Clinical application of a hemoglobin-based oxygen-carrying solution

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The number of red blood cell (RBC) transfusions administered to small animals has increased dramatically during the past 15 years. Establishment of national commercial animal blood banks as well as large volunteer blood donor programs at veterinary teaching hospitals has increased the availability of blood products; yet, blood remains a limited resource and may not be immediately accessible. In addition, despite extensive screening of blood donors for infectious diseases, transmission of infections via blood transfusions remains a risk [1]. Also, there are situations where, despite a large supply of blood products, compatible RBCs may not be available (eg, a previously transfused dog may have been sensitized to a common RBC antigen, making it nearly impossible to find compatible blood for subsequent transfusions) [2]. Limited availability of blood and concerns regarding transmission of infectious disease have heightened interest in possible alternatives to RBC transfusions in human and veterinary medicine. The use of a hemoglobin-based oxygen carrier (HBOC) could be life saving when compatible RBCs are not immediately available for transfusion. The quest for a safe and efficacious HBOC for use in human beings has been ongoing for more than 60 years. Currently evaluated RBC substitutes can be grouped into HBOCs and fluorocarbon emulsions, but only HBOCs are considered here.

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Development of hemoglobin-based oxygen-carrier solutions

Early attempts in the development of HBOCs met with failure because of nephrotoxicity and short intravascular half-life [3]. The nephrotoxicity had been attributed to contamination with RBC stroma rather than with the hemoglobin (Hb) itself; thus, all HBOCs manufactured today undergo ultrapurification to remove RBC stromal elements [4]. The short half-life of the early HBOCs was caused by dissociation of the $\alpha_2\beta_2$ tetramer to $\alpha\beta$ dimers that can be filtered through the renal glomeruli. Therefore, the Hb is now chemically modified (eg, polymerized with glutaraldehyde [Oxyglobin and Hemopure; Biopure Corporation, Cambridge, MA] or *o*-raffinose [Hemolink; Hemosol, Inc., Mississauga, Ontario, Canada] or conjugated with polyethylene glycol (PEG) [Hemospan, Sangart, Inc.; San Diego, CA]) to provide a longer half-life [3]. The sources of Hb for these oxygen-carrying solutions include outdated human RBC units (eg, Hemolink and Hemospan), bovine RBCs (Oxyglobin and Hemopure), and recombinant human Hb (rHb2.0, Baxter Health Care Corporation, Boulder, CO) [3]. Although no HBOC has yet been approved for use in human beings in the United States, Hemopure has recently been approved in South Africa for use as an alternate to RBC transfusions in general surgical patients [3]. Oxyglobin is the only HBOC approved for use in dogs; therefore, the remainder of this article focuses on Oxyglobin.

Oxyglobin solution

Characteristics

Oxyglobin solution (Hb glutamer-200 [bovine]) is a sterile solution of purified, polymerized bovine Hb (13 g/dL) in a modified lactated Ringer's buffer, having an osmolality of 300 mOsm/kg and a pH of 7.8. It has a lower viscosity (1.3 versus 3.5 cp) than whole blood, potentially permitting better blood flow. Oxyglobin consists of a distribution of Hb polymers with less than 5% of the Hb as unstabilized tetramers, approximately 50% with molecular weights between 65 and 130 kd and less than 10% having molecular weights greater than 500 kd; the average molecular weight is 200 kd. By comparison, native Hb is tetrameric with an approximate molecular weight of 65 kd. The colloid osmotic pressure (COP) of Oxyglobin is 43 mm Hg, which is considerably higher than that of canine packed red blood cells (PRBCs; 5 mm Hg) and fresh-frozen plasma (17 mm Hg) and slightly greater than 6% hetastarch (HES, Abbott Laboratories, North Chicago, IL) (33 mm Hg), suggesting that Oxyglobin could be helpful in maintaining vascular volume [5]. Oxyglobin has a partial pressure of oxygen at which Hb is 50% saturated (P_{50}) of 37 mm Hg (in comparison to 27 mm Hg for canine RBCs), enhancing the offloading of oxygen. Oxyglobin's oxygen affinity is regulated by physiologic chloride concentration rather than by 2,3-diphosphoglycerate

(DPG) as for canine RBCs. Because chloride is an abundant ion in plasma, Oxyglobin is able to offload oxygen immediately on infusion.

