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Insensitivity of cerebral oxygen transport to oxygen affinity of hemoglobin-based oxygen carriers

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Abstract

The cerebrovascular effects of exchange transfusion of various cell-free hemoglobins that possess different oxygen affinities are reviewed. Reducing hematocrit by transfusion of a non-oxygen-carrying solution dilates pial arterioles on the brain surface and increases cerebral blood flow to maintain a constant bulk oxygen transport to the brain. In contrast, transfusion of hemoglobins with P_{50} of 4–34 Torr causes constriction of pial arterioles that offsets the decrease in blood viscosity to maintain cerebral blood flow and oxygen transport. The autoregulatory constriction is dependent on synthesis of 20-HETE from arachidonic acid. This oxygen-dependent reaction is apparently enhanced by facilitated oxygen diffusion from the red cell to the endothelium arising from increased plasma oxygen solubility in the presence of low or high-affinity hemoglobin. Exchange transfusion of recombinant hemoglobin polymers with P_{50} of 3 and 18 Torr reduces infarct volume from experimental stroke. Cell-free hemoglobins do not require a P_{50} as high as red blood cell hemoglobin to facilitate oxygen delivery.

Keywords

Anemia; Blood substitute; Cerebral blood flow; Hemoglobin; Oxygen affinity; Oxygen transport

1. Introduction

The use of cell-free hemoglobin (Hb) as a transfusion fluid that carries oxygen has been investigated for many decades. A major problem encountered in the development of hemoglobin-based oxygen carriers (HBOCs) was the occurrence of systemic vasoconstriction (which leads to arterial hypertension) that follows infusion of cell-free Hb solutions. It has been clearly demonstrated that a factor that contributes to this phenomenon is the scavenging of nitric oxide (NO) by the Hb molecules extravasating through the endothelium [1]. To prevent Hb extravasation, Hb molecules were subjected to chemical modifications by introducing intramolecular crosslinks to prevent the dissociation of tetrameric Hb molecules into dimers. Stabilized tetrameric Hb molecules, however, also extravasate in specific vascular beds. Sustained NO scavenging will depend on the balance of continued Hb permeation across the endothelium versus clearance of interstitial Hb in the lymph. Physical and mathematical models also infer that plasma-based Hb more readily scavenges endothelial-derived NO than Hb concentrated within the red blood cell membrane [2–5].

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Subsequently, intermolecular crosslinking was used to obtain large polymeric forms of Hb molecules that would be less permeable. A problem associated with this approach is the heterogeneity of the products obtained through chemical modifications and the possible persistence in solution of physiologically significant amounts of non-modified Hb molecules. Surface decoration using polyethylene glycol derivatives is another procedure used to increase the molecular volume of the Hb molecules and to limit filtration through the endothelium [6, 7]. These products are endowed with high oncotic pressure and viscosity, which may limit the concentration of Hb that can be administered. Alternatively, recombinant molecular engineering is used to obtain Hb polymers of different sizes in the absence of chemical modification [8,9]. Although the amount of recombinant HBOCs that can be prepared is currently limited, the continuous development of molecular engineering could result in large-scale production capacity of recombinant oxygen carriers that (a) are free from infectious agents that could be present in mammalian blood and (b) have functional and conformational characteristics that are geared to various clinical applications.

Another factor that contributes to vasoconstriction is the presence of autoregulatory mechanisms that balance changes in blood viscosity and maintain oxygen delivery to prevent the potential damage that is associated with either excessive or inadequate tissue oxygenation. Thus, it is important to understand and correlate the various factors that participate in regulating vasoactivity and oxygen delivery.

Half a century of investigation on HBOCs has resulted in the development of several compounds that vary in molecular size, viscosity, oncotic activity, and oxygen affinity. It is obvious that oxygen affinity and efficacy of oxygen delivery to tissues may be correlated. It is not obvious whether the oxygen affinity and oxygen transport characteristics of Hb encapsulated in red cells are reproducible or desirable in cell-free HBOCs dissolved homogeneously in plasma.

The history of the development of HBOCs in our laboratories over the past 15 years includes experimentation with HBOCs with the partial pressure of oxygen at 50% oxyhemoglobin saturation (P_{50}) ranging from 3 to 34 Torr. In this review, we address the question of the relationship between the oxygen affinity of HBOCs and their ability to deliver oxygen to tissues. We report data obtained in conditions (a) of partial oxygen deprivation during anemia induced by exchange transfusion, and (b) of extreme hypoxia during cerebral ischemia.

