stream due to involuntary contraction of the urethral sphincter.[116] Patients with reflex dyssnergia will initially have a normal urine stream, followed by short spurts, and then a complete cessation of urine flow.[116] Lower motor neuron bladder dysfunction occurs due to lesions involving the sacral spinal cord or nerve roots, or those involving the pelvic or lumbosacral plexus.[114] Patients experiencing lower motor neuron bladder dysfunction have decreased tone of both the detrusor and urethral musculature resulting in a flaccid, easily expressible bladder that overflows with minimal intraluminal pressure.[2,114]

Loss of voluntary micturition requires supportive measures to prevent complications including bladder distension, urine scalding, bladder atony, and urinary tract infection. Pharmacologic therapy may be utilized to facilitate urethral relaxation and bladder emptying. Alpha-adrenergic antagonists such as prazosin or phenoxybenzamine may be used to decrease internal urethral sphincter tone in patients with upper motor neuron

bladder dysfunction. Phenoxybenzamine is a nonspecific alpha-adrenergic antagonist; its major side effects include hypotension, lethargy, gastrointestinal upset[115] and also has a slow onset of action and may take several days to achieve therapeutic effect.[2] Prazosin is a selective alpha-one adrenergic antagonist that has a more rapid onset of action than phenoxybenzamine; its major side effect is marked hypotension[2,115] and close blood pressure monitoring should be implemented if utilized. Diazepam is a benzodiazapene that may be used to relax the skeletal muscle of the external urethral sphincter to facilitate bladder emptying. Side effects are minimal and include sedation; its usage should be avoided in patients with impaired hepatic metabolism. Bethanechol is a parasympathomimetic used to stimulate detrusor muscle contraction via cholinergic stimulation. It may be used in both lower motor neuron and upper motor neuron bladder dysfunction but should not be used as a single agent in upper motor bladder dysfunction due to stimulation of detrusor contraction in patients with increased urethral sphincter tone.[115] Concurrent therapy with medications to reduce urethral sphincter tone should be considered in these patients. Side effects of bethanechol are due to cholinergic stimulation and include anorexia, salivation, vomiting, diarrhea, and abdominal discomfort.[115]

Manual expression, intermittent catheterization, or indwelling catheterization may be used to facilitate bladder emptying during hospitalization. Manual expression has been historically preferable due to the risk of urinary tract infection associated with catheterization. Intermittent or indwelling urinary catheterization may also be utilized in bladder management in patients where manual expression is not possible. Intermittent catheterization is associated with a decreased risk of infection when compared to indwelling catheterization.[2,117] Prolonged indwelling catheterization has been associated with a higher risk of urinary tract infection with bacterial resistance after 4 days.[118] A recent study found that the incidence of urinary tract infection in patients with type I thoracolumbar disk herniations did not differ between patients managed via manual expression or intermittent catheterization.[119] In this study, it was determined that the primary predisposing factor to the development of urinary tract infections was the length of time that assisted evacuation occurred prior to the patient regaining voluntary micturition.[119] Due to the risk of the development of microbial resistance, prophylactic antibiotic therapy to prevent urinary tract infection is not recommended[2,117–120] unless a documented infection via culture and sensitivity testing is present. A recent study revealed that a majority of urinary tract infections in dogs with intervertebral disk herniation occurred between 1 to 6 weeks postinjury.[121] A significant percentage (15%) of the patients evaluated, however, had infections present 3 months following injury and close monitoring of urine culture up to this point is recommended even in those patients that regain neurologic function.[121] As voiding difficulties and the inability to completely empty bladders may be present in recovering patients; the risk of the development of urinary tract infections may be present long after the time of initial injury. The propriety of treating urinary tract infections in the absence of severe complications such as pyelonephritis and sepsis remains controversial due to the possible risk of resistance development in patients that cannot void urine normally. However, not treating these patients with appropriate antibiotic therapy may lead to the development of these severe complications.

Recumbency

Managing recumbency is crucial toward achieving a positive outcome. The potential complications due to prolonged recumbency include aspiration pneumonia, pressure ulceration, limb contraction, urine scalding, and fecal soiling. Proper management can prevent these complications. High-quality bedding provides both comfort and prevents decubital ulcers from excessive pressure, particularly on bony protuberances. Thick blankets and absorbent material increase the surface area so the patient's weight is distributed in a uniform manner and absorb and dissipate moisture. For patients that are in a prolonged state of recumbency, water mattresses or other soft surfaces such as a foam or air mattress provide an ideal environment. Recumbent patients should be turned at least once every 4 hours to prevent congestion and atelectasis in dependent lung lobes. Proper hygiene to prevent dermatitis and urine scalding is imperative. Patients may need periodic bathing and blankets and bedding changed when needed. Particular attention should be paid toward keeping surgical sites and traumatic wounds clean. Patients experiencing spinal trauma may also have fecal incontinence. Voluntary and involuntary control of defecation requires a mechanically functional colon, rectum, and anal sphincter. Nervous system innervation is provided by the parasympathetic nervous system (ie, vagus and pelvic nerves), sympathetic nervous system (via the hypogastric nerves), and somatic efferent innervation to external anal sphincter through the pudendal nerve.[122] Dysfunction of any of these systems may result in fecal incontinence. Proper hygiene of these patients to prevent dermatitis from fecal soiling is important. Patients that are recumbent for an extended period of time are at risk of developing muscle atrophy from disuse and contracture. Other complications from prolonged recumbency include periarticular fibrosis, cartilage atrophy, and osteopenia.

Physiotherapy and active massage therapy has been described as a useful adjunct in paralyzed patients.[123] Passive range of motion exercises should be performed in recumbent patients to prevent these complications. Passive range of motion exercises are important for maintaining joint range of motion, maintaining cartilage health, minimizing muscle contracture, and maintaining muscle elasticity.[124] These exercises should be continued until the time when the patient is able to self achieve range of motion. Further rehabilitation exercises including assisted walking through slings, harness, or carts, swimming exercises, and hydrotherapy may be instituted at that point.

Prognosis

Determining the course of a patient's functional recovery and outcome is difficult at the time of presentation. The data in the veterinary literature in regards to patient outcome are varied and many factors including concurrent injuries, location and extent of the primary injury, and progression of secondary injury prior to intervention are all contributing factors affecting outcome. Loss of deep nocioception is generally accepted as a negative prognostic indicator. Patients with loss of deep nocioception following spinal fracture or luxation carry a poor prognosis for recovery.[125,126] In a study that evaluated patients with loss of deep nocioception and thoracolumbar injury, patients with traumatic spinal injuries had a worse prognosis (11.8% regained motor function) than those with intervertebral disk herniations (64% regained motor function).[126] Patients with traumatic cervical injuries amendable to nonsurgical management have a favorable prognosis with functional recovery of 90% reported.[127] Cervical spinal injuries requiring surgical management have a perioperative mortality of 36%; however, functional recovery is high (100%) in patients that survive the perioperative period in 1 study.[127] In the same study, delayed referral of greater than 5 days or longer was associated with a reduction in functional recovery for both surgical and nonsurgical patients.[127] MRI has also been shown to be an important independent prognostic indicator in both intervertebral disk herniation and fibrocartilaginous embolic myelopathy. In a study published evaluating MRI findings with intervertebral disk herniation; patients with T2-weighted intramedullary hyperintensity and those with elevated compressive length ratios (in relation to the length of the second lumbar vertebrae) were associated with a less favorable outcome.[128] The extent of hyperintensity on T2-weighted images in patients with suspected fibrocartilaginous embolic disease has also been shown to be a negative predictor in patient outcome.[10,129] A recently published study evaluated the measurement of MBP as an independent prognostic biomarker in patients with intervertebral disk herniation.[17] Patients with significantly increased MBP concentration in the CSF (>3 ng/mL) had a worse prognosis for functional outcome.[17] Although the recovery rate of patients that do not lose deep nocioception is varied in the literature; the overall recovery rates and prognosis is much more favorable. Recovery rates of up 86–100% in canine patients with surgically managed thoracolumbar disk herniation with intact deep nocioception have been reported.[2,130–134]

Conclusions

Managing acute spinal cord patients is challenging, but properly managing these patients starting at presentation could positively impact the ultimate outcome of these patients. Research in the field of spinal cord injury is ongoing in both human and veterinary medicine with the hope of improving current therapies and developing potentially new therapies that will improve the outcome of patients.

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INTEGRATIVE MEDICINE: THE EVIDENCE, ECONOMICS, & LOGISTICS OF AN EMERGING FIELD

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Veterinary clients are increasingly concerned about maximizing the health and wellness of their pets. An estimated $16 billion is spent annually on veterinary care for companion animals,[1] and owners and veterinary insurers are pursuing therapies traditionally regarded as alternative and complementary. These therapies may also be increasingly recommended by the veterinary care team.

No comprehensive surveys have assessed the economic impact or prevalence of such therapies. However, a survey of veterinary students and colleges suggested that recent graduates frequently encounter questions from clients about these areas, and veterinary schools are increasingly responding with formal instruction in many of the relevant modalities.[2]

Such trends in veterinary medicine mirror those extensively documented in human health care in which consumer demand has driven an increase in complementary or integrative medicine.[3] The reasons are multifactorial but include distrust of aspects of conventional medical care, a view of natural or less invasive interventions as safer, and, in humans, a recognition of mind–body relationships.[3]

TABLE 1.
Complementary Therapies Commonly Integrated in Veterinary Medicine

Acupuncture
Nutrition, nutraceuticals, and herbal medicine
Laser therapy
Rehabilitation and sports medicine
Regenerative medicine (ie, stem cell or platelet therapy)
Hyperbaric oxygen
Homeopathy
Therapeutic massage
Veterinary spinal manipulation

WHAT'S IN A NAME?

The term **integrative medicine** was adopted by leading human clinical and research programs in recognition of the significant overlap between conventional specialties and other therapies. The National Institutes of Health defines integrative medicine as *the incorporation of complementary (nonmainstream) approaches into "mainstream" health care* and, as such, the list of potential therapies within the field is extensive.

Integrative medicine services in veterinary hospitals include various combinations of therapies (**Table 1**).

Other terms, such as **holistic** and **alternative** medicine, have been used to describe integrative therapies. However, *holistic medicine* suggests that conventional medical approaches do not account for the whole of a patient's health, while *alternative medicine* implies that its therapies are outside, and separate, of conventional medicine. Therefore, neither term describes a system of medicine that incorporates efficacious aspects of conventional and complementary care.

INTEGRATIVE MEDICINE IN PRACTICE

Human patients have increasingly used integrative medical approaches in the past 2 decades,[4] and it is theorized that these patients may also pursue integrative approaches for their pets.

In humans, the use of integrative therapies has particularly increased in certain patient populations: nearly 50% of human oncology patients use complementary therapies as part of their treatment protocols, and a veterinary study found a similar prevalence in pets nearly a decade ago.[5,6] Increased research is necessary to better define the demand and current utilization of such therapies in veterinary medicine.

A recent small survey examined the benefits of offering acupuncture in small animal practices.[7] Practices that offered acupuncture indicated that:

- A mean of 14% of appointments were for this service
- About ⅓ of their acupuncture clients used the practice for routine care after initially visiting the practice for their first acupuncture appointment.

Both acupuncture and randomly selected primary care clients indicated that:

- They were more likely to use a veterinarian who offered acupuncture
- The mean pet expenditures of both types of clients were similar, refuting the idea that only those with increased discretionary income elect acupuncture treatment.

PREPARING YOUR PRACTICE

Many practices have effectively managed to incorporate integrative therapies, while others have struggled even after a significant investment of time and resources. Therefore, each practice must evaluate a number of variables before offering integrative therapies.

Acupuncture

Evidence. The increasing use of acupuncture in veterinary medicine remains controversial, primarily due to the inclusion of aspects of Traditional Chinese Medicine by some practitioners.[8]

A complex system of acupuncture meridians, originally designed for humans, has been transposed to dogs and cats. The complete system has not been evaluated, but there is documented evidence for clinically relevant physiologic responses to needle insertion at specific acupoints (eg, GV-20, PC-6, ST-36) (**Figure 1**).[9-11]

Research in a number of species suggests that electroacupuncture—the application of low level (mA) current to acupuncture needles—releases endogenous opioids that modulate pain (**Figure 2**, page 80).[12] However, clinical studies in veterinary medicine have been mixed and more are needed.[13-16]

Training. While no certification is legally required to perform acupuncture as a veterinarian, there are three major training and certification programs in the United States:

FIGURE 1. An acupuncture needle has been placed in this Weimaraner at GV-20, a common acupuncture point that is associated with sedation.

Additional Complementary Therapies

Homeopathy: A medical system in which dilute remedies are given, based on an underlying principle that "like treats like" and that more dilute agents are more potent. The stock solutions are generally derived from agents that, in high doses, would cause the clinical signs a patient displays. The science of homeopathy is subject to significant controversy as many of the most potent remedies contain no molecular trace of the stock compound. More information is available from the Academy of Veterinary Homeopathy (theavh.org).

Herbal medicine: Herbs are often included in many traditional medical systems, and are scientifically studied in an emerging field known as *ethnopharmacology*. Controversies surround the safety and efficacy of such products. Several private organizations certify veterinarians in different types of herbal medicine. Additional information is available from the Veterinary Botanical Medicine Association (vbma.org) and Chinese medical organizations, such as the American Association of Traditional Chinese Veterinary Medicine (aatcvm.org) and International Veterinary Acupuncture Society (ivas.org).

Therapeutic massage: This modality is commonly considered part of rehabilitation and sports medicine, but it may be practiced by nonveterinarians in some states. Several private certifications for nonveterinarians and veterinarians exist, and many rehabilitation certification programs discuss this modality.

Veterinary spinal manipulation therapy (VSMT): This modality is similar in some ways to human chiropractic treatments, although in many states that name is reserved for licensed chiropractic professionals. There are different schools and styles of VSMT practice, but many promote the concept of joint subluxation to explain reported effects. Several scientific veterinary reviews are published, but much of this focuses on equine practice. Additional information is available from the American Veterinary Chiropractic Association (animalchiropractic.org).

Note: *The author does not practice or possess training in veterinary homeopathy or veterinary spinal manipulation.*

- Chi Institute, tcvm.com
- International Veterinary Acupuncture Society, ivas.org
- Medical Acupuncture for Veterinarians, onehealthsim.org.

These certifications are issued by private organizations and are not regulated. Tuition costs for the training programs listed above range from approximately $6000 to $8500. The AVMA does not regulate acupuncture certifications as they do with specialty board certifications, such as surgery or internal medicine.

Staffing. Acupuncture can be offered without additional staffing. Acupuncture needles are generally left in situ for 15 to 30 minutes, during which time a practice may or may not have a staff member with the patient and client. Most practices reserve a room for clients to relax with their pets after needle placement by the veterinarian.

Patient & Client Population. In small animal practices, acupuncture is most commonly employed for osteoarthritis and intervertebral disk disease.[7] Therefore, small animal practices with a large geriatric pet population will likely support an acupuncture caseload if the clinic staff and veterinarians are proactive in offering the service. Practices with a rehabilitation or pain management focus will also likely derive benefit. Acupuncture anecdotally appears most successful in practices with longer appointment times and clients residing in certain geographic locations (eg, urban areas, East and West coasts). Clients will often need to return at a routine interval for regularly scheduled follow-up treatments, and these visits may increase the client's bond to the veterinarian.

Facilities & Equipment. Acupuncture requires little additional equipment. Acupuncture needles are sold in different sizes, with 32- to 34-gauge needles being most commonly used in small animals. Cost per treatment for acupuncture needles is estimated at $1, and electroacupuncture units are available for about $300. The total treatment cost to practices in Central Florida was estimated as $50.65 per visit, including amortized education and administrative costs, with a reported average client fee of $95.80.[7]

Practices should be prepared to have a room occupied for 40 to 45 minutes. This may be disruptive if it exceeds the average appointment time or if examination rooms are limited. Additionally, many practices use more comfortable furniture and examination areas for acupuncture rooms given that both patient and client will be relaxing during needle administration.

Summary. Acupuncture provides adjunctive treatment for management of chronic musculoskeletal and neurologic abnormalities, with few significant costs apart from initial training. Local market demographics are likely to heavily influence success of acupuncture in any practice.

Nutrition & Nutraceuticals

The number of commercial manufacturers of pet foods and supplements is increasing, and owners frequently inquire about novel strategies for feeding, including frozen, raw, grain-free, organic, home prepared, and sustainable diets.[17] Dietary supplements are administered by some owners and may include vitamins, minerals, antioxidants, essential fatty acids, food extracts, chondroprotectants, and herbal medications, among others.

Guidelines for nutritional assessment have been published,[18] and nutrition is a cornerstone of wellness examinations and programs. Discussion of nutrition with the client reinforces the veterinarian as the expert in this field and prevents nutritional outsourcing to other entities. Such discussions are likely to strengthen the owner–veterinarian bond, which has positive implications for the practice.

Nutrition and nutraceuticals are extensively discussed in the articles, **Novel Trends in Small Animal Nutrition: A Practical Guide** (January/February 2013) and **Surveying**

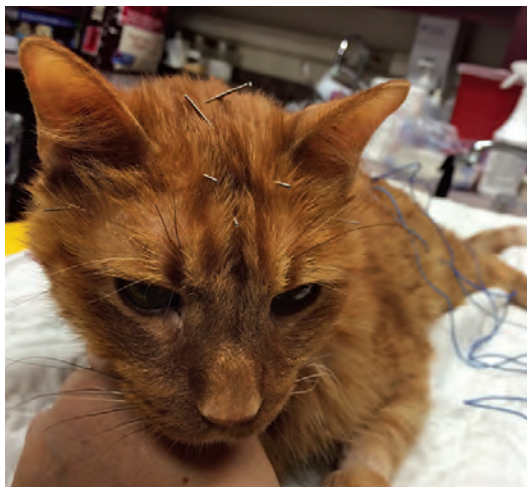

FIGURE 2. A cat receives electroacupuncture stimulation for osteoarthritis and diffuse muscle atrophy. The electrical stimulation of acupuncture needles may increase the analgesic effects of the treatment.

Supplements: Current Trends, Research, and Recommendations (May/June 2014), available at tvpjournal.com.