Pharmacokinetics

The terminal elimination half-life of Oxyglobin is dose dependent (Table 1). Administration of Oxyglobin to healthy dogs as a top-loading dose of 8.5 mL/kg (low), 21 mL/kg (middle), and 42.5 mL/kg (high) at a rate of 7 mL/kg/h yielded a mean elimination half-life of 22, 30, and 38 hours, respectively [6]. Volume of distribution and plasma clearance were also dose dependent, and the elimination half-life and clearance of individual plasma Hb components of Oxyglobin (dimer, tetramer, octamer, > octamer) were inversely proportional to molecular size, with the larger molecular components being cleared from the plasma more slowly [7]. At the high end of the recommended dose, 30 mL/kg, more than 95% of Oxyglobin is eliminated within 5 to 9 days. Oxyglobin is metabolized by the reticuloendothelial system, except for the less than 5% that exists as an unstable tetramer and is excreted by the kidneys.

Oxygen delivery

Traditionally, RBC Hb carries 98% of the circulating oxygen, and plasma carries only 2%. Administration of Oxyglobin increases the plasma Hb concentration ([Hb]). At low hematocrits (eg, <10%), most of the oxygen content of the blood may be shifted to the plasma. Oxyglobin is expected to increase oxygen delivery to tissues as a result of an increased oxygen content in the blood. Because the P_{50} of Oxyglobin was modified to be higher than that of Hb in canine RBCs, oxygen uptake and offloading occur more readily by Oxyglobin than by RBCs, facilitating delivery of oxygen to tissues. One study compared the effects of RBCs and HBOC-201 (Hemopure), a related HBOC that has a similar P_{50} as Oxyglobin, on muscular tissue oxygenation after acute and almost complete isovolemic hemodilution in dogs. HBOC-201

Table 1
Expected plasma hemoglobin concentration and duration of effect at multiple dose levels after a single infusion of Oxyglobin in dogs

Dose (mL/kg)	Immediate post-infusion plasma hemoglobin concentration (g/dL)	Duration (hours) of plasma hemoglobin concentration > 1 g/dL	Terminal half-life (hours)
10	1.5–2.0	11–23	18–26
15	2.0–2.5	23–39	19–30
21	3.4–4.3	66–70	25–34
30	3.6–4.8	74–82	22–43

Data from Oxyglobin package insert.

demonstrated a higher oxygen extraction ratio, resulting in a relative tissue oxygenation potential that was three- to fourfold higher than that of stored autologous RBCs [8]. In other words, 1 g Oxyglobin Hb delivers the equivalent amount of oxygen as 3 to 4 g RBC Hb. Similar findings were noted in a study involving healthy human men, in which 1 g HBOC-201 was similar to 3 g RBC Hb in preserving submaximal exercise capacity [9]. In addition, the low viscosity of Oxyglobin enhances perfusion of tissues and allows oxygen delivery to tissues despite disruption of blood flow. In a study investigating the effect of HBOC-201 on skeletal muscle oxygenation during 95% artificial arterial stenosis in dogs, tissue oxygen tension increased to nearly baseline values after treatment with HBOC-201 [10].

Oxygen delivery, however, is dependent on cardiac output and oxygen content in the blood. Decreased cardiac output leading to decreased systemic oxygen delivery despite evidence of adequate tissue oxygenation has been noted experimentally after Oxyglobin administration in dogs [11–13]. In canine models of acute hypovolemia, a decrease in cardiac output after resuscitation with Oxyglobin was attributed to increased systemic vascular resistance [11–13]. Despite a low cardiac output, however, Oxyglobin reconstituted oxidative metabolism, as evidenced by resolution of metabolic acidosis and lactic acidemia [11–13]. The investigators postulated that Oxyglobin may have improved oxygen transport in the microvasculature as a result of hemodilution and its high efficiency in the uptake and release of oxygen [11]. Further support for improving tissue oxygenation by Oxyglobin is provided by a study in which brain tissue oxygen was monitored directly in swine undergoing acute hemorrhage; small-volume resuscitation with HBOC-201 (6 mL/kg) rapidly restored hemodynamic parameters without detrimental vasoactive effects and improved brain tissue oxygen tension [14].

Resuscitation from hemorrhagic shock

Because of its colloidal properties and ability to carry oxygen, investigators have been interested in understanding the use of Oxyglobin in the treatment of shock. One of the most impressive studies performed using an HBOC was a complete ($\geq$95%) exchange transfusion of eight conscious splenectomized sheep with an early formulation of Oxyglobin to reach a final hematocrit of less than 4% (final plasma [Hb] was 6.1 g/dL) with stable hemodynamics and oxygen transport parameters and no clinical evidence of distress [15]. All sheep undergoing exchange transfusion with the HBOC survived long term, and a marked reticulocytosis was noted as early as 3 days after exchange transfusion, leading to an increase in hematocrit to approximately 20% by day 10.