2. Methodologies of hemoglobin design and evaluation

2.1. Recombinant hemoglobin

Optimal expression of recombinant Hb was obtained using a hybrid protein, Hb Minotaur, which contained α -human and β -bovine chains [8]. Polymerization of Hb Minotaur spontaneously occurred through the oxidation of thiol groups that were introduced on the surface of the β -chains. Two polymers have been prepared: Hb Polytaur and Hb (Polytaur) $_n$ [8,9]. Like Hb Prisca [10], Hb Polytaur is a homogeneous globular polymer with a molecular weight (MW) of ~500 kDa, P_{50} of 18 Torr, and oxygen-binding cooperativity of $n=1.8$. Hb (Polytaur) $_n$ is a large, heterogeneous polymer with average MW of ~1.0 MDa, a P_{50} near 3 Torr, and no oxygen-binding cooperativity.

2.2. Chemically modified hemoglobins

Stabilized bovine or human tetrameric Hb was prepared by intramolecular crosslink of lysine residues at β -82 by the reagent bis(3,5-dibromosalicyl) fumarate [11] or bis(3,5-dibromosalicyl)sebacate [12,13] to produce non-dissociable tetrameric Hbs with a P_{50} of 18 Torr and cooperativity of $n=1.7$ or a P_{50} of 34 Torr and cooperativity of $n=2.1$, respectively.

Polymerization of Hb bovine has been described [1]. In essence, the surface carboxyl groups were activated by treatment with 1-ethyl-3-(dimethyl-amino-propyl)carbodiimide to form pseudopeptide bonds with the amino residues on the surface of adjacent molecules. Diafiltration at 300 kDa was used to eliminate residual non-modified Hb molecules and small, polymerized forms. The resulting Hb-Zerolink had an average MW of ~25 MDa, P_{50} near 4 Torr, and no oxygen-binding cooperativity.

2.3. Measurements

Physical properties of the various Hb solutions are summarized in Table 1. Oxygen equilibria curves were measured in phosphate buffer at pH 7.4 at 37 °C using either a hemoxanalyser or the thin-layered Gill cells.

For infusion, all HBOCs were dissolved in lactated Ringer solutions. The solutions were sterile filtered (0.45 μ m, 0.8 μ m), and endotoxins were removed using Detoxo-gel (Pierce). Proteins were stored at -80 °C.

Cerebral blood flow (CBF) was measured using microsphere technology in anesthetized cats [11]. Pial arteriolar diameter was measured by intravital microscopy through closed cranial windows in anesthetized cats and rats [14]. Focal cerebral ischemia was produced in mice by occlusion of the middle cerebral artery, as described [8].

3. Cerebrovascular responses to HBOC transfusion

3.1. Oxygen delivery in anemic conditions (partial oxygen deprivation)

Anemia was obtained by isovolumic exchange transfusions to reduce the hematocrit by 40%–50%. Oxygen transport of non-dissociable fumaryl and sebacyl crosslinked tetrameric Hbs was investigated in the brain, where extravasation is prevented by the tight endothelial junctions of the vascular endothelium (blood–brain barrier).

In a first group of experiments [11], CBF was measured in anemic cats after isovolemic exchange transfusion with either 6% albumin or 7% fumaryl crosslinked Hb (P_{50} =18 Torr, n =1.7) solutions possessing equivalent colloid osmotic pressure. The plasma concentration of fumaryl crosslinked Hb was approximately 2.5 g/dl. CBF is known to increase upon reduction of the hematocrit so as to maintain adequate oxygen transport. The data in Fig. 1 indicate an increase in CBF that was larger upon albumin transfusion than upon Hb transfusion at equivalent reductions in hematocrit. Convective oxygen transport, defined as the product of CBF and arterial oxygen content, was nearly identical in the two cases, indicating adjustment of the respective CBF to the oxygen content of arterial blood. After inhibition of NO synthase, differences in CBF persisted between albumin and fumaryl crosslinked Hb groups, whereas oxygen transport remained similar in both groups [11]. Thus, the lower CBF in the Hb group was not primarily the result of NO scavenging.

In subsequent sets of experiments [14,15], CBF was measured in anesthetized cats after isovolemic exchange transfusion of 7% sebacyl crosslinked tetrameric Hb (P_{50} =34 Torr, n =2.1) and 12% polymeric Hb-Zerolink (P_{50} =4 Torr). As mentioned, Hb-Zerolink is a large polymer (average MW~25 MDa) that does not extravasate into renal lymph and does not produce arterial hypertension [1]. In each case, the decrease in hematocrit was accompanied by very little change in arterial oxygen content and CBF (Fig. 2). Consequently, cerebral oxygen transport was unchanged from baseline, and maintenance of bulk oxygen transport was seen whether the P_{50} of the cell-free Hb was 4, 18, or 34 Torr.