Laser Therapy

Evidence. Several classes of therapeutic lasers for pain, inflammation, and wound healing are marketed to veterinary professionals. Laser stands for *light amplification by stimulated emission of radiation*. Light in the visible red and infrared spectra (600–1000 nm) exerts the biologic effects of photobiomodulation, which include[19]:

1. Increased adenosine triphosphate (ATP) production through activity on mitochondrial cytochrome C oxidase
2. Induction of cellular antioxidant production due to a sublethal increase of free oxygen radicals
3. Vasodilation as a result of nitric oxide release from proteins.

The clinical effects on increased dermal healing are well established in other animal models and in humans.[20,21] The effects on tendons and osteoarthritis are mixed or unclear. Few clinical trials in dogs or cats have been performed. A postoperative protocol for dogs after decompressive hemilaminectomy documented decreased time to ambulation.[22] The benefits in other conditions are unclear. The potential side effects at high doses include thermal burns and cellular apoptosis, and the optimal doses for most conditions are not yet established.

Training. Laser manufacturers typically provide training to the purchasing veterinary practice. For example, the American Institute of Medical Laser Applications (aimla.org) provides additional training on laser theory, types, and protocols. Independent training may be provided by rehabilitation and sports medicine training programs and continuing education conferences.

FIGURE 3. A dachshund receives class IIIb laser therapy and electroacupuncture 24 hours after a hemilaminectomy for intervertebral disk protrusion.

Staffing. Veterinary technicians can be trained to provide laser therapy based on the attending veterinarian's prescription. Knowledge of anatomy and laser principles is helpful.

Patient & Client Population. Therapeutic lasers are used most frequently for wound or incisional healing, osteoarthritis, soft tissue injury (tendon, ligament), and intervertebral disk disease. There is likely significant overlap with the patient population for which acupuncture and rehabilitation is suitable. Some practices use laser therapy on all cases postoperatively to increase their laser caseload and return on investment.

Facilities & Equipment. The cost of a laser is generally proportional to its power. Lower-powered lasers (class IIIb, **Figure 3**) may be purchased for around $5000, whereas those with a higher power (class IV) range upwards of $20,000. The power (W) describes the amount of energy (J) delivered over time (s). A higher-powered laser can deliver a dose of photonic energy in a shorter period of time, saving labor costs. The benefits of low-power versus high-power lasers are heavily debated. Practices will require a significant laser caseload to achieve an adequate return on investment with most class IV lasers.

Summary. Laser energy exerts potentially advantageous cellular effects. The precise dose needed in the treatment of veterinary patients to reach targeted cells remains unknown for most conditions, and only limited clinical trials are available. Practices should carefully evaluate their potential caseload before committing to an expensive laser.

Rehabilitation

Evidence. Canine rehabilitation has experienced significant growth in recent years. Rehabilitation protocols rely on a combination of physical exercises, hydrotherapy (**Figures 4 and 5**, page 82), therapeutic ultrasound, thermal modalities, laser, electrostimulation, and shockwave therapy. Many specialty centers have established rehabilitation practices to routinely assist in postsurgical recovery and reconditioning. The literature in this area has

primarily focused on questions of basic mechanisms and responses,[23] whereas controlled clinical data are comparatively sparse.[24]

Training. Two training certifications are presently available for canine rehabilitation: one offered by the University of Tennessee Rehabilitation program (ccrp.utvetce.com) and another by the Canine Rehabilitation Institute (caninerehabinstitute.com). Cost of tuition ranges from $5000 to $6500. Certification is available for veterinarians, veterinary technicians, and physical therapists.

Similar to acupuncture training, certification is not legally required to incorporate rehabilitation modalities into a clinical practice. Specialist certification is regulated by the American College of Veterinary Sports Medicine and Rehabilitation (vsmr.org) and requires considerable practice experience or residency training.

Staffing. Rehabilitation is labor intensive and veterinarians will be unable to charge effectively for their time if they are performing the modalities themselves. As a result, a trained and dedicated technician is necessary in most practices. Two to three rehabilitation technicians are utilized for every evaluating veterinarian in many dedicated rehabilitation programs.

Patient & Client Population. Practices with a robust orthopedic surgery caseload and/or sports medicine patients are most likely to benefit. Rehabilitation can also be of value in geriatric and neurologic patients, but the caseload may be insufficient if reliant on this population alone. Most clients who invest in surgical repair of an injury, or who prefer to avoid surgery, appear willing to pay the comparatively low cost for rehabilitation. Many clients are receptive to a fee structure in which individual modalities are included in a package price rather than itemizing specific charges for each intervention, which also avoids any appearance that items are being oversold to increase clinic production.

Facilities & Equipment. The breadth of rehabilitation services offered is directly related to the initial costs. Many rehabilitation techniques require sufficient space, and an area of about 500 square feet is the suggested minimum if active exercises are performed. This space requirement can be a challenge for existing practices, most of which were not designed with rehabilitation in mind. Passive modalities can be provided with less space, but this may limit the type of cases that can be managed.

Equipment costs vary considerably based on the modalities offered. **Table 2** provides a list of equipment and suggested applications.

Summary. Rehabilitation medicine incorporates many different therapies and can

FIGURE 4. A Labrador retriever receives hydrotherapy following a forelimb amputation for osteosarcoma. Swimming increases the active range of motion of some joints and provides low-impact activity. *Courtesy University of Florida College of Veterinary Medicine*

FIGURE 5. A geriatric Labrador retriever with diffuse osteoarthritis walks on an underwater treadmill, which provides exercise and muscle conditioning with reduced weight-bearing.

TABLE 2.
Rehabilitative Equipment: Suggested Uses & Costs

EQUIPMENT	USE	APPROXIMATE COST
Underwater treadmill and/or pool	Low-impact activity	$20,000+
Therapeutic ultrasound	Deep thermal stimulation, collagen remodeling	$1500+
Therapeutic laser	Tissue healing	$5000–$30,000+
TENS/NMES unit	Electrostimulation	$50
Shockwave therapy	Tissue remodeling	$15,000+
Exercise aids/equipment	Therapeutic exercises	$1000

NMES = neuromuscular electrical stimulation; TENS = transcutaneous electrical nerve stimulation

be tailored to the practice. However, dedicated technical staff will be required, and sufficient caseload is best achieved with an existing population of geriatric, performance, or postsurgical patients.

Regenerative Medicine

Evidence. A patient's endogenous cells can be harvested, processed, and then employed to stimulate tissue repair and to reduce inflammation. The primary regenerative veterinary medical techniques are platelet therapy and stem cell therapy. The hemostatic effects of platelets are well known, but platelets also contribute to cellular signaling, immunity, and tissue regeneration. The latter is primarily mediated by a number of growth factors found in alpha granules (**Table 3**).[25] Dogs have shown clinical response when treated with platelet products for osteoarthritis, postoperative cranial cruciate ligament rupture, and tendinopathy, although such studies are preliminary and often characterized by low study numbers and lack of a control group.[26-28] Moreover, studies in other species have been mixed.

TABLE 3.
Growth Factors in Platelet Alpha Granules

GROWTH FACTOR	FUNCTIONS
PDGF-BB (and other isoforms)	• Mitosis of fibroblasts and smooth muscle cells • Angiogenesis and connective tissue production • Influences expression and coordination of other growth factors
TGF-beta	• Extracellular matrix synthesis • Type 1 collagen production • Mesenchymal stem cell proliferation • Granulation tissue formation • Immunoregulation (local)
bFGF	• Angiogenesis • Proliferation of cells of mesodermal origin
VEGF	• Angiogenesis • Endothelial cell proliferation
EGF	• Angiogenesis • Mitosis of fibroblasts, osteoblasts, and epidermal cells • Keratinocyte locomotion and cutaneous collagen production
IGF-1	• Cell proliferation and survival • Platelet signaling and activation • Myoblast proliferation
CTGF	• Fibrosis • Platelet adhesion • Angiogenesis • Developmental chondrogenesis

Various platelet collection and processing systems are available and described in previous scientific studies (**Figure 6,** page 84).[29]

Autologous stem cells are typically processed from adipose tissue, obtained from surgical collection of falciform ligament or subcutaneous fat, and variably described as mesenchymal or adipose-derived stem cells. These cultured multipotent cells can then theoretically differentiate into adipocytes, chondrocytes, or osteoblasts.

Initial clinical studies in dogs with osteoarthritis demonstrate a modest and temporary reduction in clinical signs.[30,31] Some authors suggest that any

For further information

on integrative medical therapies, read the following articles, available at tvpjournal.com:

- **Canine Rehabilitative Nutrition** (January/February 2015)
- **Laser Therapy in Companion Animals** (May/June 2014)
- **Platelet Rich Plasma: Its Place in Cranial Cruciate Ligament Repair** (November/December 2012)
- **Physical Rehabilitation for Veterinary Practices** (March/April 2012)
- **Advances in Stem Cell Therapy: Application to Veterinary Medicine** (July/August 2011)

underlying structural abnormalities should first be repaired for best results and that cartilage scaffolding is necessary for persistent cartilage repair.[32]

Training. Most platelet products can be processed in-house with minimal additional training beyond the information provided in product instructions. Autologous stem cells require surgical biopsy and processing by an outside laboratory. Intra-articular injection of both products requires knowledge of basic musculoskeletal anatomy.

Staffing. Additional staffing is not required.

Patient & Client Population. Practices with a geriatric, sports medicine, orthopedic surgery, or rehabilitation caseload have the best opportunity to use these techniques. The cost of platelet processing and injection to the client will be greater than $150, and the cost of stem cell injections is generally greater than $1000 per dose.

Facilities & Equipment. Platelet processing requires centrifugation equipment obtained from the manufacturer of the separation system. Stem cell processing is typically done off-site. The initial investment in equipment is low, so these therapies can be offered on an intermittent basis.

Summary. Regenerative medicine is a growing area within integrative therapeutics. Clients find the concept of promoting endogenous healing to be appealing, but treatments are more expensive than other interventions. Practices with other integrative modalities are best positioned to use these treatments given the overlap in indications for this and other techniques.

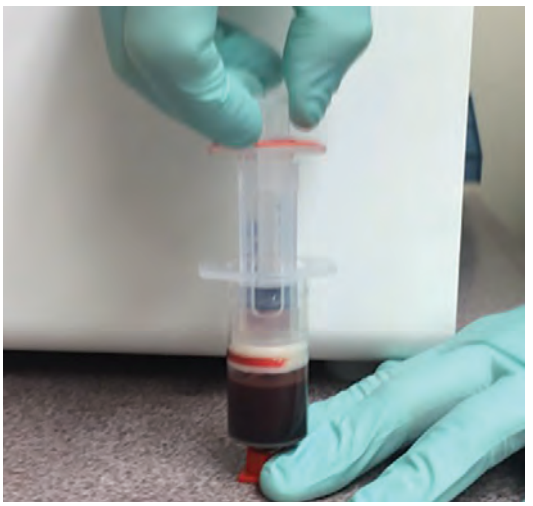

FIGURE 6. Multiple systems are available to extract platelet containing plasma from a patient's blood. The system shown here uses a double-barreled syringe and a specific centrifugation technique.

Hyperbaric Oxygen

Evidence. Hyperbaric oxygen chambers (**Figures 7 and 8**) have been extensively studied and utilized in human medicine, but two of the most common applications in human health—decompression sickness and carbon monoxide toxicity—are rarely encountered in veterinary patients. However, they are also approved in humans for treatment

FIGURE 7. A young German shepherd is treated with a session of hyperbaric oxygen for oomycosis.

FIGURE 8. Most veterinary hyperbaric chambers can accommodate all but the largest of canine patients, and most sessions last 1 to 2 hours.

of extensive wounds and burns, radiation injury, refractory osteomyelitis, compartment syndrome, and severe anemia. Hyperbaric chambers are used clinically, but not approved, for spinal and head trauma and stroke.

Physiologic effects of hyperbaric oxygen are well documented and similar to laser therapy; the advantage of hyperbaric oxygen is its systemic, rather than localized, effect. Hyperbaric oxygen induces[33-35]:

1. ATP production by providing additional oxygen for phosphorylation
2. Compensatory increase in intracellular antioxidant production due to sublethal doses of free oxygen radicals
3. Post-treatment vasodilation due to nitric oxide release.

Veterinary research studies are mixed regarding effects of hyperbaric oxygen. Experimental studies in cats demonstrated positive effects on wound healing of skin flaps but minimal effects on short-term fracture healing.[36,37] A standardized treatment protocol for dogs was shown to be well-tolerated in a clinical rehabilitation center, and oxygen toxicity—characterized by intrasession seizures and reported in many species, including humans and dogs—was not observed.[38] The most common conditions treated included intervertebral disk disease and extensive wounds, although efficacy was not reported.

Training. The manufacturers of chambers provide initial training. There are independent organizations that certify hyperbaric technicians for human medical practice. A certified hyperbaric technologist program for veterinary staff is now available from several continuing education providers (nbdhmt.org/chtv.asp).

Specific guidelines for safe chamber use must be rigidly followed because oxygen chambers can be the source of injury or death, as was the case when an equine unit exploded in a private rehabilitation center in Florida. Adverse events of this severity have not been reported with small animal chambers, but veterinarians should discuss safety and liability with the chamber manufacturer.

Staffing. Hyperbaric chamber sessions require constant monitoring and, therefore, a dedicated hyperbaric technician is required unless the chamber is installed in a central treatment area where observation will be constant.

Patient & Client Population. Hyperbaric oxygen sessions are generally reserved for severe injuries, and only those facilities with a large emergency caseload or specialty referral population are likely to support the costs and technical staff required. Hyperbaric chamber sessions are charged at an average rate of approximately $150 per hour in the Southeast United States and, therefore, practices that can support a hyperbaric chamber are likely to be those with a higher average transaction charge.

Facilities & Equipment. Used or new hyperbaric oxygen units manufactured for humans can be purchased for animal use, while veterinary-specific hyperbaric chambers are available from Veterinary Hyperbaric Oxygen (vhbo2.com) and Hyperbaric Veterinary Medicine (hvmed.com). Companies may provide lease options to reduce initial costs, but modification of an existing building is required to provide a true earth ground, an outside exhaust line, and dedicated oxygen input.

The outright purchase cost of a hyperbaric chamber is quite variable, and veterinary manufacturers either exclusively lease or do not provide exact costs publicly. Human hyperbaric chambers start at around $75,000 for a reconditioned unit that is about 10 years old. New units with advanced monitoring and/or safety features for human practice may cost more than $150,000.

Summary. Only several hyperbaric chambers were in use for small animals nearly a decade ago, but now dozens of chambers have been installed in large practices in the U.S. The hyperbaric veterinary field is expected to continue to grow, but practices should carefully evaluate whether there is adequate caseload to justify the operating and startup costs. Moreover, additional information is required to better refine the clinical benefits of hyperbaric oxygen.

THE PRACTICE OF THE FUTURE

Integrative medicine has experienced significant owner-driven growth. As a result, it is unlikely the profession will witness a reduction of these therapies in the future.

Clients are increasingly searching for comprehensive treatment protocols, and clinics that can provide the best combination of therapeutic modalities are likely to improve market share and patient care. Practices investing in one or more integrative modalities now are also likely to gain the experience necessary to adapt to future trends and implement the best evidence-based complementary approaches with conventional care.

In the future, and as additional research becomes available, many therapies considered "outside mainstream treatment" today will likely become standard treatment in the future and provide a critical component of veterinary care.

NMES = neuromuscular electrical stimulation;
TENS = transcutaneous electrical nerve stimulation;
VSMT = veterinary spinal manipulation therapy

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Review of Equine Hyperbaric Medicine

Nathan Slovis, DVM, Dipl. ACVIM, CHT

ABSTRACT

Hyperbaric oxygen therapy appears to be a promising adjunctive treatment for a variety of equine disorders, including laminitis and other ischemic injuries. Hyperbaric oxygen (HBO) is a high-dose oxygen inhalation therapy that is achieved by having the patient breathe 100% oxygen inside a pressurized hyperbaric chamber. The delivery of oxygen to the tissues is through respiration because there is insufficient absorption of oxygen through the skin. The benefits of HBO are derived from both the physiologic and pharmacologic effects of high-dose oxygen. HBO is based on two physical factors related to the hyperbaric environment: mechanical effects of pressure and increased oxygenation of tissues. The use of HBO by veterinary medical hospitals is in its infancy. Our clinic has currently treated more than 250 patients in our HBO chamber. Patients included pregnant animals as well as neonatal foals, with no adverse effects noted. Patients have been pressurized from 1.5 to 3 ATA (ATM absolute) ranging from 60 to 90 minutes at treatment pressure (depth). Hagyard Equine Medical Institute has used HBO as adjunctive therapy for fungal disease (fungal pneumonia); thermal burns, carbon monoxide, smoke inhalation; closed head injuries; ileus; central nervous system edema/perinatal asphyxia; peripheral neuropathies; sports injuries (exertional rhabdomyolysis); cellulitis; compartment syndrome; and ischemic injuries (laminitis). In carefully selected patients, the addition of HBO therapy to standard measures may improve clinical outcomes. Further research is needed in the field of equine HBO medicine.

Keywords: Hyperbaric oxygen; Oxygen therapy; Hyperbaric chamber; Boyle's law; Equine

INTRODUCTION

Hyperbaric oxygen (HBO) is a high-dose oxygen inhalation therapy that is achieved by having the patient breathe 100% oxygen inside a pressurized hyperbaric chamber (Figure. 1). The delivery of oxygen to the tissues is through respiration because there is insufficient absorption of oxygen through the skin.[1,2] The principal source of oxygen transport is the red blood cell in the form of oxyhemoglobin. At normal sea level pressure where alveolar oxygen pressure is at 100 mmHg, hemoglobin is approximately 97% saturated and yields an oxygen content of approximately 19.8 mL oxygen per deciliter blood. When alveolar oxygen pressure is at 200 mmHg, hemoglobin becomes fully saturated with oxygen. After hemoglobin is fully saturated, additional oxygen is carried to the tissues in physical solution in plasma. HBO does not significantly increase hemoglobin's transport of oxygen but elevates the capillary plasma oxygen transport.[3,4] The benefits of HBO are derived from both the physiologic and pharmacologic effects of high-dose oxygen. HBO is based on two physical factors related to the hyperbaric environment: mechanical effects of pressure and increased oxygenation of tissues. This paper reviews scientific and clinical literature regarding HBO therapy in laboratory animals and humans and introduces to the practitioner the potential use of this treatment modality for equine patients.