In an experimental model of feline hemorrhagic shock, autologous blood, 6% hetastarch, and Oxyglobin were compared for their ability to restore cardiovascular function and oxygen transport; each cat received 20 mL/kg of

one of the resuscitation fluids infused over 60 minutes and was monitored for 5 hours after resuscitation [16]. Oxyglobin and autologous blood were equally effective in restoring oxygen transport variables and seemed to improve peripheral perfusion as evidenced by increasing arterial pH and decreasing blood lactate levels, whereas oxygen transport variables remained decreased in cats receiving HES. Oxyglobin produced rapid increases in mean arterial pressure and central venous pressure (CVP) within minutes of beginning infusion (peaks of approximately 120 mm Hg and 6 mm Hg, respectively, 1 hour after resuscitation); the pressures remained high for 2 to 3 hours and gradually declined, although remaining greater than prehemorrhage levels. All cats were euthanatized 5 hours after resuscitation, and necropsy revealed pulmonary edema in three of three cats receiving autologous blood and two of three cats receiving Oxyglobin. Dose or rate of infusion was postulated as a potential cause of the pulmonary edema, yet none of the three cats receiving HES developed pulmonary edema [16].

Animal models have been developed to assess the potential role of HBOCs in hemorrhagic shock with tissue injury. One such model of hemorrhagic shock after blunt abdominal trauma with liver injury was established by Manning et al [17]. This model may be relevant to a small animal that has been hit by a car. Uncontrolled exsanguinating liver injury was induced in swine using multiple liver lacerations, and at 9 minutes after the onset of bleeding, animals received 10 mL/kg/min of either lactated Ringer's solution or HBOC-201 to achieve and maintain a mean aortic pressure of 60 mm Hg for up to 2 hours [17]. All swine were initially successfully resuscitated, but only 1 of 10 treated with lactated Ringer's solution survived to 2 hours, whereas 7 of 7 administered HBOC-201 were alive at 2 hours. Hematocrit was less than 1% in 9 of 10 animals in the lactated Ringer's group and in 6 of 7 swine in the HBOC-201 group. In a similar study of hemorrhagic shock in swine comparing the effects of no fluid, HES, and HBOC-201, all animals receiving HBOC-201 (n = 8) survived 60 minutes after the initial liver injury and then underwent liver repair, with 7 of 8 surviving 96 hours with good functional recovery [18]. None of the swine in the no fluid or HES groups survived until 60 minutes. Both studies indicate that use of HBOC-201 in exsanguinating shock stabilizes hemodynamic and metabolic parameters and improves early survival in comparison to use of non-oxygen-carrying fluids (crystalloids and colloids) and when compatible blood is not immediately available for transfusion.

Vasoreactivity

Vasoconstriction resulting in an increase in systemic and pulmonary arterial pressures has been noted in various studies evaluating HBOCs. Proposed mechanisms include nitric oxide scavenging, endothelin release, and sensitization of peripheral α-adrenergic receptors [19]. Nitric oxide is

produced by endothelial cells and causes vasodilation; therefore, scavenging of nitric oxide by Hb shifts vascular tone toward vasoconstriction. Support for the role of nitric oxide scavenging leading to hypertension includes the finding that nitric oxide donors, such as nitroglycerin and L-arginine, decrease Hb-induced hypertension [19]. A study comparing responses of mean arterial pressure with a 50% isovolemic exchange transfusion in rats with six different cell-free Hb solutions yielded three blood pressure responses: an immediate and sustained increase, an immediate yet transient increase, and no significant change [20]. Differences in blood pressure responses did not correlate with nitric oxide reaction rates and inversely correlated with nitric oxide affinities. These results suggest that blood pressure increases observed with administration of cell-free Hb solutions are not the result of nitric oxide scavenging reactions by Hb [20].

Although there are different theories regarding potential HBOC-induced vasoconstriction, there are also different study results, in part dependent on the particular HBOC evaluated and the animal model used. In the rodent model mentioned previously, the cross-linked tetramer Hb solutions had a sustained vasopressor effect, whereas the PEG-conjugated Hb solution had no effect on blood pressure [20]. Also, it has been suggested that the vasopressor property of cell-free Hb is species dependent (rodent > pig > human being) [21]. Even among studies in human beings evaluating the same product, HBOC-201, there are conflicting results. In a study evaluating hemodynamics after HBOC-201 administration in patients undergoing preoperative hemodilution for elective abdominal aortic surgery, systemic and pulmonary vascular resistance indices increased by 71% to 121% and 53% to 70%, respectively, after infusion of HBOC-201 at two doses (approximately 7 mL/kg and 9 mL/kg); there was a resulting net decrease in cardiac index and oxygen delivery index (approximately 30%) [22]. In a dose-escalation study (total dose approximately 8 mL/kg) evaluating the hemodynamics of HBOC-201 in adult patients undergoing elective surgery, however, there was no increase in mean arterial blood pressure [23]. Similarly, there was a negligible increase in blood pressure ($\leq$20 mm Hg) after infusion of HBOC-201 in healthy volunteers [9].