Cerebral metabolic rate of oxygen (CMRO₂) is a major factor that regulates cerebral oxygen transport. The level of anemia used in these studies did not reduce CMRO₂, and CMRO₂ was

not impaired after exchange transfusion with fumaryl or sebacyl crosslinked Hb (Fig. 3). Fractional oxygen extraction (arteriovenous oxygen content difference/arterial oxygen content) is equivalent to the ratio of CMRO₂/cerebral oxygen transport and provides a means by which to normalize for interanimal variation in arterial oxygen content and CMRO₂. Using arterial and sagittal sinus oxygen content measurements, cerebral fractional oxygen extraction was not altered after exchange transfusion with fumaryl or sebacyl crosslinked Hb (Fig. 3). In contrast, previous work in near-term fetal and one-week-old lambs found that replacing part of the red cell fetal sheep Hb ($P_{50} \sim 17$ Torr) with adult sheep Hb ($P_{50} \sim 44$ Torr) decreased CBF and cerebral oxygen transport over a wide range of arterial PO₂ and oxygen content [16,17]. Cerebral fractional oxygen extraction increased as red blood cell Hb P_{50} increased from 17 to 32 Torr in unanesthetized fetal sheep and from 26 to 37 Torr in unanesthetized postnatal lambs (Fig. 3). Therefore, increasing red cell Hb P_{50} , which is predicted to increase tissue PO₂ [18], resulted in an active decrease in CBF and oxygen transport to the microcirculation that limits overoxygenation of the tissue. In contrast, replacing red cell Hb ($P_{50} \sim 37$ Torr) with plasma-based Hb in the cat did not alter the relationship of oxygen transport to CMRO₂. Thus, regulation of cerebral oxygen transport to the microcirculatory exchange site was not influenced by the P_{50} of the HBOC.

We also tested whether the relationship of CBF and oxygen extraction to arterial oxygen content would be altered by plasma-based Hb during hypoxic hypoxia. Over a range of 50%–100% arterial oxyhemoglobin saturation, CBF remained greater in anesthetized cats that were exchange-transfused with a 6% albumin solution than in those that were exchange-transfused with a solution of 7% sebacyl crosslinked Hb [19]. However, at a particular arterial oxyhemoglobin saturation, arterial oxygen content after albumin exchange transfusion is less than after Hb exchange transfusion. Interestingly, when CBF was plotted against arterial oxygen content, a single relationship was obtained (Fig. 4). Thus, cerebral oxygen transport remained equivalent among albumin- and Hb-transfused groups when hypoxic hypoxia was superimposed at similar levels of decreased hematocrit but with different levels of oxygen-carrying capacity. CMRO₂ was maintained over this range of hypoxic hypoxia in the albumin group (3.1 ± 0.2 to 3.2 ± 0.3 ml O₂/min/100 g; $\pm$ SE) and the Hb sebacyl group (3.5 ± 0.2 to 3.8 ± 0.3 ml O₂/min/100 g). Consequently, fractional oxygen extraction remained similar among the albumin and Hb groups over a wide range of hypoxia, although some increase in extraction started to become evident in the Hb group at 50% oxyhemoglobin saturation (Fig. 4). Hence, cerebrovascular resistance is precisely adjusted to preserve cerebral oxygen transport with various combinations of anemic hypoxia, hypoxic hypoxia and oxygen-carrying capacity sustained by HBOC.

3.2. Cerebral pial arterioles

Changes in CBF are the result of changes in blood viscosity and active regulation of vascular tone. Vascular resistance in pial arterioles on the brain surface comprises ~40% of the total vascular resistance in the cerebrovascular bed. To directly assess changes in vascular tone, the diameter of pial arterioles was measured *in vivo* through closed cranial windows. In a study on anesthetized cats [20], exchange transfusion with an albumin solution produced an increase of the pial arteriolar diameter, whereas exchange transfusion with a solution of the low-affinity sebacyl crosslinked Hb produced a decrease in diameter (Fig. 5A). When the window was superfused with N^ω-nitro-L-arginine (L-NNA), an inhibitor of NO synthase, baseline diameter decreased ~10% [15]. On this new baseline, in the absence of NO, subsequent exchange transfusion with albumin produced dilation, whereas subsequent transfusion with sebacyl crosslinked Hb produced constriction (Fig. 5B). Therefore, differences between the vascular responses to albumin and Hb transfusion were not dependent on NO scavenging. Exchange transfusion with the high-affinity Hb-Zerolink also caused constriction (Fig. 5C). When co-infusion with polyvinylpyrrolidone (PVP) was used to increase plasma viscosity, the

vasodilation produced by albumin solutions was only marginally increased [14], whereas the constriction seen with Hb-Zerolink was reversed and the pial arterioles markedly dilated (Fig. 5C). Thus, the presence of HBOC in the plasma does not impair the ability of arterioles to dilate in response to an increased viscous load. The constriction seen with cell-free Hb exchange transfusion alone is likely the result of decreased blood viscosity associated with decreased hematocrit. The constriction offsets the decrease in blood viscosity and results in little change in CBF and oxygen transport. This constriction with constant oxygen transport infers that the high-affinity HBOC delivers oxygen to tissues at reduced hematocrit.