HISTORY OF HYPERBARIC CHAMBERS

In 1662, a British clergyman named Henshaw, without scientific basis thought it would be a good idea to raise the ambient pressure around a patient for therapeutic purposes. He later built the "domicilium," which was a sealed chamber that could either raise or lower pressure depending on adjustment of the valves. Henshaw reported that acute diseases of all kinds would respond to increased ambient pressure. In the 19th century, following Henshaw's ideology, pneumatic institutes began to spread throughout Europe. These large chambers were often able to accommodate more than one person and could sustain pressures of 2 or more atmospheres. These pneumatic institutes started to rival the popularity of the mineral water spas.[5]

It was not until 1879 that semi-scientific efforts were made in regard to atmospheric pressure. A French surgeon names Fontaine built a mobile operating room on wheels that could be pressurized. He performed over 20 surgeries in the unit, using nitric oxide as the anesthetic. Dr. Fontaine noted that he could achieve deep surgical anesthesia because it increased the effective percentage of nitrous oxide in the patient's body accompanied by a higher oxygen partial pressure (ie, compressed air at 2 atmospheres gives an effective level of 42% inhaled oxygen). Dr. Fontaine also noted that hernias were seen to reduce

From the Hagyard Equine Medical Institute, McGee Medical Center, Lexington, KY. Reprint requests: Nathan Slovis DVM, Dipl. ACVIM, CHT, Hagyard Equine Medical Institute, McGee Medical Center, 4250 Iron Works Pike, Lexington KY 40511.

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Fig. 1. The current chamber that Hagyard Equine Medical Institute uses is similar to a round stall, 10 feet in diameter and 9-1/2 feet tall. It is recessed into a concrete pad, so the horse does not have to walk up a ramp. The horse enters through a 42-inch door, where you can turn the horse loose and allow it to walk around in the chamber. A horse with laminitis can even lie down to be more comfortable.

more easily (Boyle's law—pressure–volume relationship), and the patients were not their normal cyanotic color when coming out of anesthesia.[5]

Compressed air therapy was first introduced into the United States in 1871 by Dr. J.L. Corning. In the early 1900s, Dr. Orville Cunningham, a professor of anesthesia at the University of Kansas, noted that patients with heart disease and other circulatory disorders had difficulties acclimating at high altitudes when compared with sea level. With these observations, Dr. Cunningham postulated that increased atmospheric pressure would be beneficial for patients with heart disease. To test his hypothesis (1918), he placed a young resident physician suffering from the flu into a chamber used for animal studies. The physician was successfully oxygenated during his hypoxic crisis when compressed to 2 atmospheres (ATM). Dr. Cunningham, realizing that his concepts were sound, built an 88-foot-long chamber, 10 feet in diameter, in Kansas City and began treating a multitude of diseases, most of them without scientific rationale.[5] The American Medical Association (AMA) and the Cleveland Medical Society, failing to receive any scientific evidence for his rationale, forced him to close his facility in 1930.

The advent of the use of HBO in modern clinical medicine began in 1955 with the work of Churchill-Davis, who helped to attenuate the effects of radiation therapy in cancer patients using high oxygen environments. That same year, Dr. Ite Boerema, a professor of Surgery at the University of Amsterdam in Holland, proposed using HBO in cardiac surgery to help prolong the patient's tolerance to circulatory arrest. He conducted surgical operations under pressure, including surgical corrections of transposition of the great vessels, tetralogy of Fallot, and pulmonic stenosis. In 1960, Dr. Boerema published a study on "life without blood." The study involved exsanguinating pigs and removing their erythrocytes before exposing them to 3 ATM HBO. These pigs were noted to have sufficient oxygen in the plasma to sustain life when they where given HBO at 3 ATM absolute (ATA).[6]

It has frequently been said that the history of "hyperbaric oxygenation" goes back "over 300 years," probably referring to the work of Henshaw. This is incorrect, as oxygen was not discovered until 1775 by Joseph Priestley. All the early chambers were pressurized with compressed air, and oxygen was not a consideration. Clinical hyperbaric oxygen goes back only about 50 years, beginning with the work of Churchill-Davidson and Boerema.[5]

In 1967, the Undersea Medical Society (UMS) was founded by six U.S. Navy Diving and Submarine medical officers as an organization dedicated to diving and undersea medicine. The UMS was renamed Undersea and Hyperbaric Medical Society in 1986. This professional society was established for those practicing hyperbaric medicine or diving medicine. They are responsible for publishing approved indications for HBO treatments.

The American Board of Preventive Medicine started to offer board certification in Undersea and Hyperbaric Medicine in 1999, which was later co-sponsored by the American Board of Emergency Medicine in 2001. The National Board on Diving and Hyperbaric Medical Technology began offering board certification in hyperbaric technology in 1991 and for hyperbaric nursing in 1995.

Numerous human fellowships are available in the United States in Clinical Hyperbaric Medicine.

PHYSIOLOGIC EFFECTS

Pressure of gases is defined as a force per unit area. The pressure of 1 atmosphere (ATM) is equal to 14.7 pounds per square inch (PSI). This pressure results from the weight of the air producing a force on the surface of the earth.

Weathermen usually refer to this pressure as "barometric pressure," which is measured in inches of mercury (29.9 inches of mercury = 760 mm mercury = 1 atmosphere). The term "atmospheres," when used, refers to atmospheres absolute. Absolute pressure equals the gauge pressure plus the ambient air pressure on the surface at sea level (ie, 1 ATM). For example, if one descends 33 feet in seawater (FSW), one is at an absolute pressure of 2 ATM. This is demonstrated by the fact that 33 feet is equal to a gauge pressure of 14.7 pounds per square inch as read on the gauge. Absolute pressure equals gauge pressure plus atmospheric pressure (ie, 1 ATM + 1 ATM = 2 ATM).[7]

Absolute Pressures		Gauge Pressures	
ATA	mmHg	FSW	PSI
1	760	0	0
2	1,520	33	14.7
3	2,280	66	29.4
4	3,040	99	44.1

Terms Applicable to Hyperbaric Exposures[8]

1. *Surface*: The normal atmospheric pressure from which a hyperbaric exposure begins (ie, ground level or sea level)
2. *Dive*: Any exposure to hyperbaric pressure, either in water or in a chamber
3. *Descent*: Increase in pressure, either by going down under water or by adding pressure to a chamber. May be referred to as compression
4. *Depth*: The maximum pressure achieved during a hyperbaric exposure. Typically measured in ATA, FSW, or PSI. Also referred to as treatment pressure
5. *Ascend*: Decrease in pressure. May be referred to as decompression

Gas Laws

Boyle's Law (Table 1): Pressure–volume relationship. With pressure constant, the volume of gas is inversely proportional to the pressure ($P_1/P_2 = V_2/V_1$).

Table 1. Boyle's Law - Pressure-volume relationship

Pressure (psi)	Feet Sea Water	Atmospheres Absolute	Relative Volume	mmHg
14.7	Sea level	1 ATA	1	760
29.4	33 fsw	2 ATA	1/2	1520
44.1	66 fsw	3 ATA	1/3	2280
58.8	99 fsw	4 ATA	1/4	3040
73.5	132 fsw	5 ATA	1/5	3800

When a chamber is pressurized, the volume of gas in enclosed body areas, such as the ears, sinuses, lungs, and gastrointestinal tract, respond to increased pressure by contracting. Doubling the pressure reduces the gas volume to half, and tripling the pressure reduces it by a third.

Dalton's Law: Total pressure exerted by a mixture of gases is equal to the sum of the pressure of each of the different gases making up the mixture (partial pressure of oxygen $[PO_2] = P_{tot} \times$ forced inspiratory oxygen $[FiO_2]$, where FiO_2 is the fractional concentration of oxygen expressed as a decimal).

Using Dalton's Law, we would be able to determine the PO_2 in mmHg in the chamber while breathing 100% oxygen at 66 FSW:

66 FSW = 3 absolute atmospheres

$PO_2 = P_{tot} \times FiO_2$

$PO_2 = 760(3) \times 1.0$

$PO_2 = 2{,}280$ mmHg

Henry's Law: Gas in Solution. The amount of gas dissolved in a liquid is directly proportional to the partial pressure of the dissolved gas ($P_1/P_2 = A_1/A_2$).

To help illustrate Henry's law, think about a carbonated drink containing 20 cc dissolved gas at 2 ATA. How much gas remains in solution when the beverage reaches sea level?

$P_1/P_2 = A_1/A_2$

$2/1 = 20/A_2$

$2A_2 = 20$

$A_2 = 10$ cc

MECHANICAL EFFECTS

Bubbles and gas-containing cavities within the body are subject to the mechanical effects of changing pressure, which follows Boyle's Law. Volume is changed in a geometric progression related to pressure change; large reductions take place near the surface, with subsequent reductions becoming smaller at higher pressure (Table 1). These mechanical effects are responsible for unwanted barotraumas that may result in middle-ear squeeze, sinus squeeze, and burst of lung if the patient holds their breath during decompression. If a patient is suffering from gaseous distention of the bowel, compression in the chamber eases the discomfort while the inhalation of oxygen forms a high gradient for the removal of nitrogen from the distended gut. Gas trapped in the bowel decreases by approximately 50% when a patient breathes oxygen over a 6-hour period at 2 ATM.[3,9,10]

OXYGEN SOLUBILITY

As chamber pressure increases, PO_2 in the breathing media also increases. For instance, using Dalton's Law, air at sea

level pressure (760 mmHg) contains 21% oxygen with a PO_2 of 160 mmHg. When the chamber is pressurized with air to 3 ATA, PO_2 is 479 mmHg, which is equivalent of breathing 63% oxygen at sea level. As the chamber is pressurized with air of 5 ATA, PO_2 exceeds 798 mmHg, which is greater oxygen pressure than can be attained breathing 100% oxygen at sea level!

Oxygen is transported by the blood from the lungs into the tissue by two methods: bound to hemoglobin and physically dissolved in the plasma. At normal sea level pressure, where alveolar oxygen pressure is about 100 mmHg, hemoglobin is already 97% saturated (oxyhemoglobin) and yields an oxygen content of approximately 19.8 mL of oxygen per dL blood. When alveolar oxygen partial pressure (PAO_2) reaches 200 mmHg, hemoglobin then becomes fully saturated with oxygen. Therefore, further increases in pressure will not increase the amount of oxyhemoglobin; thus, oxygen transport via hemoglobin is not improved with HBO therapy. Instead, oxygen is dissolved into the plasma and carried to the tissues in physical solution. A person breathing air at sea level pressure has only 1.5% of the oxygen physically dissolved in plasma. Oxygen transport by plasma is the key to HBO therapy, for even poorly perfused tissue can receive oxygen as the hyperoxygenated plasma seeps across it.[4] As the chamber is pressurized, the elevated alveolar oxygen tension in the lungs drives oxygen into the plasma of the pulmonary circulation and is subsequently transported throughout the body. Unlike hemoglobin saturation, which has an S-shaped curve, the amount of dissolved oxygen increases linearly as PO_2 increases.[4]

Oxygen solubility is defined by Henry's Law, which describes the relative quantity of gas entering solution as related to the PAO_2, but does not define the absolute amount of gas in solution. The absolute amount of gas varies with different fluids and is determined by the solubility coefficient of gas in fluids, which is temperature dependent. Oxygen solubility in whole blood at 37°C is 0.0031 mL of O_2 per deciliter blood per millimeter of mercury PAO_2. Breathing air at sea level, arterial oxygen tension is approximately 100 mmHg; therefore, the blood carries approximately 0.31 mL dissolved oxygen per deciliter whole blood. When breathing 100% oxygen at sea level, the amount of dissolved oxygen increases to approximately 2.1 mL O_2 per deciliter blood. Breathing 100% oxygen at 2 ATA results in a PAO_2 of 1,433 mmHg (4.4 mL dissolved oxygen per deciliter blood), and at 3 ATA provides a PAO_2 of approximately 2,200 mmHg and adds approximately 6.8 mL O_2 to each deciliter blood. A healthy adult human at rest uses approximately 6 mL oxygen per deciliter circulating blood. Thus, HBO at 3 ATA provides sufficient plasma oxygen to exceed the body's total metabolic requirement. The dissolved content of 6 mL oxygen per deciliter blood is equivalent to the sea level oxygen capacity of 5 g hemoglobin. This phenomenon is the reason Dr. Boerema was able to sustain a pig's life without blood in his study "Life Without Blood."[4,6]

GAS EXCHANGE AND OXYGEN DIFFUSION

An increase in oxygen tension causes oxygen to diffuse further from the functioning capillaries. Tissue oxygen content depends on three factors:

1. Distance from the functioning capillaries
2. Oxygen demand of the tissue
3. Oxygen tension of the capillary

Using the Krogh Erlang mathematical model, breathing air at 1 ATA, oxygen diffuses approximately 64 μm (about the thickness of 1 sheet of typing paper) at the arterial end of the capillary. During oxygen breathing at 3 ATA, oxygen diffuses approximately 250 μm (about the thickness of three sheets of typing paper).[4,11-13] In a hypoxic environment, HBO may be able to restore PO_2 to normal or slightly elevated levels (depending on the severity of the injury); it enhances epithelization, collagen deposition, fibroplasia, angiogenesis, and bacterial killing. In the presence of tissue hypoxia, some or all of these processes are impaired. Human fibroblasts can survive in 3 mmHg, but cannot migrate in <10 mmHg. Fibroblasts also do not divide in <22 mmHg and do not form collagen in <28 mmHg. Interestingly, it has been reported that if oxygen tension is held continuously at 290 to 560 mmHg, fibroblastic replication is halted.[4] When oxygen tension returns to normal, the replication process continues. Therefore, daily high doses are needed to correct the hypoxic environment but must be delivered in an intermittent pattern to avoid possible side effects to the cells.

THERAPEUTIC EFFECTS OF HBO

1. Reverse hypoxia[14]
   - Increases the amount of dissolved oxygen in the plasma
2. Alter ischemic effect
3. Influence vascular reactivity
   - Decrease adherence of neutrophils to the microvasculature.
4. Reduce edema[15,16]
   - Hyperoxygenation will cause vasoconstriction. Although vasoconstriction may be present, there is more oxygen delivered to the tissues.
5. Modulate nitric oxide production[4,17,18]
   - An increase of nitric oxide leads to vasodilation, whereas a decrease of nitric oxide (NO) leads to vasoconstriction. Carbon dioxide increases NO production and oxygen decreases NO production by the endothelial cells.
6. Modify growth factors and cytokine effect by regulating their levels or receptors[19,20]

- *Vascular Endothelial Growth Factor (VEGF)* is important for the growth and survival of endothelial cells and is found in plasma, serum, and wound exudates. Under normobaric conditions, VEGF is stimulated by hypoxia, lactate, nitric oxide, and nicotinamide adenine dinucleotide. HBO induces production of VEGF, thereby stimulating more rapid development of capillary budding and granulation formation within the wound bed.

7. Induce changes in membrane proteins affecting ion exchange and gaiting mechanisms
8. Promote cellular proliferation[2,4,11-13]
9. Accelerate collagen deposition
10. Stimulate capillary budding and arborization
11. Accelerate microbial oxidative killing
12. Improve select antibiotic exchange across membranes[21-23]
    - Anoxia decreases the activity of several antibiotics (aminoglycosides, sulfonamides, fluoroquinolone). Raising the pO_2 of ischemic tissue to normoxic levels may normalize the activity of these antimicrobials. In addition, HBO may potentiate the activity of certain antimicrobials by inhibiting biosynthetic reactions in bacteria.
13. Interfere with bacterial disease propagation by denaturing toxins
14. Modulate the immune system response
15. Enhance oxygen radical scavengers, thereby decreasing ischemia–reperfusion (I-R) injury[24,25]
    - HBOT increases the amount and activity of the free radical scavenger superoxide dismutase
    - Decreased neutrophil adhesion and subsequent release of free radicals is an important early event leading to endothelial damage and microcirculatory failure associated with I-R injury. HBO reversibly inhibits the $ß_2$ integrins, therefore inhibiting neutrophil–endothelial adhesion

COMPLICATIONS AND SIDE EFFECTS

Although any therapeutic application of hyperbaric oxygenation is intrinsically associated with the potential for producing mild to severe side effects,[26] the appropriate use of hyperoxia is one of the safest therapeutics available to the practitioner. Central nervous system (CNS) oxygen toxicity can occur at levels of 3 ATA for 1 to 2 hours. Signs in humans include convulsions, nausea, dizziness, muscle twitching, anxiety, and confusion. Pulmonary oxygen toxicity is usually associated with prolonged exposure to HBO. Onset of symptoms has been noted to occur 4 to 6 hours at 2.0 ATA. Symptoms include dyspnea, shortness of breath, chest tightness, and difficulties inhaling a deep breath. Possible causes for pulmonary toxicity include thickening of the alveolar membrane and pulmonary surfactant changes. Prevention of side effects includes removal from the oxygen source when first signs occur and no 100% oxygen at pressures greater than 3 ATA.

Doxorubicin has been shown to cause severe cutaneous necrosis if extravasation occurs during treatment. In human medicine, researchers looked to see whether HBO therapy can help decrease severity of the tissue necrosis. To the researchers' surprise, there was an 87% mortality noted in rats when HBO therapy was used.[27] Cardiotoxicity was also noted when doxorubicin was combined with HBO therapy. It is therefore strongly recommended that horses treated with doxorubicin do not have HBO therapy.

Contraindications for HBO therapy are unknown for horses but may include untreated pneumothorax, high fevers (predispose to oxygen toxicity), emphysema, and upper airway obstructions. In cases of severe necrotizing pneumonia, a bronchopleural fistula may develop that, when exposed to HBO therapy, can result in a tension pneumothorax.

It is unknown whether HBO therapy will cause congenital defects in horses. In human studies, it has not been shown to have adverse effects. During the late 1970s and early 1980s, Russian scientists looked at 700 pregnant woman at all different stages of their gestation who were treated with HBO therapy and failed to identify any maternal or fetal complications or mortality.[28,29] In our hyperbaric center, we do not hesitate to treat a mare with HBO therapy, especially when the benefits outweigh the risks. It is not unusual in our clinic to allow the mare of a patient (foal) to be allowed in the chamber during treatments to aid in the relaxation of the foal.