Similar conflicting results regarding vasoconstrictive effects of Oxyglobin are also found in experimental canine models, potentially as a result of differences in study design. In a study evaluating the acute effects of large-dose (30 mL/kg) infusion of an early HBOC formulation in a canine model of hemorrhagic shock, there was no significant increase in mean arterial pressure or mean pulmonary arterial pressure from baseline [24]. In contrast, in the canine hypovolemia studies by Driessen et al [12], systemic vascular resistance index increased significantly after Oxyglobin administration in comparison to baseline as well as to the control group (autologous blood); however, there was no increase in pulmonary vascular resistance index [12]. The clinical significance of potential Oxyglobin-induced vasoconstriction in the dog is uncertain, because despite a resultant decrease

in cardiac output, Oxyglobin restored systemic and regional tissue oxidative metabolism after low-volume resuscitation [11].

Clinical efficacy and safety of Oxyglobin in dogs and cats

Oxyglobin gained U.S. Food and Drug Administration (FDA) approval for use in dogs in 1997 after its efficacy and safety were established in a multicenter study. Sixty-four dogs with moderate to severe anemia (packed cell volume [PCV]: 6%–23%) as a result of blood loss (n = 25), hemolysis (n = 30), or ineffective erythropoiesis (n = 9) were studied [25,26]. Dogs with immune-mediated hemolytic anemia (IMHA) accounted for 47% of the study population. Thirty dogs were randomized to the treatment group and received Oxyglobin (30 mL/kg at an infusion rate of 10 mL/kg/h) immediately, and 34 dogs were randomized to the untreated control group, with an option to receive Oxyglobin if the dog's condition worsened. The dogs were monitored for a change in [Hb] and physical condition score (PCS). The PCS was determined based on an assessment of the dog's attitude, activity, and change in heart rate and ranged from 1 to 5, with 1 corresponding to a depressed, minimally responsive, and recumbent patient and 5 corresponding to a bright and responsive patient able to walk without tiring. Dogs demonstrating a decrease in [Hb] (>0.3 g/dL) or deterioration of physical condition received additional oxygen-carrying support in the form of PRBCs in the treatment group or Oxyglobin in the control group. Treatment success was defined as the lack of need for additional oxygen-carrying support for 24 hours. The success rate was 95% for Oxyglobin-treated dogs and 32% for untreated control dogs; there was a significant difference between treated and control dogs independent of the cause of anemia. Mean total [Hb] in the treated group increased from a baseline measurement of 4.3 g/dL to 6.8 g/dL immediately after infusion of Oxyglobin. Likewise, the mean PCS improved immediately after infusion of Oxyglobin (2.5 at baseline, 3.7 after infusion) and remained improved for at least 24 hours.

Although Oxyglobin is approved by the FDA for use only in the dog at this time, it has been administered to several other species, including the cat. In a retrospective study evaluating the use of Oxyglobin in 72 cats at the Veterinary Hospital of the University of Pennsylvania, Oxyglobin was administered to 70 cats as a supportive measure in the treatment of anemia caused by hemorrhage (n = 29), ineffective erythropoiesis (n = 24), and hemolysis (n = 11) (unknown cause in 6 cats) [27]. The most common reason for choosing Oxyglobin rather than feline PRBCs or whole blood was the lack of readily available compatible feline blood. Although most cats received only one Oxyglobin infusion, 6 cats received two Oxyglobin infusions (>24 hours apart) and 1 cat received three infusions. Mean dose administered per infusion event and mean rate of infusion were 14.6 mL/kg and 4.8 mL/kg/h,

respectively. Positive responses noted after administration of Oxyglobin included improvement in hemoglobin concentration [Hb], body temperature, heart and respiratory rates, blood pressure, appetite, and activity.