Accepted indications for HBO therapy in humans that are covered by health insurance include[1]:

1. Air or gas embolism
2. Carbon monoxide poisoning
3. Clostridial myositis and myonecrosis
4. Crush injury, compartment syndrome, and other acute ischemias
5. Decompression sickness
6. Enhancement of healing in selected wounds
7. Exertional anemia
8. Intracranial abscess
9. Necrotizing soft tissue infections
10. Refractory osteomyelitis
11. Delayed radiation injury (soft tissue and bony necrosis)
12. Skin grafts and flaps
13. Thermal burns

The use of HBO in veterinary medicine is in its infancy. Our clinic has currently treated hundreds of equine patients in our HBO chamber. Patients included pregnant animals as well as neonatal foals, with no adverse effects noted. Patients have been pressurized from 1.5 to 3 ATA, ranging from 60 to 90 minutes at treatment

pressure (depth). We have used HBO as adjunctive therapy for:

- Fungal disease (fungal pneumonia)
- Thermal burns, carbon monoxide, smoke inhalation
- Closed head injuries
- Ileus
- CNS edema/perinatal asphyxia
- Peripheral neuropathies
- Sports injuries (exertional rhabdomyolysis)
- Cellulitis, compartment syndrome
- Ischemic injuries (large colon torsions or small intestine volvulus, laminitis)

In carefully selected patients, the addition of HBO therapy to standard measures may improve clinical outcomes. Further research is needed in the field of equine HBO medicine.

The current cost for HBO is $400 to $500 per treatment.

Example Treatment Protocols for Equine Patients at the Hagyard Equine Medical Institute's Hyperbaric Facility:

Fracture Healing

3 ATA for 60–90 minutes 1× daily
15–20 treatments may be required

Septic Arthritis

2.5 ATA for 60 minutes 1× daily
10–15 consecutive treatments

Osteomyelitis

2.5–3.0 ATA for 60–90 minutes 1–2× daily
15–25 consecutive treatments
- Improve tissue oxygenation, salvage potential medullary necrosis, improve antimicrobial delivery, and enhance antimicrobial effects

Tendon/Suspensory Injury

2.5 ATA for 90 minutes 1× daily
10–20 treatments. Best if started on the acute injury.[30]

Pneumonia

2 ATA for 60 minutes
5–20 treatments 1× daily in conjunction with appropriate antibiotics
- Lower airway pressure because of concern for respiratory depression with hyperoxia in severe cases
- HBOT is used for pneumonias that do not appear to be resolving with appropriate antimicrobial therapy. WE DO NOT USE THIS AS A PRIMARY THERAPEUTIC OPTION.

Lung Abscesses

3 ATA for 60 minutes
10–20 treatments 1× daily
- MUST NOT HAVE RESPIRATORY COMPROMISE (SEE ABOVE STATEMENT ON HYPEROXIA)

Exercise-Induced Pulmonary Hemorrhage

2.0 ATA for 60 minutes
1× daily for 5 days and then increase to 2.5 ATA every other day for 2 weeks[31]
- Effect of HBOT on pulmonary pathophysiology is unknown in this condition. HBOT may work by mobilizing stem cells into circulation, which move to injured lung parenchymal sites. HBOT also enhances angiogenesis and improves connective tissue repair.

Exertional Rhabdomyolysis

2.5 ATA for 60 minutes 1–2× daily (depending on severity)
3–5 treatments
Best if done <48 hours after injury
- Anti-inflammatory effects, reduction of tissue swelling, improved oxygen delivery/circulation to injured muscles, decreased recovery time

Postoperative Gastrointestinal Surgery/I-R Injury

2.0–2.5 ATA for 60 minutes
3–10 consecutive treatments 1–2× daily immediately postoperatively[32,33]
- Reduce edema, accelerates enterocyte turnover, and improves intestinal recovery after I-R injury. Reduce the expression of intercellular adhesion molecule 1 and integrin β2, reduce the production of interleukins, tumor necrosis factor alpha, and Platelet Activating Factor (PAF).

Closed Head Injuries[34]

1.5–2.0 ATA for 60 minutes
- Decreased intracerebral pressure
- Improve the mitochondrial redox function
  - Improve glucose metabolism
- Injured brain could not tolerate exposures >2 ATA

Cellulitis

2.0–3.0 ATA every 24 hours or every 12 hours for 60 minutes
- Continue until resolution. If diagnosed early, the resolution can occur within 3–5 days

Perinatal Asphyxia Syndrome

1.5–2.0 ATA for 60–90 minutes for 4–10 consecutive treatments

Neuroprotective, maintain tissue adenosine triphosphate, protect mitochondrial function, inhibit conversion of xanthine dehydrogenase to xanthine oxidase, inhibit arachidonic acid cascade, and avoid production of reactive oxygen species. [35]

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Expression of T helper cell–associated inflammatory mediator mRNAs in cells of bronchoalveolar lavage fluid samples and oxygen concentration in arterial blood samples from healthy horses exposed to hyperbaric oxygen

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OBJECTIVE
To evaluate the mRNA expression of T helper (Th)1, Th2, and Th17 cell–associated inflammatory mediators in cells of bronchoalveolar lavage fluid samples collected from healthy horses exposed to hyperbaric oxygen (HBO) and to monitor blood oxygen concentration during and following HBO therapy.

ANIMALS
8 healthy horses.

PROCEDURES
In a randomized controlled crossover design study, each horse was exposed (beginning day 1) to 100% oxygen at a maximum of 3 atmospheres absolute (304 kPa) daily for 10 days or ambient air at atmospheric pressure in the HBO chamber for an equivalent amount of time (control). Bronchoalveolar lavage fluid samples were collected on days 0 and 10. After validation of candidate reference genes, relative mRNA expressions of various innate inflammatory, Th1 cell–derived, Th2 cell–derived (including eotaxin-2), Th17 cell–derived, and regulatory cytokines were measured by quantitative PCR assays. For 3 horses, arterial blood samples were collected for blood gas analysis during a separate HBO session.

RESULTS
The optimal combination of reference genes was glyceraldehyde-3-phosphate dehydrogenase, hypoxanthine ribosyltransferase, and ribosomal protein L32. Compared with day 0 findings, expression of eotaxin-2 mRNA was significantly lower (0.12-fold reduction) and the percentage of neutrophils in bronchoalveolar lavage fluid samples was significantly lower on day 10 when horses received HBO therapy. Values of Pao_2 rapidly increased (> 800 mm Hg) but immediately decreased to pretreatment values when HBO sessions ended.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that HBO therapy does not increase mRNA expression of inflammatory cytokines, but reduces eotaxin-2 mRNA transcription. The Pao_2 increase was transient with no cumulative effects of HBO. (*Am J Vet Res* 2016;77:1148–1156)

Hyperbaric oxygen therapy involves breathing 100% oxygen at higher-than-normal atmospheric pressure, which is 1 ATA (101.3 kPa).[1] The pressurized environment creates a greater oxygen molecule density in the air, which increases the partial pressure gradient of oxygen between the alveoli and capillaries. Breathing 100% oxygen at sea-level pressure (1 ATA) results in complete saturation of hemoglobin as well as an increase in the amount of oxygen dissolved in the plasma. For each increase in absolute pressure > 1 ATA, more oxygen becomes dissolved in the plasma.[1–3]

Results of several studies[1,3–5] have indicated that HBO therapy exerts various effects that impact immunity and oxygen and cellular metabolism. Indeed, HBO causes vasoconstriction,[1] reduces neutrophil adherence to endothelial cells,[6] and inhibits proinflammatory cytokine production in mononuclear cells.[7–9]

ABBREVIATIONS

ATA	Atmosphere absolute
BAL	Bronchoalveolar lavage
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HBO	Hyperbaric oxygen
HPRT	Hypoxanthine ribosyltransferase
IL	Interleukin
RPL-32	Ribosomal protein L32
SDHA	Succinate dehydrogenase complex subunit A
Th	T helper
TNF-α	Tumor necrosis factor-α

On the other hand, it is also known that macrophages isolated from the blood, spleen, and lungs of rats exposed to HBO produce more proinflammatory mediators (both spontaneously and following lipopolysaccharide stimulation) than do macrophages from rats exposed to air at atmospheric pressure.[10] In leukocytes, HBO therapy enhances bacterial-killing capacity.[1,11] Furthermore, high concentrations of oxygen increase the production of reactive oxygen species, which can cause lipid peroxidation, protein and DNA oxidation, and enzyme inactivation.[1,12] At pressures > 2 ATA (202.6 kPa), HBO therapy induces oxidative stress on humans and laboratory animals, causing adverse systemic effects; the extent of damage increases with prolonged exposure.[12–14] Toxic effects of oxygen often manifest in the CNS or the pulmonary system.[15–17] Pulmonary toxic effects often develop after prolonged exposure to HBO.[15] High concentrations of oxygen cause pulmonary damage, including thickening of the alveolar wall, development of interstitial and intra-alveolar edema, and extensive infiltration of inflammatory cells into the lungs.[15–17] However, at levels < 2 ATA, these effects are typically not observed unless the individual's intrinsic antioxidant defense mechanisms are either compromised or overwhelmed.[13,18]

Hyperbaric oxygen therapy has been used in a variety of clinical cases where hyperoxic conditions would likely provide positive effects. In human medicine, HBO therapy is beneficial under conditions of ischemia or hypoxia, promotes wound healing, and protects against certain infections.[1,3] However, the use of HBO as a therapeutic intervention in equine medicine has only gained interest in more recent years.[2] Consequently, evidence to support most of the proposed applications in horses is lacking. To date, HBO therapy studies in horses have been limited to assessments of its effects on skin grafts, wound healing, endotoxemia, platelet function, and stem cell proliferation.[19–22]

Although HBO therapy is currently used in equine practice, its physiologic effects are poorly understood and may actually be deleterious for the cells lining the lungs. In addition, the appropriate duration and levels of blood oxygenation are unknown for HBO therapy in horses. Therefore, the purpose of the study reported here was primarily to assess the effects of HBO on the expression (at the mRNA level) of Th1, Th2, and Th17 cell–specific cytokine profiles in pulmonary cells of healthy horses as a means to determine whether the HBO protocol used altered inflammatory responses in the lungs. Concurrently, a second aim was to monitor blood oxygenation in a small number of horses during and after HBO therapy. Our hypotheses were that HBO therapy would increase blood oxygen concentration when horses were within the hyperbaric chamber but only for a short period after cessation of each treatment session and that HBO therapy would induce lung inflammation in healthy horses.

Materials and Methods

The study protocol was approved by the Animal Care Committee of the Health Science Centre at the University of Calgary. The study had a randomized controlled crossover design and was conducted and presented in accordance with the REFLECT statement guidelines.[23] The data were collected at Bar None Ranches, De Winton, AB, Canada (altitude, approx 1,000 m); owner consent was obtained for use of horses on the premises. The number of horses for the mRNA experiments (n = 8) was calculated with a power of 80% on the basis of the effects of HBO therapy on cells in BAL fluid samples from the first 3 horses. Horses had no history of respiratory tract disease and were kept in the same environment with the same management and diet. The study was performed over the minimum number possible of consecutive days, which was 76. For each horse, results of a general physical examination, CBC, serum biochemical analysis, and cytologic examination of a BAL fluid sample[24–26] performed within a 2-week period prior to the study were all within reference ranges. Exclusion criteria were any abnormality revealed by those assessments including evidence of lung inflammation in the BAL fluid sample.

Study procedures

Horses were randomly assigned to receive HBO therapy or no HBO therapy (control) once daily for 10 days (beginning on day 1). When horses did not receive HBO therapy, they were exposed to ambient air at atmospheric pressure in the same hyperbaric chamber for a period equivalent to that needed to complete the HBO therapy. After a washout period of 8 weeks, each horse underwent the other experimental protocol. For each horse, a BAL fluid sample was collected the day before each 10-day experimental period (day 0) and after the last chamber session on day 10. For all 8 horses, arterial blood gas analysis was performed prior to and immediately after exiting the hyperbaric chamber on days 1 and 10, respectively. Arterial blood gas monitoring was performed for 3 horses during HBO therapy on days 1 and 10. Horses were returned to the breeding program after the study.

Assessment of the effect of HBO therapy on blood oxygen concentration

For the blood oxygenation experiments, arterial blood gas analysis was performed on all 8 horses prior to and immediately after exiting the hyperbaric chamber (within 10 minutes after coming out of the HBO chamber) on days 1 and 10, respectively, of the HBO and control treatments. By use of an ultrasound-guided technique, a sample of arterial blood (2 mL) was collected from the right or left (alternate side was used on day 10) common carotid artery with a 23-gauge, 1.5-inch needle. Analysis was immediately performed on-site with a handheld analyzer.[a] In addition, arterial blood gases were monitored in 3 other

horses while they were undergoing HBO therapy within the chamber during a separate session. For these horses, an arterial catheter was placed in the left transverse facial artery without sedation before the HBO therapy session. A threaded plug on one of the HBO vessel ports was replaced by a threaded adapter (sterilized prior to the experiment) machined with arterial line connectors on both the outside and inside of the chamber. The arterial line was flushed with saline (0.9% NaCl) solution before being connected to the arterial catheter. An arterial blood sample (2 mL after priming the line by removal of 25 mL of blood) was withdrawn through the port from outside the HBO chamber prior to pressurization (baseline), when O_2 pressure reached 3 ATA (303.9 kPa; 0 minutes), 10 and 20 minutes later, during decompression when O_2 pressure reached 2 ATA and again at 1 ATA, and 10 minutes after the horse was exposed to ambient air. Blood gas analysis was performed immediately after collection of samples with a handheld analyzer[a] following manufacturer's recommendations. The arterial catheter was removed immediately after collection of the last sample when horses had been exposed to ambient air for 10 minutes. Owing to the technical challenges of this method, this extensive data collection was only performed on 3 horses.

Assessment of the effect of HBO therapy on inflammatory mediators

For the inflammatory mediator experiments, the primary outcomes measured were the findings of cytologic examination of BAL fluid samples and inflammatory gene expressions in the BAL fluid samples. Healthy horses underwent exposure to HBO or no exposure (control treatment) once daily for 10 days and subsequently received the alternate treatment after an 8-week interval. Horses receiving HBO therapy were exposed to 100% oxygen in a horizontally oriented pressurized hyperbaric chamber.[b] Horses were not sedated for each chamber session. Over a period of 60 minutes, a slow ramping pressurization protocol was used to increase the pressure in the chamber to 3 ATA. This pressure was maintained for 20 minutes and then decreased to ambient pressure over a period of 15 minutes. With once-daily treatments, the horses were exposed to HBO conditions (100% oxygen at > 1 ATA) for a total of 90 minutes over a period of 10 consecutive days. Previously published reports describing HBO therapy protocols for horses typically have a total pressurization time of 60 to 90 minutes at a maximum pressure of 1.5 to 2.6 ATA (152 to 263 kPa).[19–21] Thus, a long exposure time at > 1 ATA pressure was chosen for the present study protocol to determine whether a change in inflammatory factors would be elicited under HBO therapy conditions that exceed those of standard protocols. For the control treatment, horses were exposed to ambient air at atmospheric pressure in the same HBO chamber for a period equivalent to that needed to complete the HBO therapy. Each horse received the HBO and control treatment (8-week interval). A BAL fluid sample was collected from each horse before (day 0) and after the HBO or control treatment (day 10) for cytologic examination and quantitative PCR analysis; on day 10, the BAL fluid sample was collected immediately following the completion of the hyperbaric chamber session. For collection of the BAL fluid samples, horses were sedated, as previously described by Wasko et al.[27] Briefly, two 250-mL volumes of sterile endotoxin-free saline (0.9% NaCl) solution were delivered and aspirated via an appropriately positioned video-endoscope.[c] A 250-µL aliquot of fluid underwent cytologic examination, and a differential count was performed on 400 nucleated cells after staining the slides with a modified Wright-Giemsa solution[28]; the percentage of each cell type was calculated. Fifty-milliliter aliquots were centrifuged at 2,200 × *g* for 10 minutes; supernatants were discarded, and the cell pellets were resuspended in 1.5 mL of RNA preservative solution.[d] Samples were stored at −80°C until analyzed.

Messenger RNA analyses

The RNA extraction and cDNA synthesis were performed as previously described.[29,30] Briefly, BAL-isolated cells were thawed on ice and homogenized,[e] and RNA was then extracted.[f] Synthesis of cDNA was done with 500 ng of RNA mixed in solution with reverse transcriptase,[g] RNase inhibitor,[h] and oligo (dT) primers.[i] Yield and purity of RNA and cDNA were assessed with a spectrophotometer.[j] Only RNA samples with ratios of absorbance at a wavelength of 260 nm to absorbance at a wavelength of 280 nm between 1.80 and 2.00 were used for DNA synthesis.

Expressions of mRNA transcripts were determined by quantitative PCR procedures.[k] The reaction solution contained 13 µL of *Taq* DNA polymerase solution,[l] 1.5 µL (40nM) of forward and reverse primers, 2 µL of cDNA, and 7 µL of nuclease-free water. Negative control samples contained sterile water. The cycling conditions were as follows: initial denaturation (95°C for 5 minutes), denaturation (45 cycles at 95°C for 1 minute), annealing (64°C for 30 seconds), and extension (70°C for 30 seconds), followed by a melting curve (60° to 95°C). Reactions were always executed in triplicate. Reaction specificity was also verified by gel electrophoresis of the PCR products.

The primer sequences of the candidate reference genes and 9 of the 10 inflammatory genes used in this study have been described.[29,30] The primer set for equine TNF-α was designed according to Giguère et al.[31] Reference genes were GAPDH, RPL-32, HPRT, and SDHA. Target genes were innate inflammatory (TNF-α and IL-8), Th1 cell–derived (interferon-γ and IL-1β), Th2 cell–derived (IL-4, IL-5, IL-6, and eotaxin-2), Th17 cell–derived (IL-6 and IL-12p35), and regulatory (IL-10) cytokines.

Stability and relative quantification analyses of target genes were assessed with a primer efficiency correction, with a baseline correction, by use of previously validated[32] window-of-linearity method

software.[m] Validation of the reference genes was performed with a software program.[n] The latter software uses a pairwise comparison model that calculates 2 parameters (M and V) for optimal stability and normalization of the data set. The M value ranks the candidate reference genes according to their stability; a low M value represents high expression stability. The V value determines the optimal number of reference genes required for accurate normalization by analyzing pairwise variation between sequential normalization factors containing an increasing number of reference genes. A value of $V < 0.15$ is required for accurate normalization.[33] A relative expression software tool was used to assess the relative amounts of target inflammatory genes in horses under control and HBO-exposure conditions by performing a pairwise comparison; those relative amounts which were then compared between treatments. The relative expression software tool calculates expression ratios (ratios of mRNA expression before and after HBO therapy in this study) with 95% confidence intervals and determines significance with a statistical randomization algorithm.[34] Differences in arterial blood gas variables and percentages of cells in BAL fluid samples before and after HBO therapy were assessed with a Wilcoxon signed rank test. A value of $P < 0.05$ was considered significant for all measurement comparisons.