Hemoglobin-based oxygen-carrier solution–related effects

A predictable adverse event noted during the canine clinical trial [25,26] as well as in the feline retrospective study [27] was a transient discoloration (yellow to red) of mucous membranes, sclera, urine, and, occasionally, skin. Potentially, the most worrisome of the adverse events was the development of circulatory overload. Because of the plasma expanding properties of Oxyglobin, increased CVP (5–13 cm H_2O) was observed in many dogs and was sometimes associated with pulmonary edema. This effect was rate dependent, being most marked at rates greater than 10 mL/kg/h in the dog and greater than 5 mL/kg/h in the cat. CVP was not measured in the cats receiving Oxyglobin, but the most common adverse events noted in the feline study were pleural effusion, pulmonary edema, or both (25 cats). Preexisting cardiopulmonary disease was observed in most of the cats that developed circulatory overload, however. Conservative rates of administration (<5 mL/kg/h in the dog and <2–3 mL/kg/h in the cat) are advised if preexisting cardiopulmonary disease is present or the animal has other predisposing factors that could lead to overexpansion of the circulating volume. Based on pharmacokinetics, continuous rate infusion (CRI) has also been suggested [7], using the following equation: CRI = Desired Plasma Concentration of Oxyglobin × Clearance.

It should be pointed out that allergic reactions to Oxyglobin, a bovine protein, were not observed in the canine clinical trial or in the retrospective feline study. Another important note is that nephrotoxicity was not observed in any dogs or cats (with necropsy examinations available for 23 cats) receiving Oxyglobin in these studies. In addition, a study evaluating potential toxic side effects of Oxyglobin on the kidney in dogs undergoing blood exchange with Oxyglobin did not identify any adverse effects on the ultrastructural integrity of the kidney [28].

Veterinary case reports

The first report on the clinical use of an earlier formulation of Oxyglobin describes treatment of endoparasite-induced anemia in dogs [29]. Five of seven dogs administered the HBOC (30 mL/kg at a rate of 7 mL/kg/h) demonstrated a sustained improvement in PCS and had an increase in plasma [Hb] greater than or equal to 1 g/dL at 2 hours after infusion [29]. The clinical effect of the HBOC was similar to that observed in dogs with endoparasite-induced anemia treated with whole blood [29]. Another clinical case report describes use of this same HBOC in a miniature horse

with cyclic ovarian hemorrhage and a PCV of 11%; the mare (A-negative) had been previously transfused (Aa-positive donor), and, subsequently a compatible blood donor could not be found [30]. Therefore, the mare received the HBOC at a dose of 30 mL/kg at a rate of 8 mL/kg/h before surgery without complication; the total [Hb] increased to 5.1 g/dL, and additional blood products were not necessary during the ovariectomy and postoperative recovery. Oxyglobin has since been safely administered to a miniature horse and pony with presumed red maple leaf toxicosis and to a foal with neonatal isoerythrolysis [31,32].

One case report describes the use of Oxyglobin in a cat with acetaminophen toxicosis. A young exotic shorthaired cat with methemo-globinemia, cyanosis, and dyspnea was administered Oxyglobin (11.6 mL/kg at a rate of 6.6 mL/kg/h) in an attempt to increase the oxygen-carrying capacity of the blood (45% PCV), because initial treatment with *N*-acetylcysteine and oxygen did not result in rapid clinical improvement; the cat responded and recovered without complication [33].

Administering and monitoring Oxyglobin infusions in clinical practice

Indications

Oxyglobin is indicated as a temporary oxygen-carrying supportive measure in the treatment of anemia caused by hemorrhage, hemolysis, or ineffective erythropoiesis. If the cause of the blood loss or hemolysis can be promptly controlled or treated, repeated infusions of Oxyglobin or sub-sequent RBC transfusions may not be necessary. Because of its relatively short half-life in comparison to RBCs, Oxyglobin is not the ideal choice for management of patients with ineffective erythropoiesis, but it may be life saving in such patients until compatible RBCs become available.

Although any anemic patient may benefit from Oxyglobin, a clinical situation in which the use of Oxyglobin may be favored by some (but not all) clinicians over PRBCs is IMHA. A widely held belief is that administration of RBCs to patients with IMHA will "fuel the fire" or lead to accelerated destruction of RBCs and worsen that patient's overall condition. Transfused RBCs are not more likely attacked by autoantibodies than the patient's own cells, however. During initial hospitalization, the reported mortality rate associated with IMHA in dogs is variable but averages 30% to 40% [34,35]. In a retrospective study of RBC transfusions in dogs, IMHA accounted for 67% (29/43) of the dogs with hemolysis receiving RBC transfusions; 76% of the dogs with IMHA receiving RBC transfusions survived hospitalization [36]. In the study by Klag et al [34], 57% (24/42) of the dogs with IMHA received at least one RBC transfusion and no hemolytic transfusion reactions were observed. Also, the incidence of mortality was not significantly different between dogs that received blood and those that did not, a remarkable finding given that dogs receiving RBC transfusions were more likely severely affected