Results

Of 14 horses examined within a 2-week period prior to the study, only 8 had no notable physical examination findings, and the count and cytologic appearance of cells in a BAL fluid sample were considered normal. The 8 horses were therefore included in the study. The horses were Thoroughbred broodmares (mean age, 14 years; age range, 12 to 16 years).

Effect of HBO therapy on arterial blood gas concentrations

The results of the arterial blood gas analyses for all 8 horses were summarized **(Table 1)**. Among the horses, there was no difference in any blood gas analysis variable prior to and immediately after exiting the hyperbaric chamber on days 1 and 10 of the HBO or control treatment. Values of $Paco_2$ generally decreased after HBO therapy, but the changes were not significant.

The 3 horses monitored within the hyperbaric chamber during a separate HBO session had similar changes in Pao_2 **(Table 2)**. At 3 ATA (0 minutes), 2 of the 3 horses had values of Pao_2 greater than the maximum limit of the analyzer (800 mm Hg); this change was detected in the other horse at the 10-minute time point at 3 ATA. These high values of Pao_2 persisted until decompression was started. Values of Pao_2 were measurable in all 3 horses at 1 ATA, and the decrease in Pao_2 to baseline values was extremely rapid (within 10 minutes) once horses were exposed to ambient air. Therefore, no cumulative effects of oxygenation were observed. The values by the end of the decompression period were not significant. Similarly, no significant effect of HBO therapy on $Paco_2$ or blood Hco_3^- concentration was evident from the data collected on these 3 horses.

Effect of HBO therapy on cells in BAL fluid samples

When the 8 horses underwent HBO therapy, the mean neutrophil cell count in BAL fluid was significantly ($P = 0.042$) lower at day 10 than at day 0 **(Table 3)**. Determination of differential cell counts on the BAL fluid samples collected before and immediately after the last day of treatment (HBO therapy or control) revealed no therapy-induced changes in the percentages of macrophages, lymphocytes, eosinophils, and mast cells.

Table 1—Median (25th to 75th percentile) arterial blood pH, $Paco_2$, and Pao_2 in 8 healthy horses before and after once-daily HBO therapy or control treatment for 10 days (treatment beginning day 1).

Time point	Variable	Before HBO therapy session	After HBO therapy session	Before control session	After control session
Day 1	pH	7.454 (7.421–7.472)	7.458 (7.434–7.487)	7.417 (7.398–7.434)	7.455 (7.406–7.468)
	$Paco_2$ (mm Hg)	43 (38.5–47.3)	38.1 (34.4–40.3)	43.1 (39.7–45.8)	36.2 (35.7–44.3)
	Pao_2 (mm Hg)	79.0 (75–84)	76.0 (70–86)	76.5 (62.7–82)	75.0 (65–85)
Day 10	pH	7.472 (7.435–7.49)	7.472 (7.442–7.484)	7.427 (7.409–7.452)	7.446 (7.423–7.451)
	$Paco_2$ (mm Hg)	40.3 (39.43–42.6)	37.5 (37.2–42)	41.1 (39.4–47)	43.3 (41.1– 45)
	Pao_2 (mm Hg)	76.5 (69.7–91.5)	79.0 (66–87)	79.0 (64.2–84.5)	78.0 (74–95)

Horses were randomly assigned to receive HBO therapy or no HBO therapy (control) once daily for 10 days (treatment beginning day 1) in a crossover study. When horses did not receive HBO therapy, they were exposed to ambient air at atmospheric pressure in the same hyperbaric chamber for a period equivalent to that needed to complete the HBO therapy. After a washout period of 8 weeks, each horse underwent the other experimental protocol. For HBO therapy, horses were exposed to 100% oxygen in a horizontally oriented pressurized hyperbaric chamber. For each chamber session, horses were unsedated. Pressure in the chamber was increased to 3 ATA over a 60-minute period; this pressure was maintained for 20 minutes and then decreased to ambient pressure over a period of 15 minutes. With once-daily treatments, the horses were exposed to HBO conditions (100% oxygen at > 1 ATA) for a total of 90 minutes over a period of 10 consecutive days. Arterial blood gas analysis was performed on all 8 horses prior to and immediately after exiting the hyperbaric chamber (within 10 minutes after coming out of the HBO chamber) on days 1 and 10, of the HBO and control treatments. By use of an ultrasound-guided technique, a sample of arterial blood was collected from the right or left (alternating side) common carotid artery and analyzed on-site with a handheld analyzer. There was no difference in pH, $Paco_2$, and Pao_2 prior to and immediately after exiting the hyperbaric chamber on days 1 and 10 of the HBO or control treatments. Reference values for arterial blood variables in horses (at room temperature, not corrected for altitude [study was performed at an altitude of approx 1,000 m]) are as follows: pH, 7.394 to 7.442; $Paco_2$, 39.5 to 44.4 mm Hg; and Pao_2, 85.8 to 104.9 mm Hg.

Table 2—Individual temperature-corrected values of arterial blood pH, $Paco_2$, and Pao_2 for 3 of the 8 horses in Table 1 obtained during an HBO therapy session.

Variable	Baseline	3 ATA (0 minutes)	3 ATA (10 minutes)	3 ATA (20 minutes)	Decompression (2 ATA)	End of decompression (1 ATA)	Ambient air conditions (10 minutes)
pH	7.46	7.48	7.49	7.50	7.57	7.45	7.45
	7.46	7.48	7.49	7.52	7.52	7.45	7.45
	7.40	7.42	7.43	7.43	7.43	7.44	7.44
$Paco_2$	41.7	45.9	51.6	49	35.6	45.7	40.5
	45.8	52.6	51.8	46.7	44.8	41.8	43.7
	50.9	53.4	41.8	42.5	48.1	38.5	44.7
Pao_2	96	530	> 800	> 800	> 800	381	89
	76	> 800	> 800	> 800	> 800	368	87
	87	> 800	> 800	> 800	614	363	77

For these horses, an arterial catheter was placed in the left transverse facial artery without sedation before the HBO therapy session. A threaded plug on one of the HBO vessel ports was replaced by a threaded adapter (sterilized prior to the experiment) machined with arterial line connectors on both the outside and inside of the hyperbaric chamber. The arterial line was flushed with saline (0.9% NaCl) solution before being connected to the arterial catheter. An arterial blood sample was withdrawn through the port from outside the hyperbaric chamber prior to pressurization (baseline), when O_2 pressure reached 3 ATA (0 minutes), 10 and 20 minutes later, during decompression when O_2 pressure reached 2 ATA and again at 1 ATA (end of decompression), and 10 minutes after each horse was exposed to ambient air. Arterial blood samples were analyzed on-site with a handheld analyzer; the analyzer's maximum limit for Pao_2 < was 800 mm Hg.

See Table 1 for remainder of key.

Table 3—Mean ± SD differential cell counts (%) in BAL fluid samples obtained from the 8 healthy horses in Table 1 before (day 0) and after once-daily HBO or control treatment for 10 days (beginning day 1).

Cell type (%)	Day before first HBO therapy session	After tenth HBO therapy session	Day before first control session	After tenth control session
Macrophage	56.2 ± 9.5	59.0 ± 14.5	63.4 ± 9.9	62.9 ± 7.9
Lymphocyte	35.6 ± 10.8	36.6 ± 14.5	31.3 ± 11.9	33.2 ± 7.5
Neutrophil	5.3 ± 3.1	1.8 ± 0.8*	2.1 ± 1.6	2.1 ± 1.5
Mast cell	2.7 ± 1.5	2.4 ± 2.2	3.1 ± 1.5	1.7 ± 1.2
Eosinophil	0.2 ± 0.3	0.2 ± 0.3	0.1 ± 0.1	0.1 ± 0.2

A BAL fluid sample was collected from each horse before (day 0) and after (day 10) the HBO or control treatment; on day 10, the BAL fluid sample was collected immediately following the completion of the hyperbaric chamber session. For collection of the BAL fluid samples, horses were sedated, and two 250-mL volumes of sterile endotoxin-free saline (0.9% NaCl) solution were delivered and aspirated via an appropriately positioned video-endoscope.[c] A 250-µL aliquot of fluid underwent cytologic examination, and a differential count was performed on 400 nucleated cells after staining the slides with a modified Wright-Giemsa solution; the percentage of each cell type was calculated.

*Value before the first HBO therapy session was significantly (P = 0.042) different from the value after the tenth HBO therapy session. Reference values for BAL fluid cell counts in horses are as follows: macrophages, 44.4% to 74%; lymphocytes, 16.8% to 49.2%; neutrophils, ≤ 5%; mast cells, ≤ 2%; and eosinophils, ≤ 1%.

See Table 1 for remainder of key.

Validation of reference genes

Data analysis with the specialized software model indicated that GAPDH and SDHA were the most and least stable reference genes, respectively. The software program ranked the most to least stable genes as follows: GAPDH (0.307) then HPRT (0.344), RPL-32 (0.361), and SDHA (0.547). The software program indicated that the top gene combination for generating optimal normalization was GAPDH, HPRT, and RPL-32, yielding the lowest V value (0.124; **Figure 1**). The addition of SDHA to this combination increased the V value to > 0.15, indicating that the inclusion of this gene did not allow for accurate normalization.

Effect of HBO therapy on mRNA transcription of inflammatory mediator genes in pulmonary cells

The control treatment (atmospheric air) had no significant effect on the mRNA expression of the tested genes (data not shown). Eotaxin-2 mRNA expression (relative to expression of the reference genes GAPDH, HPRT, and RPL-32) was significantly (P = 0.031) lower (0.12-fold decrease; SE range, 0.76 to 1.02) in BAL fluid cells isolated from the horses following HBO therapy, compared with findings before treatment **(Figure 2)**. There were no significant effects of HBO therapy on mRNA transcrip-

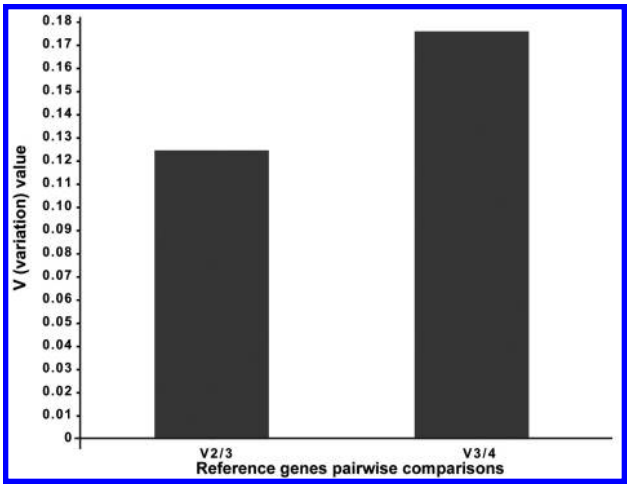

Figure 1—Calculation of normalization factors by a specialized software program from an increasing number of genes evaluated to allow assessment of mRNA expression of Th1, Th2, and Th17 cell–associated inflammatory mediators in cells in BAL fluid samples collected from healthy horses exposed to HBO. The software uses a pairwise comparison model that calculates 2 parameters (M and V) for optimal stability and normalization of the data set. The V value determines the optimal number of reference genes required for accurate normalization by analyzing pairwise variation between sequential normalization factors containing an increasing number of reference genes (2, 3, and 4 in this instance). The data shown represent the pairwise variations between 2 sequential normalizations factors. Values of V < 0.15 are most suitable for data analysis. V3/4 indicates that addition of the fourth (least stable) reference gene to the previous 3 (more stable) reference genes increases the V to > 0.15. Therefore the combination of the 3, but not 4, reference genes was suitable for data analysis.

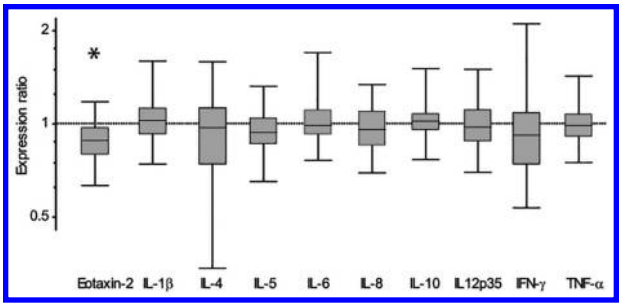

Figure 2—Box-and whisker plot of the ratios of pretreatment and posttreatment mRNA expressions of eotaxin-2, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p35, interferon (IFN)-γ, and TNF-α in lung cells isolated from BAL fluid samples collected from 8 healthy horses before (day 0) and after once-daily HBO therapy for 10 days (treatment beginning day 1). Horses were exposed to 100% oxygen in a horizontally oriented pressurized hyperbaric chamber. For each chamber session, horses were unsedated. Pressure in the chamber was increased to 3 ATA over a 60-minute period. This pressure was maintained for 20 minutes and then decreased to ambient pressure over a period of 15 minutes. With once-daily treatments, the horses were exposed to HBO conditions (100% oxygen at > 1 ATA) for a total of 90 minutes over a period of 10 consecutive days. A BAL fluid sample was collected from each horse for quantitative PCR analysis on days 0 and 10; on day 10, the BAL fluid sample was collected immediately following the completion of the hyperbaric chamber session. Expression of each mRNA was compared with the expression of a combination of the reference genes GAPDH, HPRT, and RPL-32. A value of 1 indicates no change in expression; values < 1 indicate a downregulation in gene expression, and values > 1 indicate an upregulation in gene expression. For each box, the horizontal line represents the median and the upper and lower boundaries represent the third and first quartiles, respectively. Whiskers represent the maximum and minimum values. *Eotaxin-2 mRNA expression (relative to expression of the reference genes GAPDH, HPRT, and RPL-32) was significantly (P = 0.031) lower in BAL fluid cells isolated from the horses following HBO therapy, compared with findings before treatment.

tion of TNF-α, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, or IL-12p35.

Discussion

Numerous studies[14–18,35,36] have revealed that oxidative stress secondary to HBO therapy can trigger localized pulmonary inflammation in various species; however, little is known about the effects of HBO therapy in horses. In addition, the biological effect of HBO therapy, including the duration of blood hyperoxygenation, in horses is unknown. The aim of the present study was therefore primarily to assess the effects of HBO therapy on inflammatory mediator mRNA expression, as a means to determine whether HBO therapy alters inflammatory responses in healthy equine lungs. A second objective was to describe the effect of HBO therapy on arterial blood gases in horses. With quantitative PCR methods, the mRNA expression of a variety of Th1-, Th2-, and Th17-associated cytokines in BAL fluid cells from horses that underwent once-daily HBO therapy for 10 days was evaluated. The study findings indicated that mRNA transcription of a variety of proinflammatory mediators was unaltered by HBO therapy. Interestingly, expression of eotaxin-2 in pulmonary cells isolated from the horses when they were exposed to HBO was significantly reduced following treatment. Differential cell counts of the BAL fluid samples collected indicated a significant decrease in the percentage of neutrophils following HBO therapy; however, there were no significant changes from pretreatment findings for the populations of macrophages, lymphocytes, eosinophils, and mast cells in horses when they received the HBO or control treatment. The mechanism that underlies the actions of HBO therapy on gene expression remains unclear. Collectively, the results of the present study have suggested that the HBO protocol used, which provided a long exposure to 100% oxygen at > 1 ATA, does not activate inflammatory genes in healthy horses. The arterial blood oxygen data obtained indicated that there was no cumulative effect of HBO therapy in horses because the pretreatment values (at day 0) were similar to the posttreatment values (at day 10) when horses were exposed to HBO. In addition, the present study involved an innovative arterial sampling technique that allowed monitoring of arterial blood gases in a small number of horses while they were undergoing HBO therapy in the

hyperbaric chamber as well as immediately after the HBO therapy session. Interestingly, there was not only a rapid (and expected) increase in the oxygenation of the blood beyond the measurement capabilities of the analyzer (upper limit, 800 mm Hg) during HBO therapy, but also a rapid decrease to baseline values during decompression. A nonsignificant increase in blood pH was also observed. All changes rapidly returned to baseline values during decompression; measuring blood gases from samples collected following decompression when horses had been in ambient air for 10 minutes revealed no changes on variables, compared with findings prior to HBO therapy.

Eotaxin is a potent eosinophil chemoattractant and chemotactic for basophils, mast cells, and Th2 lymphocytes.[37] Additionally, eotaxin promotes degranulation in both eosinophils and basophils.[37] Eotaxin-2 is a functional homolog of eotaxin with similar cellular selectivity and actions.[38] Data have indicated that eotaxin and eotaxin-2 promote recruitment of equine eosinophils in vitro[39,40] and that CCR3, the receptor for eotaxin-2, is expressed in equine lung tissue.[41] Both mediators have been implicated in allergic lung diseases in humans and horses.[37,39,42] Furthermore, we have previously shown that eotaxin-2 has a role in the pathogenesis of inflammatory airway diseases in horses.[30]

Other studies[8–10,43–45] have revealed attenuation of cytokine expression with HBO therapy in laboratory animals as well as in humans. The HBO therapy protocols used in those studies varied, with pressures from 1.5 to 8 ATA, durations from 60 to 90 minutes, frequencies from once to twice daily, and treatment periods from 3 to 20 days. In contrast, pulmonary and CNS toxic effects have been reported at as little as 1.5 ATA[43]; severe lung edema as an oxygen-induced toxic effect was observed in rats following 200 to 400 minutes at 4 ATA.[45] The protocol in the present study used slow (1 hour) pressure ramping up to a maximum pressure of 3 ATA, which was then maintained for 20 minutes, once daily for 10 days. Such a protocol did not trigger the expression of proinflammatory cytokines in the BAL fluid cells of the study horses. Plafki et al[36] suggested that oxygen-related toxic effects in the lungs are due to either very intense or prolonged courses of HBO therapy. It has been proposed that pulmonary damage caused by oxidative stress worsens at higher pressures and shorter exposure times, but with less of an associated inflammatory response.[15] In light of a study[14] in which oxidative stress variables appeared to be directly proportional to the extent of HBO exposure, it is probable that the duration of the horses' exposure to pressurized oxygen in the present study was sufficiently short to avoid inducing pulmonary inflammatory responses or damage. This was also supported by the fact that the HBO exposure did not affect the percentages of macrophages, lymphocytes, eosinophils, and mast cells in BAL fluid samples.