than those not receiving blood products. RBC transfusions are thus not contraindicated in patients with IMHA. Persistent autoagglutination precludes the ability to blood type the patient and perform a crossmatch; therefore, it is safest to assume that the canine patient is dog erythrocyte antigen (DEA) 1.1-negative and to administer DEA 1.1-negative RBCs. Because of the many canine blood types, however, the potential for RBC incompatibility reactions increases with repeated blood transfusions 1 week after the first transfusion. Situations in which Oxyglobin may be preferable to PRBCs in patients with IMHA include rapid destruction of transfused RBCs resulting in a negligible increase in PCV within a few hours of transfusion and persistent autoagglutination in previously transfused patients to avoid the risk of an RBC incompatibility reaction. A recent retrospective study evaluating the influence of oxygen-carrying support (ie, RBCs and Oxyglobin versus Oxyglobin alone) on survival in dogs with IMHA did not detect a difference among treatment groups, and the overall survival rate was 62% [37]. No data are currently available to comment on the efficacy of Oxyglobin in comparison to RBCs in the treatment of dogs with IMHA.

Because of its colloidal properties, Oxyglobin may be particularly useful in patients with anemia and hypovolemia, such as in trauma-induced hemorrhagic shock. In comparing Oxyglobin with other non-oxygen-carrying fluids commonly used for resuscitation, only dextran-70 with saline has a higher COP (Table 2). Oxyglobin is the least viscous of the synthetic colloids and is close to a physiologic pH. There are many other clinical situations (eg, heatstroke, gastric-dilatation-volvulus) in which hypotension and tissue hypoxia are apparent, and one could speculate that Oxyglobin's colloidal properties and oxygen-carrying abilities would be beneficial in such cases [38,39]. Although Oxyglobin's colloidal effects may be advantageous in many clinical situations, caution must be exercised in the administration of Oxyglobin to normovolemic anemic patients with advanced heart disease or oliguric/anuric renal failure that are predisposed to circulatory overload, particularly cats [27].

Table 2
Characteristics of fluids commonly used in resuscitation

	Viscosity (cp)	COP (mm Hg)	Electrolytes	Osmolality (mOsm/L)	pH
LRS	0.85	NA	Na, K, Cl	275	6.7
0.9% NaCl	NA	NA	Na, Cl	308	5.0
Hypertonic saline (7.5%)	NA	NA	Na, Cl	1712	5.0
6% hetastarch	4.3	33	Na, Cl	310	5.5
Dextran-70 with saline	3.7	66	Na, Cl	310	3–7
Whole blood	3.5	25–30	Na, K, Cl	300	Variable
Oxyglobin	1.3	43	Na, K, Cl	300	7.8

Abbreviations: COP, colloid osmotic pressure; K, potassium; LRS, lactated Ringer's solution; NA, not applicable; NaCl, sodium chloride.

Dosage and administration

The recommended dosage of Oxyglobin (for use in dogs) is a one-time dose of 10 to 30 mL/kg administered intravenously at a rate of less than or equal to 10 mL/kg/h, with the increase in plasma [Hb] dependent on the dose (see Table 1). Because Oxyglobin is not approved for use in the cat, the manufacturer does not provide recommendations for this species, but our clinical experience suggests that a dose of 10 to 15 mL/kg at a rate of less than 5 mL/kg/h is tolerated by most normovolemic cats. The ideal total dose and rate of administration vary from patient to patient depending on the degree of anemia and circulatory volume (eg, a dog presenting with hemorrhagic shock after trauma requires and tolerates a greater total dose and rate of administration than a dog with flea-induced anemia). A laboratory study evaluating restoration of muscular tissue oxygenation after profound isovolemic hemo-dilution in dogs established that an increase in total [Hb] by as little as 0.7 g/dL with HBOC-201 restored normal tissue oxygenation [8]. Therefore, many normovolemic anemic dogs may benefit from a lower dose (10 mL/kg) of Oxyglobin, given that the plasma [Hb] is expected to be greater than 1 g/dL for almost 1 day at this dose (see Table 1). Although Oxyglobin is labeled for single-dose use because of concerns regarding the antigenicity of bovine Hb in other species, antibovine Hb antibodies that developed in dogs experimentally administered repeated doses of Oxyglobin over several months did not result in anaphylactic or other hypersensitivity reactions or interfere with Oxy-globin's oxygen-carrying abilities [40].