An increase in the number of neutrophils in BAL fluid samples from horses is often associated with development of clinical or subclinical pulmonary disease. In the present study, evidence of lung inflammation in the screening BAL fluid sample was an exclusion criterion, and in the study horses, the percentage of neutrophils in BAL fluid samples was within reference range at all times.[24,25,27] However, despite randomization of the crossover design, horses seemed to have a higher percentage of neutrophils in BAL fluid samples prior to HBO therapy, compared with findings prior to control treatment. Nevertheless, because there was a significant decrease in the percentage of neutrophil after horses were exposed to HBO, it is possible that HBO therapy could exert a beneficial effect in horses with pulmonary disease, and further research is indicated.

Quantitative PCR methods were used in the present study to assess changes in gene expression. Interpretation of PCR quantification data can be misled by changes in reference gene expression induced by the treatment[46]; therefore, a strength of the present study was that we first identified the most suitable reference genes in the cells isolated from the BAL fluid samples. We previously reported[29] reference genes and validation methods to assess gene stability. Results of studies[29,47–49] have validated reference genes for equine skin and equine sarcoids, peripheral blood mononuclear cells, and BAL fluid in horses with inflammatory airway disease or recurrent airway obstruction; however, the stability of reference genes following HBO therapy had not yet been reported. In the present study, it was demonstrated that GAPDH was the reference gene of choice. However, to increase the validity and robustness of the data, a combination of reference genes is more appropriate; in the present study, GAPDH, HPRT, and RPL-32 were identified as the most stable combination of reference genes by use of the specialized software program. Also, efficiency correction of the PCR reactions was applied as another important methodological precaution[32] in the present study. Reaction efficiency varies among samples, and small differences in PCR efficiency can significantly impact the interpretations of the data. A software program[m] for the analysis of quantitative PCR data that allows calculation of the efficiency of individual PCR reactions without the assumptions involved with other methods, such as extrapolating data from a standard curve, was used in the present study. In addition, a relative expression software tool was used to perform the quantitative PCR quantification analysis and included a correction for the differences in efficiency among PCR reactions.[50] An advantage of the relative expression software tool is that it determines whether changes in relative gene expression are significant and provides an SE error and a confidence interval range for calculated relative gene expressions.[34]

A quantitative PCR assay is a powerful tool for detecting changes at the level of gene transcription. In the present study, we were able to inves-

tigate the effects of HBO exposure on inflammatory gene expression in lung cells of healthy horses. The facts that the change in eotaxin-2 expression was moderate and that we did not observe changes for the other inflammatory factors evaluated decrease the relevance of verifying whether these changes in mRNA expression directly reflect changes at the protein level. Another limitation of the study was that the expression of inflammatory factors was measured for the entire lung cell population, without sorting inflammatory cell types. This is a common approach in the study of lung cell inflammatory gene expression that allows screening of inflammatory factor production. Lastly, a better understanding of the effects of HBO therapy on lung physiology would require the study of both antioxidant status and signs of oxidative stress, which were outside the scope of the present study. Although these factors have been investigated in humans and laboratory animals[4,14,15,22,51–53] as well as in horses with recurrent airway obstruction,[54,55] the effects of HBO therapy in horses have yet to be fully elucidated.

The results of the present study indicated that the HBO protocol used did not lead to development of lung inflammation in horses. To the contrary, expression of eotaxin-2 mRNA was reduced in the pulmonary cells isolated from horses following HBO exposure, whereas the control procedure had no effect on pulmonary cell cytokine expression. A limitation of the study was that these results were not confirmed at the protein level. However, in the context of allergic pulmonary diseases, HBO therapy may be a viable therapeutic option for affected horses.

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Footnotes

a. Abbott-iStat and CG4+ cartridges, Abbott Laboratories, Abbott Park, Ill.
b. Equineox Technologies Ltd, Maple Ridge, BC, Canada.
c. Optomed, Les Ulis, France.
d. Qiagen, Mississauga, ON, Canada.
e. Onmi International, Kennesaw, Ga.
f. RNeasy Mini kit, Qiagen, Mississauga, ON, Canada.
g. OmniscriptRT, Qiagen, Mississauga, ON, Canada.
h. RNase-OUT, Qiagen, Mississauga, ON, Canada.
i. Invitrogen, Burlington, Ontario, Canada.
j. Nanodrop ND-1000, Thermo Fisher Scientific, Wilmington, Del.
k. MX3005P, Stratagene, La Jolla, Calif.
l. PerfeCta TM SYBR Green Supermix, Quanta BioSciences, Gaithersburg, Md.
m. LinRegPCR, version 110, Heart Failure Research Center, Academic Medical Center, Amsterdam, Netherlands. Available at: LinRegPCR.nl. Accessed Feb 28, 2012.
n. geNorm^{plus} software. Available at: medgen.ugent.be/~jvdesomp/genorm. Accessed Feb 28, 2012

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The Use of Hyperbaric Oxygen Therapy in Small Animal Medicine

By Diane Levitan, VMD, ACVIM

(*continued from front cover*)
size chamber; however, the risks and benefits of treating multiple patients simultaneously must be assessed on a case-by-case basis.

Understanding how HBOT works starts with a review of some of the important laws of physics and their resultant effects on the body. [**FIGURE**].

DIRECT EFFECTS OF PRESSURE

Pressure dissolves gas into solution, shrinks bubbles and decreases diffusion distances. At normal atmospheric pressure (measured at sea level as 1 atmosphere absolute or 1 ATA), arterial oxygen tension is 100 mm Hg and tissue oxygenation is 55 mm Hg. In 100% oxygen at 3 times atmospheric pressure, arterial oxygen tension is 2000 mm Hg and tissue oxygenation is 500 mm Hg. Oxygen dissolved in plasma results in oxygen transport and tissue survival without the need for hemoglobin.[1]

High oxygen gradients result in diffusion of oxygen into areas of low oxygen, which provides immediate help to ischemic and compromised tissue even with marginal or no blood flow. At elevated pressures, oxygen, nitrogen, and other gases will diffuse into solution and then be exhaled from the lungs.

Compression from HBOT reduces all gas volumes, thereby relieving pressure from ileus, bloat, intraluminal gas accumulation, perioperative gastrointestinal obstruction, gas gangrene, emphysematous biliary or urinary bladder tissues and subcutaneous emphysema due to a reduction in gas volume, nitrogen diffusion into tissues and blood and a high gradient for nitrogen removal via respiration. The decrease in pressure from gas in tissues or cavities results in less injury from vascular compromise, and decreased bacterial translocation, necrosis, pain and swelling.[2-8]

EFFECTS OF 100% OXYGEN DELIVERY VIA PLASMA

Infections, injury, and disease increase tissue demands for oxygen while such problems as anemia, toxins and hemorrhage can decrease the body's ability to transport oxygen via hemoglobin. Additionally, conditions resulting in swelling and edema or vessel blockages increase the distance oxygen must travel to the tissues.

Delivery of 100% oxygen under pressure allows plasma to carry much more oxygen and reduces the importance of hemoglobin-based delivery.[1] 100% oxygen dissolved in plasma can be delivered from capillaries to tissues at least three times farther than delivered when carried by hemoglobin alone.[1,9] And, increasing barometric pressure from 1.0 ATA to between 2.0 and 2.5 ATA increases the dissolved oxygen in plasma approximately 3-fold compared with a patient breathing room air. When the inhaled oxygen concentration is increased to 100% under the same increased pressure, the plasma oxygen concentration increases by almost 17-fold. In theory, with 100% oxygen at 2.5 ATA, enough oxygen can be dissolved in plasma to meet the normal requirements of the body at rest without the need for hemoglobin.[1,9]

Oxygen under pressure causes vasoconstriction by inducing smooth muscle contraction in all muscular vessels (arterial and venous), but not capillaries or lymphatics, and decreasing bleeding/oozing from vessels while allowing lymphatic channels to continue to clean up and remove edema. The increased partial pressure of oxygen in plasma and the increased CO_2 in damaged tissues (CO_2 is a more potent vasodilator than oxygen is a vasoconstrictor), offset the vasoconstriction so that tissue oxygenation remains high and microvascular blood flow improves.[3]

DIANE LEVITAN, VMD, ACVIM

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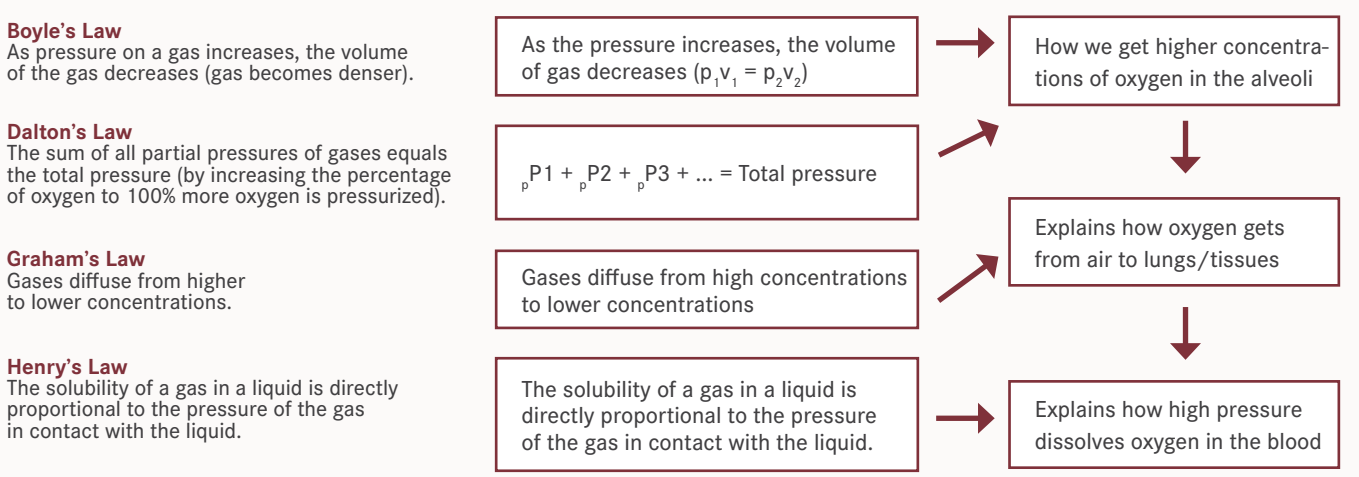
FIGURE 1: PHYSICAL LAWS BEHIND HBOT



How HBOT provides therapeutic benefits via the delivery of 100% oxygen at an elevated atmospheric pressure.

MEDICAL BENEFITS OF HIGH OXYGEN TISSUE LEVELS

High oxygen levels reduce inflammation by mediating cytokines, prostaglandins, and nitric oxide, and have been shown to have direct bacteriostatic and bactericidal effects against gram positive, gram negative, aerobic and anaerobic microorganisms.[3] High tissue oxygen levels have a synergistic antibiotic effect by aiding in the efficacy and action of aminoglycosides, sulfonamides, and cephalosporins, among others. Inhibiting the growth of many fungal organisms; reducing free radical damage and reperfusion injury; and inhibiting toxins.[10,11]

ENDORSEMENT OF HBOT IN HUMAN MEDICINE

Although HBOT fell out of favor in the mid-1990s due to misuse and false claims, in the past 40 years, thousands of evidence-based scientific articles have elucidated its therapeutic value and the underlying cellular and physiologic mechanisms at work.[12,13] The value of hyperbaric oxygen is now well appreciated in human medicine and accepted as treatment for many indications, 15 of which are routinely approved by Medicare and Medicaid [**SIDEBAR 1**].[12] The Undersea and Hyperbaric Medical Society endorses its use for other conditions as well.

HBOT is also used to treat disease states not routinely endorsed by insurance even though varying degrees of efficacy have been demonstrated. These include severe sepsis, cerebral edema, burns, hepatic necrosis, pancreatitis, clostridial infections, head trauma, stroke and many more. There are also more controversial uses, not likely to be recommended by all physicians. These include treatment of nonvascular causes of chronic brain issues such as autism, Alzheimer's disease and cognitive dysfunction associated with age.[12,13]

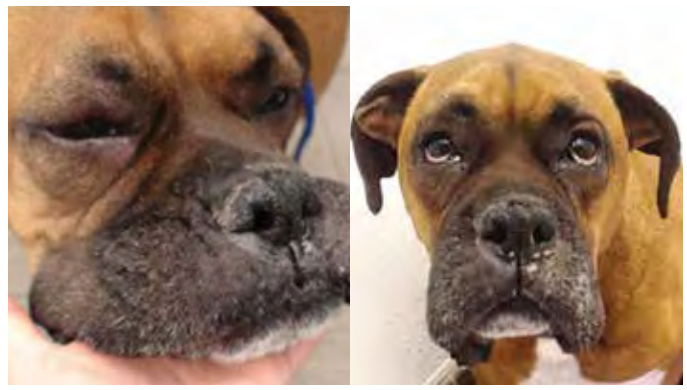
HBOT IN VETERINARY MEDICINE

HBOT has been tested on animals for many years. The earliest documentation of therapeutic use was in 1998. Since then, sporadic treatment reports have appeared in the veterinary literature. Currently, there are around 44 HBOT chambers being used for pets, and over 1000 being used for humans in the United States.

HBOT is especially useful in conditions already approved for human therapy [**SIDEBAR 1**] but, in my experience, there are other indications. Some cases detailing the use of HBOT are included below.

CASE #1:

An 8-year-old male neutered boxer recovering from surgical debridement of necrotic pancreatic tissue developed edema from an allergic reaction to a fresh frozen plasma transfusion. Instead of steroids, he received only HBOT therapy for 1 hour at 2 ATA. The result was reversal of the allergic reaction.



CASE#1: Male neutered boxer with facial edema pre and post 1-hour treatment with HBOT.

CASE #2:

A 3-year-old female mixed breed dog presented with a severe degloving wound to the left rear leg. After cleaning and debriding the wound, supplemental HBOT therapy was instituted at 2.5 ATA for 1 hour twice daily for 3 days, followed by treatment at 2 ATA for 45 minutes twice daily for 2 days before being reduced to every other day for 2 days, along with other supportive care. The end result was rapid migration of healthy granulation tissue and new skin and hair growth.

CASE#2: Mixed breed with degloving injury on Day 1, Day 5, and Day 17 after treatment with HBOT.

SIDEBAR 1: These are endorsed and recommended by Medicare/Medicaid—that list has not been updated since 2006.

- Acute carbon monoxide intoxication
- Decompression illness
- Gas embolism
- Gas gangrene
- Acute traumatic peripheral ischemia
- Crush injuries and suturing of severed limbs
- Progressive necrotizing infections (necrotizing fasciitis)
- Acute peripheral arterial insufficiency
- Preparation and preservation of compromised skin grafts (not for primary management of wounds)
- Chronic refractory osteomyelitis, unresponsive to conventional medical and surgical management
- Osteoradionecrosis as an adjunct to conventional treatment
- Soft tissue radionecrosis as an adjunct to conventional treatment
- Cyanide poisoning
- Actinomycosis, only as an adjunct to conventional therapy when the disease process is refractory to antibiotics and surgical treatment
- Diabetic wounds of the lower extremities

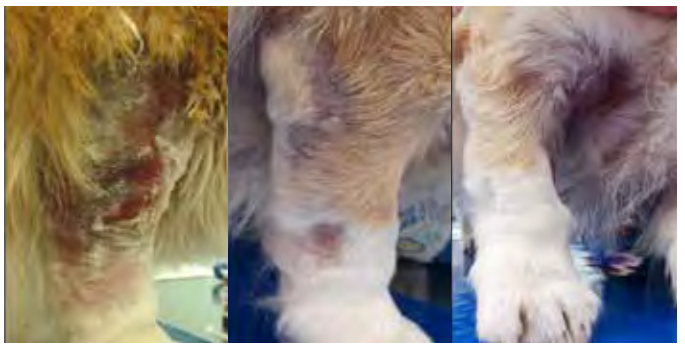
These are approved by the Undersea & Hyperbaric Medical Society but not yet paid for by Medicare/Medicaid:

- Acute retinal artery occlusion
- Idiopathic sudden sensorineural hearing loss

Check with your patient's insurance plan to ascertain coverage for animal medical conditions.

PHOTOS COURTESY OF **HYPERBARIC VETERINARY MEDICINE, DR. JULIO ESCALANCE AT MEDICAR HYPERBARTIC OXYGEN, MEXICO CITY, MEXICO**

CASE #3:
A 7-year-old male intact corgi with lymphoma suffered an undetected extravasation event after chemotherapy with Adriamycin. The patient presented 40 days post treatment with severe swelling, tissue necrosis and compartment syndrome resulting in nerve entrapment with knuckling of the left forepaw. It was determined that standard immediate post-extravasation event treatment with dexrazoxane and sargramostim would likely be ineffectual and so HBOT was instituted at 2 ATA for 1 hour twice daily for 10 days, followed by 36, almost daily, additional treatments at the same dose for a total of 52 days. At the end of treatment, the wounds were healed and the corgi was walking normally. No adverse effects on cancer therapy were noted as he survived for 20 additional months before succumbing to lymphoma.



CASE#3: Male intact corgi with Adriamycin extravasation tissue necrosis and compartment swelling on Day 1, Day 29, and Day 52 of HBOT therapy.

We have also seen improvement in patients affected by intervertebral disk disease, pancreatitis, burns, smoke inhalation, ischemic stroke, spider/snake bites, head trauma, and many other conditions. HBOT has also been used as adjunctive post-operative therapy in orthopedic cases in order to reduce swelling and speed healing. As a general rule, HBOT is most effective for acute conditions, although it has been shown to lessen pain and improve function in osteoarthritis, chronic intervertebral disk disease, long-term management of Aspergillosis and many others.

There are no established treatment regimens currently available, although anecdotal recommendations and guidelines abound. Many recommendations are extrapolated from human medicine. The number of treatments, frequency, and duration varies according to results, the owner's personal schedule, and cost.