Oxyglobin is supplied in 60-mL and 125-mL single-dose bags in a protective foil overwrap. The product is stored at room temperature and is stable for up to 3 years; however, once the bag is opened, any unused portion should be discarded after 24 hours to avoid bacterial contamination and oxidation to methemoglobin. Oxyglobin is usually administered at room temperature but may be prewarmed to 37°C by placing the bag in its foil overwrap in a temperature-controlled water bath. Oxyglobin should be administered using aseptic technique via a standard intravenous infusion set; filtration via a blood administration set is not necessary. Use of an infusion pump is ideal to deliver the Oxyglobin at a set rate and to avoid accidental overdosage. Medications should not be added to the Oxyglobin bag, nor should other fluids or drugs be administered through the same infusion set. Caution should be exercised if administering other fluids (crystalloids, other colloids, or blood) simulta-neously with Oxyglobin, given the risk of circulatory overload. There has been a recommendation of administering 60% of maintenance crystalloids with Oxyglobin in normovolemic patients [41].

Monitoring

Patients receiving Oxyglobin require careful monitoring during and for several hours after the infusion to determine if the patient's clinical signs are

improving in response to the initial dose of Oxyglobin or if the patient requires additional oxygen-carrying support (ie, more Oxyglobin or RBCs) and to identify any potential adverse reactions. A blood type should be performed in anticipation of administering RBCs when available or if needed. Patients are monitored as for a blood transfusion, with temperature, heart rate, and respiratory rate recorded before the infusion, within 15 minutes of the start of the infusion, and then hourly during the infusion and early after the infusion (2–4 hours). If an immediate hypersensitivity reaction (eg, dyspnea, cardiovascular collapse) occurs (possible but not documented in any clinical or research cases), infusion of Oxyglobin should immediately be discontinued and appropriate treatment administered (eg, crystalloids, epinephrine) (personal communication, Biopure Technical Services, 2003). Unlike with blood transfusion, febrile reactions are rarely observed with Oxyglobin administration. The most serious potential adverse event, circulatory overload leading to life-threatening pulmonary edema or pleural effusion, necessitates particularly careful monitoring of respiratory rate and effort and onset of coughing. Although monitoring of CVP during and immediately after infusion of Oxyglobin is recommended, clinical assessment of respiratory rate, jugular venous distention, and diuresis may be used as general indicators of expanded blood volume. If CVP can be measured and changes more than 6 cm H_2O or if dyspnea or coughing is noted, Oxyglobin infusion should be discontinued and possibly reinstituted at a slower rate when the clinical signs resolve or CVP decreases. Depending on the severity of the clinical signs, a diuretic (eg, furosemide) may be indicated.

Monitoring for adequate oxygenation is a clinical assessment. Tachycardia, poor pulse quality, pallor, lethargy, weakness, and decreased appetite are important clinical signs of poor perfusion or anemia. They may indicate that a patient is in need of additional oxygen-carrying support. Therefore, resolution of these signs is typically considered a favorable response to the infusion in a normovolemic animal. In hypovolemia, however, typical measures of resuscitation (heart rate and mean arterial pressure) used to guide transfusion therapy in patients after hemorrhage may be insufficient because of the vasopressive effects of Oxyglobin and could lead to inadequate volume repletion [12]. Direct measurement of tissue oxygenation is difficult and impractical in clinical settings. In critically ill patients, however, improvement in indices of acid-base status (pH and P_{CO_2}) and cellular metabolism (lactate) can be used as indirect evidence of improved tissue oxygenation.

Monitoring of patients receiving Oxyglobin can be aided by measuring total (RBC and plasma) [Hb] or plasma [Hb] rather than PCV, although concurrent measurement of PCV may be helpful in noting continuing blood loss or hemolysis as well as volume expansion. After infusion of Oxyglobin at a dose of 30 mL/kg, however, a decrease of approximately 25% in PCV as a result of hemodilution is expected; the dilutional effects may not be seen at doses of 15 mL/kg or less (package insert). A point-of-care hemoglobin-

ometer, the HemoCue (HemoCue AB, Angelholm, Sweden), accurately measures [Hb] in Oxyglobin [42] and the blood of various species [43]. The HemoCue is appealing for clinical practice because it is portable and simple to use, requires a small sample volume (10 μL), and provides accurate results in less than a minute. The frequency of measurement of [Hb] (± PCV) after infusion of Oxyglobin should be dictated by the clinical condition of the patient, but, in general, measurements immediately after infusion and at 12 and 24 hours may be appropriate. Because PCV is measured more frequently than [Hb] in veterinary practice, it may be helpful to think of [Hb] in terms of a comparable PCV. For example, if a patient has a total [Hb] of 8 g/dL after infusion of Oxyglobin, the oxygen-carrying capacity of the blood roughly corresponds to that of a PCV of 24% ([Hb] × 3), or greater if considering more efficient tissue oxygenation with Oxyglobin. See Appendix 1 for an example of monitoring a clinical patient.