SAFETY AND TRAINING FOR HBOT

Overall, HBOT chambers are easy to use but can be dangerous to both the patient and the operator. Patient contraindications include certain lung pathologies, fever, and predisposition to seizures. There is also danger in prolonged exposure to 100% oxygen. However, because 100% oxygen is extremely flammable, operators must pay meticulous attention to its proper use. In February of 2012, a horse-specific monoplace chamber in Florida exploded, killing the patient and the attendant. The cause was probably a spark created when the horse became agitated and kicked a metal plate on the inside of the chamber. Operators need to be aware that although rare, these accidents can happen and so proper training [**SIDEBAR 2**] and thorough adherence to safety precautions are musts.[14]

If you do decide to offer hyperbaric oxygen to patients, it should be made affordable and accessible. You should also invest in specialized training [**SIDEBAR 2**]. It is an adjunctive therapy, and you must be prepared to treat the entire patient and any underlying or comorbid conditions. For more information on how to start, visit the Veterinary Hyperbaric Medical Society website at **www.vhbot.org**, or e-mail them at **info@vhbot.org**. ■

References available at www.AmericanVeterinarian.com.

Dr. Levitan's Mantra for HBOT: The decrease in pressure from gas in tissues or cavities and decreased edema results in less injury from vascular compromise, decreased bacterial translocation, necrosis, pain and swelling. If it only did that, it would be enough, but it also does so much more!

SIDEBAR 2: HBOT Training Opportunities

International ATMO, Inc.
405 N. Saint Mary's
St. Suite 720
San Antonio, TX 78205
Education@hyperbaricmedine.com
Web: hyperbaricmedicine.com

HVM: Hyperbaric Veterinary Medicine
6400 Congress Ave, Suite 1700
Boca Raton, FL 33487
800-928-6886

Steve Reimers
Reimers Systems
300 North Lee Street Suite 201
Alexandria, VA 22314
Phone 703-684-2060
Fax 703-684-5343

Undersea and Hyperbaric Medical Society
631 US Highway 1, Suite 307
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Fax: 919-490-5149
Email: uhms@uhms.org

Effect of hyperbaric oxygen treatment on incorporation of an autogenous cancellous bone graft in a nonunion diaphyseal ulnar defect in cats

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Objective—To determine whether hyperbaric oxygen treatment (HBOT) would affect incorporation of an autogenous cancellous bone graft in diaphyseal ulnar defects in cats.

Animals—12 mature cats.

Procedure—Bilateral nonunion diaphyseal ulnar defects were created in each cat. An autogenous cancellous bone graft was implanted in 1 ulnar defect in each cat, with the contralateral ulnar defect serving as a nongrafted specimen. Six cats were treated by use of hyperbaric oxygen at 2 atmospheres absolute for 90 minutes once daily for 14 days, and 6 cats were not treated (control group). Bone labeling was performed, using fluorochrome markers. Cats were euthanatized 5 weeks after implanting, and barium sulfate was infused to evaluate vascularization of grafts. Ulnas were evaluated by use of radiography, microangiography, histologic examination, and histomorphometric examination.

Results—Radiographic scores did not differ between treatment groups. Microangiographic appearance of grafted defects was similar between groups, with all having adequate vascularization. Differences were not observed between treated and nontreated groups in the overall histologic appearance of decalcified samples of tissue in grafted defects. Mean distance between fluorescent labels was significantly greater in cats given HBOT than in nontreated cats. Median percentage of bone formation in grafted defects was significantly greater in cats given HBOT.

Conclusions—Hyperbaric oxygen treatment increased the distance between fluorescent labels and percentage of bone formation when incorporating autogenous cancellous bone grafts in induced nonunion diaphyseal ulnar defects in cats, but HBOT did not affect revascularization, radiographic appearance, or qualitative histologic appearance of the grafts. (*Am J Vet Res* 2000;61:691–698)

Autogenous cancellous bone grafts are commonly used in animals to stimulate bony union after arthrodeses and in animals with multifragmented, delayed, or nonunion fractures.[1-9] Cancellous bone grafts possess 3 properties that augment osseous union: some donor cells survive transplantation and produce bone directly (osteogenesis), the graft acts as a scaffold for ingrowth of new bone from the recipient (osteoconduction), and the graft releases factors that cause transformation of undifferentiated recipient cells into bone-forming cells (osteoinduction).[10] The sequence of graft incorporation is influenced by vascularity of the recipient bed, stability of the repair, and whether there is infection.[2,11] Unfortunately, despite appropriate conventional surgical and medical treatment, including use of autogenous cancellous bone grafts, not all fractures and arthrodeses achieve union. Additional therapeutic modalities that enhance osseous union would be of benefit.[9]

Hyperbaric oxygen treatment (HBOT) consists of providing an environment of 100% oxygen in a chamber in which the pressure is maintained at > 1 **atmosphere absolute (ATA**; greater than pressure at sea level).[12,13] Therapeutic mechanisms and benefits of HBOT have been described[14,15] and include hyperoxygenation of hypoxic tissue,[13] direct stimulation of fibroblasts that causes an increase in collagen synthesis,[16] and enhancement of tissue revascularization.[17,18]

The effect of HBOT on incorporation of autogenous cancellous bone grafts has not been investigated. However, HBOT is considered to be an adjunct to the established treatment modalities of surgical debridement and systemically administered antibiotics in humans with chronic refractory osteomyelitis.[19-21] Hyperbaric oxygen treatment purportedly stimulates osteoclast function, fibroblast proliferation and collagen production, and macrophage production of angiogenesis factor by providing a steep gradient from hyperoxia to hypoxia.[22] A beneficial effect of HBOT on fracture healing in

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laboratory animals (rats, rabbits) has been reported.[23-26] In addition, investigators in another study[27] reported a beneficial effect of HBOT on incorporation of autogenous free corticocancellous grafts in rabbits.

Nonunion fracture models have been developed for the ulna of dogs and tibia of cats.[28-30] On the basis of the positive effects ascribed to HBOT for fracture healing and in the treatment of osteomyelitis, we hypothesized that HBOT would accelerate the incorporation of autogenous cancellous bone grafts in cats, using a nonunion model. The objectives of the study reported here were to duplicate a nonunion diaphyseal ulnar defect in cats that has been documented in dogs, determine whether HBOT would induce healing of a nongrafted experimentally induced nonunion diaphyseal ulnar defect, and determine whether use of HBOT in accordance with a specific protocol would accelerate incorporation of autogenous cancellous bone grafts in an experimentally induced nonunion diaphyseal ulnar defect in cats.

Materials and Methods

Animals—Twelve mature conditioned cats were randomly assigned to 2 groups: 6 cats received HBOT (treated group), and 6 cats did not (nontreated [control] group). Each cat was considered clinically normal on the basis of results of complete physical and orthopedic examinations as well as measurement of PCV, plasma total protein concentration, and concentration of BUN. The protocol used in this experiment was approved by the Louisiana State University Laboratory Animal Care and Use Committee. All cats were housed and cared for in an accredited facility (American Association for the Accreditation of Laboratory Animal Care) that conforms to guidelines established by the National Institutes of Health and the USDA.

Creation of nonunion defect—The procedure for creating a nonunion diaphyseal ulnar defect in the cats was adapted from a procedure described for dogs.[30] On day 1, food was withheld from each cat for 12 hours. Cats were given preanesthetic medication (ketamine hydrochloride; 8 mg/kg of body weight, IM). Anesthesia was induced by IV administration of a combination of diazepam (2 mg/kg) and ketamine (4 mg/kg), followed by mask administration of halothane. Anesthesia was maintained with halothane and oxygen administered via an endotracheal tube. Cats were given lactated Ringer's solution (10 ml/kg/h, IV) throughout the surgical procedure.

The forelimbs of each cat were shaved and prepared for surgery. A caudal surgical approach was used to expose each ulna.[31] Before surgery, the length of each ulna was determined from measurements obtained from radiographs. During surgery, a sterile ruler was used to identify the point at which the proximal and middle third of each ulna met. A full-thickness 1-cm segment of each ulna, including the periosteum, was removed from this point, using a hand-held oscillating saw.[a] A sterile polytetrafluoroethylene spacer,[b] 1 cm in length and 0.75 cm in diameter with a previously drilled central hole, was inserted into each defect. An intramedullary pin (1.57 or 1.93 mm in diameter, dependent on size of the cat and surgeon preference) was placed to retain the spacer and maintain alignment of the ulna. Muscle fascia and subcutaneous tissues were closed, using 3-0 polydioxanone in a simple continuous pattern, and the skin was closed, using 3-0 nylon in a Ford interlocking pattern. Butorphanol tartrate was administered (0.2 mg/kg, IM) immediately after surgery as an analgesic. Placement of spacers was evaluated on lateral radiographs of the antebrachium in each cat (**Fig 1**).

Cats were examined twice daily throughout the remainder of the study. Wounds were inspected for evidence of swelling, discharge, or dehiscence. Rectal temperature of each cat was recorded daily.

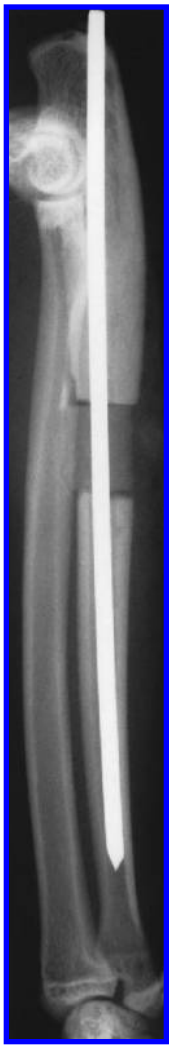

Figure 1—Lateral radiographic view of the forelimb of a cat after the initial surgery. Notice the position of the spacer and intramedullary pin.

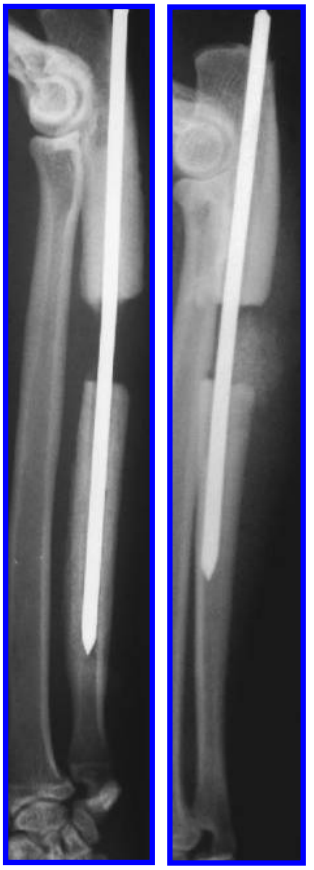

Figure 2—Lateral radiographic views of the forelimb of a cat after removal of the spacer, revealing the open (nongrafted) nonunion (left) and evidence of a cancellous bone graft in the nonunion of the contralateral ulnar diaphysis (right).

Grafting procedure—On day 20, lateral radiographs of the antebrachium of each forelimb were obtained. On day 21, cats were prepared for surgery and anesthetized as described. Cefazolin (22 mg/kg, IV) was administered immediately after induction. Both forelimbs were clipped and aseptically prepared, including the proximal portion of each humerus. The ulnas were exposed by use of the caudal surgical approach described, and intramedullary pins were removed, which allowed removal of polytetrafluoroethylene spacers. New intramedullary pins of equal or greater diameter were placed after removal of the spacers. When the ulna initially had been stabilized with a 1.57-mm-diameter intramedullary pin, a 1.93-mm-diameter intramedullary pin was placed. When a 1.93-mm diameter intramedullary pin had been used initially, a pin of identical diameter was placed. Margins of the ulnar ostectomy were not curetted. An aliquot (0.5 ml) of cancellous bone was harvested from the proximal portion of each humerus.[8] The volume of graft was quantitated by use of a modified 3-ml syringe.

Assignment of the limb in which the graft was placed was determined by use of a complete-block randomized design. An autogenous cancellous bone graft was packed around the intramedullary pin in the defect in the assigned ulna. The defect in the contralateral ulna did not receive a

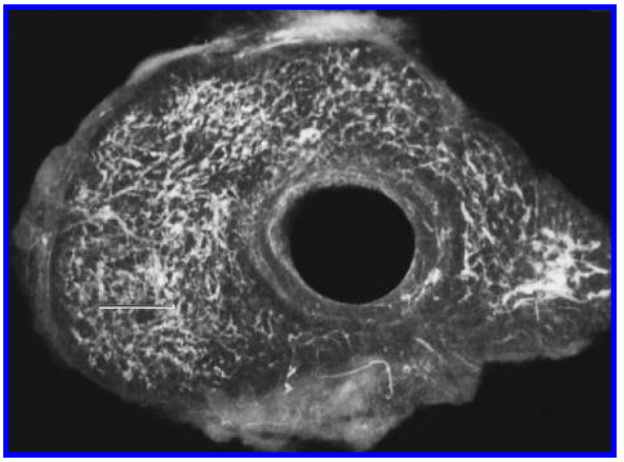

Figure 3—Microangiograph of a section of tissue obtained from the ulna of a nontreated cat 5 weeks after grafting. Notice the adequately vascularized cancellous bone. The section was obtained from the area adjacent to the distal graft-host interface. Not stained. Bar = 150 µm.

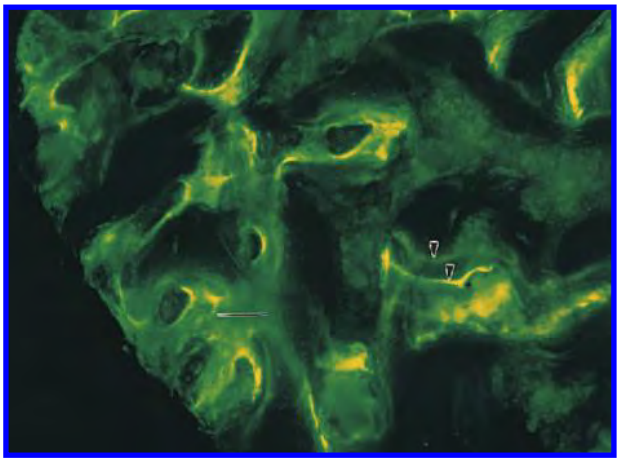

Figure 4—Photomicrograph (obtained by use of a fluorescent microscope) of a section of ulna obtained from a cat after administration of calcein green on day 35 and oxytetracycline on day 42. Two fluorochrome labels, evident as bright parallel bands (arrows), indicate uptake by mineralizing osteoid. Not stained. Bar = 20 µm.

graft. Wounds were closed as described. Butorphanol was administered after surgery as an analgesic, as described. Lateral radiographs of the antebrachium of each forelimb were obtained immediately after surgery (**Fig 2**). Cats were allowed to fully bear weight immediately after the surgeries on days 1 and 21.

Hyperbaric oxygen treatment—The HBOT were performed in an animal hyperbaric chamber (1 m^3).[c] Three cats, each in a separate cage, were treated concurrently. Treated cats were exposed to 90 minutes of 100% oxygen at 2 ATA (202.6 Pa) daily beginning on day 22 (the day after the second surgery) and continuing until day 36 (duration, 14 days). A continuous flow of oxygen (10 L/min) was maintained throughout the procedure. Sedation was not required. Nontreated cats were placed in the chamber daily for 90 minutes and exposed to room air at atmospheric pressure on days 22 to 36.

Administration of fluorochrome label—Oxytetracycline[d] (30 mg/kg, IV) and calcein green[e] (30 mg/kg, IV) were given to all cats to label newly mineralized bone. Oxytetracycline was diluted to a half-strength concentration (50 mg/ml) with sterile water and injected slowly during a 2- to 3-minute period via the cephalic vein on days 28 and 42. Calcein green was prepared as a 1% solution, using sterile buffered deionized water, and diluted to a 0.066% solution in saline (0.9% NaCl) solution; it was injected slowly during a 20-minute period via the cephalic vein on day 35.[32-34]

Radiography—Lateral radiographs of the antebrachium of each forelimb were obtained on days 0 and 20, as described. On day 56 (35 days after grafting), lateral radiographs of the antebrachium of each forelimb were made to evaluate incorporation of the graft and healing of the nongrafted defect. These radiographs were evaluated, using a scoring system reported elsewhere.[35] Briefly, a score of 7 points was possible, using a combination of scores for 3 categories (appearance of graft: 0 = resorbed, 1 = mostly resorbed, 2 = largely intact, 3 = reorganizing; quality of proximal union: 0 = nonunion, 1 = possible union, 2 = radiographic union; quality of distal union: 0 = nonunion, 1 = possible union, 2 = radiographic union). Scoring was performed by a board-certified veterinary radiologist (RDP) who was unaware of the treatment group to which each cat had been assigned.

Vascular infusion—On day 56, cats were sedated with ketamine (8 mg/kg, IM), and a catheter was placed in a peripheral vein. Heparin (300 U/kg, IV) was administered and allowed to disperse in the circulation for 5 minutes prior to induction of anesthesia. Anesthesia was induced, using sodium thiamylal (10 mg/kg, IV), and median sternotomy was used to enter the thoracic cavity. The aortic root was isolated, using a circumferential double loop of 2-0 silk, and cats were euthanatized with an overdose of thiamylal. The aorta was cannulated, using the end of an intravenous infusion set, and radiographic contrast material and formalin were infused, as described elsewhere.[36] The cranial vena cava was transected to allow exsanguination and monitoring of perfusion of the angiography solution. Vasculature was infused via the aorta with a 30% barium sulfate suspension[f] in saline solution at 120 to 140 mm Hg pressure until the venous effluent was pink as a result of the high concentration of barium sulfate. Footpads of the forelimbs were incised, and detection of pink effusion confirmed that the limbs were adequately perfused with the suspension. A second infusion of a solution of 30% barium sulfate diluted with 10% formalin[g] was performed in the same manner. After infusions were completed, forelimbs were removed from each cadaver. Muscles were removed from the ulna on each limb; care was taken to avoid disturbing the fracture callus. Ulnas were labeled for identification and placed in neutral-buffered formalin for 14 days. After removal from formalin, ulnas were deep-frozen (–80 C) to preserve fluorescent bone labels.

Sample preparation for microangiography and histologic examination—The healed callus of those ulnas that had achieved bony union, regardless of whether they had received a cancellous bone graft, was sectioned perpendicular to the long axis of the bone. Sections were cut, using a low-speed diamond-blade saw,[h] and sections were numbered in sequential order from distal to proximal. Alternating thick (approx 1 mm) and thin (approx 200 µm) sections were cut. Thick slices were slowly decalcified, using citrate-buffered formic acid.[37] Microangiographs were made of all decalcified sections, as described elsewhere (**Fig 3**).[36] Thin, undecalcified sections were stored in a solution of 80% ethyl alcohol:20% neutral-buffered formalin for evaluation of fluorescent labels.