A serum chemistry screen, complete blood cell count, and urinalysis should be performed before administering Oxyglobin. The plasma/serum of patients receiving Oxyglobin appears red and may thus result in artifactual increases or decreases in results of some chemistry tests depending on the type of analyzer and reagents used (information available from Oxyglobin Technical Services [1-888-400-0030]), but, in general, measurements of sodium, chloride, potassium, and blood urea nitrogen (BUN) are valid with most commonly used analyzers. Results of a complete blood cell count are valid, except for the RBC indices of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), which are increased. Urine dipstick measurements are inaccurate during transient discoloration of the urine because of excretion of Hb dimers. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) can be accurately determined with mechanical, magnetic, or light-scattering methods, but optical methods are not reliable for coagulation assays in the presence of Oxyglobin. Pulse oximetry measurement in the presence of Oxyglobin has been considered valid, although it is not an effective tool in monitoring response to therapy with Oxyglobin. In ex vivo studies in which canine blood was mixed with different concentrations of Oxyglobin, the Nova CO-Oximeter (Nova Biomedical Corporation, Waltham, MA) accurately measured oxygen saturation in the presence of Oxyglobin [44]. Evaluation of a Nellcor oxygen saturation monitor (Nellcor Puritan Bennett, Pleasanton, CA) in a canine model of acute blood loss and resuscitation with Oxyglobin (probe placed on the tongue) revealed that values of oxygen content calculated from the oxygen saturation monitor deviated by 9% to 16% from directly measured values, depending on the dose [45]. This difference in results may be a result of the relative dosage of HBOC and the degree of anemia or hemodilution. Because the oxygen-dissociation curve for Oxyglobin is right-shifted compared with that of canine RBCs, if the amount of plasma Hb relative to RBCs is greater, a lower saturation could be obtained with the pulse oximeter.

Summary and conclusions

Experimental and clinical experience with Oxyglobin has demonstrated that it is safe and efficacious in providing temporary oxygen-carrying support to anemic dogs. Although many cats seem to have benefited clinically from Oxyglobin (not approved for cats), prospective controlled clinical studies are necessary to determine the clinical efficacy and safety of Oxyglobin and the ideal dose and rate of administration in anemic cats. Potential advantages of Oxyglobin over RBCs include its immediate availability, lack of need for blood typing and crossmatching, and the carrying of oxygen in the plasma, allowing oxygen delivery to tissue not limited by RBC flow. In addition, the colloidal properties of Oxyglobin allow for expansion of the circulatory volume, which may be helpful in patients with hypovolemia, especially hemorrhagic shock, but which may also be a concern in normovolemic patients, potentially leading to volume overload. There are conflicting data regarding Oxyglobin's vasoconstrictive effects, the clinical significance of which is uncertain because there is no evidence of global tissue hypoxia, even in the studies noting increases in systemic vascular resistance. Although there are many proposed uses for Oxyglobin, its clearest indication is to provide oxygen-carrying support to anemic patients when compatible RBCs are not available.

Appendix 1. Case example

Signalment: 9-month-old intact female mixed-breed dog (6.7 kg)
Clinical signs: pallor, depression, lethargy
Baseline packed cell volume: 18%
Diagnosis: toxin (naphthalene)-induced hemolytic anemia
Treatment: Oxyglobin, 30 mL/kg, at a rate of 10 mL/kg/h
Monitoring: Clinical assessment of change in pulse quality, attitude, and activity

	Baseline	Immediately after infusion	6 hours after infusion	12 hours after infusion	24 hours after infusion	72 hours after infusion
Temperature (°F)	101.0	101.2	101.0	101.1	101.0	100.8
Heart rate (bpm)	140	100	96	92	96	92
Respiratory rate (bpm)	32	28	28	28	28	24
Packed cell volume (%)	18	13	14	15	16	27
Total hemoglobin concentration (g/dL)	5.4	8.2	7.9	7.4	7.1	8.4
Plasma hemoglobin concentration (g/dL)	0.1	4.9	4.8	4.3	3.5	1.1

Adverse events: yellow discoloration of mucous membranes, one episode of vomiting

Clinical assessment: improvement in pulse quality, attitude, and activity evident within 2 hours after infusion

Outcome: no need for additional oxygen-carrying support; discharged from hospital on day 3.

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