Decalcified sections (those originally used for the microangiographs) from each processed ulna were embedded in paraffin, sectioned at a thickness of 6 µm, and stained, using H&E or picrosirius red to enable correlated descriptive

histologic evaluation.[38] Specimens were evaluated qualitatively, with particular attention to appearance and activity of osteoblasts, osteoclasts, evidence of endochondral ossification, and evidence of inflammation or infection. A board-certified veterinary pathologist (JLO) performed the evaluations and was unaware of the treatment group to which each cat had been assigned.

Histomorphometric analysis—Undecalcified sections were manually ground, using 600-grit sandpaper, to a thickness of approximately 70 to 100 µm under running water[39] and mounted on glass slides by use of buffered-glycerol mounting medium.[40] A coverslip was applied, and slides were examined, using a fluorescent microscope[i] linked to a computerized image-enhancement and morphometrics program.[j] The optical-electronic system was calibrated directly by use of a stage micrometer.[k] Distance between adjacent fluorescent bone labels was measured at 20× magnification. Two hundred measurements were made per ulna.[33,41,42] We did not attempt to correct for obliqueness of the sections.[34,42] All measurements were made by the same investigator (SCK), who was unaware of the treatment group to which each cat had been assigned.

The percentage of bone in each section was calculated by examining the same slides used to measure distance between fluorescent labels. Only limbs in which a graft was placed were examined. Histomorphometric analysis was conducted by use of a bone morphometry program.[l] This was accomplished by initially calculating the total area of tissue in the measured section, which was determined on the basis of the measured peripheral circumference of the section. The space occupied by the pin was subtracted from total area to provide actual tissue area. Gaps in the section created during processing or incomplete filling of the nonunion also were subtracted from the total area. Finally, the area of bone in the section was outlined, and the percentage of bone relative to the total area of tissue in the section was calculated ([bone area/total area] – [pin area + gaps]). The remainder of the tissue was cartilage or fibrous tissue. Median percentage of bone was calculated for each group, and comparisons were made.

Statistical analysis—All statistical analyses were performed by use of nonparametric methods. For histomorphometric data, comparisons between groups were made by use of the Wilcoxon-Mann-Whitney test for independent samples. Comparisons within groups were made by use of the Wilcoxon signed-rank test for dependent samples. For radiographic data, frequency of scores was compared between treatments (HBOT vs no HBOT) for grafted and nongrafted limbs, using the Fisher exact test against a 2-sided hypothesis. Frequency of scores was compared within treatment group (between grafted and nongrafted limbs), using the Mantel-Haenszel comparison of repeated categoric data to account for paired data; a 2-sided hypothesis was tested. Mode (most frequent) score was used as a summary measurement for all scores. Significance for all tests was defined at $P \leq 0.05$. Statistical software[m] was used for all analyses.

Results

Clinical evaluation—All cats were ambulatory and able to bear weight on the day after each of the surgeries. None of the cats developed clinical signs consistent with infection (swelling, heat, discharge) at the surgical sites throughout the study. One cat broke both 1.57-mm intramedullary pins several days after the first surgery, and a second cat bent both 1.57-mm intramedullary pins several days after the first surgery. These incidents were accompanied by sudden onset of lameness in the affected limbs. These cats were anesthetized, and surgery was performed as before, except the 1.57-mm intramedullary pins were replaced with 1.93-mm intramedullary pins. Problems were not encountered in either cat after that additional surgery. Examination of lateral radiographs of the forelimbs obtained on day 20 revealed mild caudoproximal displacement (1 to 3 mm) of the proximal ulnar segment of the nonunion in 5 of the other cats in which 1.57-mm intramedullary pins were used. However, these cats used the limbs well, and surgical intervention was not required. After this experience early in the study, 1.93-mm intramedullary pins were used subsequently in all cats.

Adverse effects were not observed in association with HBOT. Three cats vomited immediately after injection of oxytetracycline, and 1 other cat vomited during infusion of the calcein green. Cats had an orange hue, particularly noticeable in the sclera, mucous membranes, and nonpigmented skin, for about 2 hours after infusion of the calcein green. Other adverse effects were not detected, and all cats maintained an excellent appetite throughout the study.

Radiographic evaluation—All pins were within the ulnar medullary canal after all surgeries. All spacers were correctly placed and completely filled each diaphyseal gap. Grafted and open defects were easily distinguishable after the second surgery. Total radiographic scores were not significantly different between treatment groups for grafted ($P = 1.000$) or nongrafted ($P = 0.455$) limbs. Radiographic scores differed significantly within treatment groups when comparing between grafted and nongrafted defects (treated group, $P = 0.014$; nontreated group, $P = 0.002$, respectively). There was 1 cat in the treated group in which the bone graft was almost completely resorbed. That cat received a radiographic score of 1 for the grafted defect and 0 for the nongrafted defect. In the nontreated group, the nongrafted nonunion was almost completely healed in 1 cat; that cat received a radiographic score of 6 for the grafted defect and 5 for the nongrafted defect. The mode of the radiographic scores was 3 for grafted defects in the treated group, 0 for nongrafted defects in the treated group, 2 for grafted defects in the nontreated group, and 0 for nongrafted defects in the nontreated group. Radiographic scores did not differ significantly between treatment groups for comparison of grafted ($P = 0.27$) and nongrafted ($P = 0.9$) defects.

Microangiography—We did not detect a qualitative difference in the appearance of the microangiographs between treatment groups. Defects that did not have sufficient bony callus to be sectioned (11/12 nongrafted defects and 1/12 grafted defects) were not examined. All sections were vascularized throughout the entire cross section of the graft. In a large proportion of the samples in both groups, there was a relatively avascular area adjacent to the pin track.

Histologic examination—Sections of callus from 5 ulnas of the treated group and 7 ulnas of the nontreated group were evaluated. Nonunions that did not have grossly visible reformed bone had a thin (1 to 3 mm)

layer of fibrous tissue surrounding the intramedullary pin and were not examined. Appreciable differences in the global histologic appearance of the decalcified samples between ulnas from cats in treated and nontreated groups were not apparent. All samples had an active osteoblast population and deposition of new bone. Osteoclastic activity was evident, but nominal, in most sections. Bone marrow elements were beginning to form in many sections, and a few sections had a large amount of cartilage undergoing endochondral ossification. A small capsule of fibrous tissue surrounded most of the bones. Additionally, there was usually a small amount of fibrous tissue surrounding the original pin track. Inflammatory change was not observed in any section, nor was there evidence of graft rejection or active bone resorption.

Histomorphometric analysis—The calcein green administered on day 35 and oxytetracycline administered on day 42 were incorporated into bone as fluorescent labels (**Fig 4**). Distance (mean ± SD) between fluorescent labels in treated cats was 35.0 ± 10.80 µm, which was significantly ($P < 0.001$) greater than the distance between fluorescent labels in nontreated cats (29.5 ± 9.17 µm).

Median percentage of bone was compared between treatment groups for the grafted defects. Median percentage of bone was 58.23% for the treated group and 47.06% for the nontreated group. A score of zero was recorded for 1 cat in the treated group that had completely resorbed the graft. Median value for percentage of bone did not differ significantly ($P = 0.07$) between treatment groups when data from all cats were included; however, when the data for the cat that completely resorbed the graft in the treated group was excluded as an outlier, median percentage of bone for the remaining cats in the treatment group was significantly ($P = 0.01$) higher than values for the nontreated group.

Discussion

The nonunion model used in the study reported here was developed on the basis of Key's hypothesis, which states that a segmental long-bone defect 1.5 times the diaphyseal diameter exceeds the regenerative capacity of bone and results in nonunion; this has been documented to be true for a tibial nonunion model in cats.[28,29,43] We were able to duplicate a convenient and reliable diaphyseal ulnar nonunion model in cats similar to that described in dogs.[30] We chose to adapt the bilateral ulnar nonunion model for dogs to cats in this study, because the surgical procedure is less complex than the tibial model, and it carries less morbidity (animals can bear weight immediately after a bilateral procedure, because the radius remains intact).[30,35,44] The spacer used in this study and a study in dogs[30] is a modification of the original ulnar diaphyseal defect model that is commonly used.[45,46] The spacer aids in development of the nonunion by physically blocking ingrowth of capillaries and soft tissues, and it may provide a more uniform diaphyseal defect into which cancellous bone can be placed. However, it may not be necessary for the success of the nonunion model used in this study.

Caudoproximal displacement of the proximal ulnar segment and breakage of intramedullary pins was observed in some cats. We stabilized ulnar ostectomies with 1.57-mm intramedullary pins on the basis of results reported in dogs,[47] but we found the stability afforded by these small-diameter pins to be inadequate. Reasons for this could include the increase in length of the ostectomy, compared with that created in dogs for correction of humeroulnar subluxation, or greater forces placed on the pins by the activities of the cats. It has been suggested that the ulna is relatively larger in cats than dogs and, thus, bears proportionately more weight.[48] Subluxation was not a problem when a 1.93-mm intramedullary pin was used to stabilize the ulnar ostectomy.

It is possible that the problems encountered with the pin breakage in this model may have affected our results, particularly with such a limited number of animals. Alternatively, the proximal ulnar segment could have been transfixed to the proximal portion of the radius by use of a cortical bone screw.[49] Finally, the nonunion could have been created more distally on the ulna, thus negating the pull of the triceps muscle; however, it was believed that the distal aspect of the diaphysis was too small to provide adequate tissue for processing. Further work evaluating and validating this nonunion model, with the above modifications, is indicated.

Union of the nongrafted defect was detected in 1 cat in the nontreated group. Union of nongrafted nonunion defects was not reported in dogs in some studies[29,30] nor in cats with a nonunion of the tibia[29]; however, Heiple et al[5] reported 1 out of 8 unions in the nongrafted limb by use of this model. Inadequate excision of the periosteum at the time of ulnar ostectomy may have been a factor resulting in union of the nongrafted defect, or it may have simply been the result of variation among cats.[50] A union failed to develop in 1 grafted defect in a cat in the treated group, with complete resorption of the graft by 5 weeks. A union also failed to develop in the nongrafted defect in that same cat. It has been reported[5] that even a fresh autogenous cancellous graft placed into a bone defect will not routinely result in successful union, and fibrous tissue and a resorptive response predominate in a small minority of animals.

The use of HBOT at 2 ATA for 90 minutes once daily appears to be safe in mature healthy cats. Adverse effects of HBOT may include neurologic abnormalities (most noticeably seizures) and barotrauma resulting in signs of pain and dysfunction from air trapped in the middle ear, sinuses, teeth, or lungs.[12] We did not observe any adverse effects in the cats treated in this study.

Radiographic evaluation was useful in this study for assessing placement and stability of implants and observing the fate of autogenous cancellous bone grafts. As expected, radiographic scores within groups differed significantly when comparing grafted defects to nongrafted defects. On the basis of these findings, we state that HBOT did not induce union of the nongrafted nonunion defects.

Examination of microangiographs of the grafts did not reveal differences between treatment groups. Autogenous cancellous bone grafts revascularize quickly,[30] and the grafts in both groups were adequate-

ly vascularized. Microangiography is a subjective evaluation of bone vascularization, difficult to quantify, and probably most helpful when there are fairly dramatic differences between treated and control groups. It functions primarily as another method of tissue description and is representative of the vascular pattern at the time of infusion.[36] It may prove more useful in future studies involving the use of allogenous cancellous grafts in which vascularization is not nearly as rapid.[30] A more quantitative technique, such as regional blood flow measurement by use of radioactive microspheres, may have documented a difference between groups.[51]

Fluorochrome markers were administered to allow quantitation of dynamic variables of bone formation.[33] Oxytetracycline and calcein green form complexes with calcium ions and, therefore, are incorporated into newly mineralizing osteoid as it is laid down by active osteoblasts.[32] Fluorochrome labels are incorporated into actively mineralizing bone and will not be incorporated when they are given before osteoid is being mineralized. Two labels were detected when we examined the undecalcified sections with a fluorescent microscope. Because we administered our first dose of oxytetracycline 7 days after the second (grafting) surgery, it is likely that the label was not incorporated. Osteoid generally is not being mineralized in appreciable amounts in cancellous bone autografts until at least 10 to 14 days after grafting.[5]

Distance between fluorescent labels was greater in the grafted defects in cats given HBOT. This reflects a greater amount of osteoid production in the autogenous cancellous bone grafts in the treated group between days 35 and 42 and indicates accelerated graft incorporation during this time. We did not attempt to calculate mineral apposition rate, because we did not measure osteoid volume, percentage of fractional-labeled osteoid, or circumference of fluorescent labels. Those variables are much more difficult to measure in cancellous bone, compared to cortical bone with discrete Haversian systems. Cancellous bone grafts are incorporated by new bone being laid down on necrotic trabeculae.[2,4] The increased distance between fluorescent labels observed in the treated cats could be a function of increased activity of osteoblasts, decreased activity of osteoclasts, or a combination of both.

Osteoblasts are responsible for producing osteoid. Energy required for calcification of osteoid is produced by aerobic conditions; however, calcium release from osteoblast mitochondria requires anaerobic conditions.[52] This implies that aerobic and anaerobic conditions are necessary for bone formation. A study[53] of spontaneously hypertensive rats treated with hyperbaric oxygen (1 hour; 2.8 ATA; 5 d/wk for 30 treatments) revealed a positive effect on osteogenesis, compared with nontreated control rats. Investigators in that study postulated that the production of energy for calcification is enhanced during exposure to HBOT, but the interval of hypoxia (time elapsed between treatments) is essential to allow critical anaerobic bone-forming activities to take place.

Previous in vivo studies[23-25] in which investigators examined the response of bone healing in rats and rabbits subjected to HBOT documented an increase in all variables studied (breaking strength of femur, calcium content of bone, bone ingrowth into a titanium chamber, healing of standardized drill hole) at lower doses of HBOT (range, 2 hours at 2.8 ATA, q 24 h for 14 days to 1 hour at 3 ATA, q 12 h for 30 days). The HBOT protocol chosen for our study was developed on the basis of results obtained by Barth et al.[26] Standardized drill holes in femurs of rats healed more rapidly when exposed to a relatively low dose of hyperbaric oxygen (2 ATA, q 24 h for 90 minutes, 5 d/wk for 20 treatments), compared with healing in nontreated control rats. The positive effects of HBOT on bone healing appear to be dose-dependent. In that same study,[26] it was documented that rats exposed to a higher dose of hyperbaric oxygen (2 ATA for 90 minutes, q 12 h, 5 d/wk for 20 treatments) had pronounced osteoclastic activity and delayed healing of the drill holes, compared with values for daily-treatment and control groups. This is similar to findings reported by Wray and Rogers,[54] who used higher doses of hyperbaric oxygen (5 hours at 2 ATA, q 24 h for 14 days), which resulted in a decrease in breaking strength of bones in a femoral fracture model in rats.

We suggest that an increase in the oxygen supply to the surviving osteoblasts in the graft in the treated group caused an increase in collagen production, which contributed to the overall increased distance between fluorescent labels on the basis that a portion of osteoid is composed of collagen.[55] Additionally, it is possible that there was an increased survival of transplanted osteoblasts in the treated group as a result of higher availability of oxygen.

Little osteoclastic activity was observed in the grafts of treated or nontreated cats in the study reported here. Most activity was overwhelmingly osteoblastic, associated with pronounced production of new bone. This is in contrast with reports about hyperbaric pressures and increased partial pressure of oxygen. It has been reported[56] that prolonged localized pressure (15 mm Hg or 0.020 atmosphere applied to the right bulla for 7 days) alone (in room air) can cause increased systemic osteoclast activity in gerbils. In another study,[57] it was reported that oxygen-derived free radicals stimulate the formation and activation of osteoclasts. Osteoclasts are derived from macrophages, which perform many of their functions via formation of superoxide radicals[57,58] and can have an increase in activity when exposed to hyperbaric oxygen.[59]

Oxygen-derived free radicals are products of normal cellular oxidation-reduction processes. Reactive oxygen species are generated when cancellous bone grafts are procured.[60,61] Under conditions of hyperoxia, oxygen-derived free-radical production increases profoundly and is believed to contribute to the development of oxygen toxicosis under prolonged hyperbaric oxygenation conditions.[62] Stimulation of osteoclast development and activity by oxygen-derived free radicals, plus the increase in pressure involved in HBOT, could explain the massive osteoclastic activity observed at high oxygen concentrations reported elsewhere.[63] It remains unexplained why osteoclast activity

was not prominent in our study or those of others[23-25] and why it appears to be dose-dependent.[26]

Differences could not be detected between treatment groups in the global histologic appearance of the decalcified sections of bony callus. As mentioned, there was active osteoblastic activity and deposition of new bone. Evidence of inflammation, graft rejection, or active bone resorption was not detected. When bone was evaluated more quantitatively, there was a trend, although not significant, toward an increased median value of percentage of bone in all sections of each grafted defect in the treated group, compared with the same value for the grafted defect in the control group. This difference became significant when data for the cat that completely resorbed the graft in the treated group was excluded.

Further investigation will be required to obtain detailed histomorphometric data on the effect of HBOT on the incorporation of cancellous bone grafts, including autogenous and allogenous grafts. Investigation at the cellular level is necessary to determine the exact effects of hyperbaric oxygen on the function of osteoblasts, whether transplanted or in situ, as well as its effects on other cells involved in the production and removal of bone.

[a]Sagittal saw with battery, Dyonics, Andauer, Mass.
[b]Teflon fluorocarbon policeman, Fisher Scientific Co, Pittsburgh, Pa.
[c]United States Air Force Type 2 animal hyperbaric chamber.
[d]Oxyvet 100, Pfizer Inc, Brooklyn, NY.
[e]Calcein, Sigma Chemical Co, St Louis, Mo.
[f]Novopaque, Picker International Inc, Highland Heights, Ohio.
[g]Micropaque, Barkley Medical Products, Anaheim, Calif.
[h]Isomet, Beuhler Ltd, Lake Bluff, Ill.
[i]Axioplan, Zeiss, Oberkochen, Germany.
[j]Image1, Universal Imaging Corp, Westchester, Pa.
[k]Stage micrometer, Leitz, Wetzlar, Germany.
[l]Bioquant, R&M Biometric Inc, Nashville, Tenn.
[m]SAS, version 6.12, SAS Institute Inc, Cary, NC.

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