When the experiments that were conducted in cats were replicated in rats, albumin exchange transfusion dilated pial arterioles and Hb-Zerolink exchange transfusion produced constriction [21]. As with sebacyl crosslinked Hb exchange transfusion in the cat, the constrictor response to Hb-Zerolink exchange transfusion in the rat remained evident after inhibition of NO synthase (Fig. 5D). However, the pial arteriolar constriction seen after exchange transfusion with Hb-Zerolink was blocked by inhibiting the production of 20-hydroxyecosatetraenoic acid (20-HETE) by cytochrome P450 with HET0016 (Fig. 4D). Moreover, another 20-HETE synthesis inhibitor and a 20-HETE antagonist also reduced the constrictor response [21]. Because 20-HETE production from arachidonic acid in vascular smooth muscle is oxygen-dependent [22], this 20-HETE-dependent vasoconstriction in response to decreased hematocrit at normal oxygen-carrying capacity can be interpreted as an autoregulatory response to prevent excess oxygenation at reduced blood viscosity.

3.3. Oxygen delivery in extreme hypoxic conditions

Oxygen delivery in the extreme hypoxic conditions of experimental stroke was investigated using recombinant polymerized Hbs in mice. A hypervolemic exchange transfusion was performed in mice over a 20-min period starting 10 min after occlusion of the middle cerebral artery. Infarct volume was reduced by transfusion of Hb Polytaur, a homogeneous polymer of seven tetrameric Hb molecules (MW~500 MDa) with an intermediate P_{50} (18 Torr) and cooperativity ($n = 1.8$) [8]. Unexpectedly, Hb (Polytaur)_n, a heterogeneous polymer with average MW of ~1 MDa, a low P_{50} (3 Torr), and no cooperativity ($n = 1$), also markedly reduced infarct volume (Fig. 6) [9]. Similar results were obtained with the chemically modified polymer Hb-Zerolink with an average MW of ~25 MDa, a low P_{50} (4 Torr), and no cooperativity (data not shown). Therefore, polymers with intermediate and high oxygen affinity are capable of delivering oxygen to ischemic tissue and reducing infarct size in the brain.

4. Interpretation of results with HBOC possessing different oxygen affinity

The oxygen transport characteristics of blood are generally used as a template for designing the functional characteristics of artificial HBOCs. Normal blood shows a P_{50} near 30 Torr, with oxygen-binding cooperativity near $n = 3$. It is a general opinion that higher oxygen affinity and lower cooperativity would decrease the ability of oxygen carriers to deliver oxygen to tissues. This view is challenged by the data that show equivalent levels of cerebral oxygen transport and pial arteriolar diameter responses to reductions in hematocrit and similar reductions in infarct volume during experimental stroke with HBOCs of differing oxygen affinities.

The simulations in Fig. 7 illustrate the P_{50} -dependent fractional delivery of oxygen by HBOCs when they bind oxygen at a partial pressure of 100 Torr (in the lungs) and release it at 2 Torr (in exercising muscles or ischemic tissue). With P_{50} between 3 and 50 Torr, fractional delivery is nearly independent of oxygen affinity. Increased oxygen-binding cooperativity increases oxygen delivery, but delivery remains substantial even in the absence of cooperativity. These simulations are supported by the experimental data presented here, which indicate that all HBOCs with P_{50} varying from 3 to 34 Torr delivered oxygen to tissues. The data include animal

trials conducted under conditions of either partial oxygen deficiency in anemic animals or the extreme hypoxia of ischemia produced by arterial occlusion. It should be appreciated that the simulation presented in Fig. 7 illustrates the potential for oxygen delivery to hypoxic tissue as a function of P_{50} . The actual delivery *in vivo* is more complex and depends on oxygen consumption, hematocrit, vascular geometry, and pre-capillary oxygen loss. More sophisticated models of oxygen transport at the capillary or whole organ level indicate that oxygen delivery in normoxic tissue is relatively insensitive to HBOC P_{50} values greater than erythrocyte Hb P_{50} values, but that P_{50} of HBOC in the range of 15 Torr can increase oxygen delivery under hypoxic conditions [23-25].

In animals made anemic by a 40% exchange transfusion, the resulting increase of CBF was lower after infusion of fumaryl crosslinked tetrameric Hb (P_{50} = 18 Torr), sebacyl crosslinked tetrameric Hb (P_{50} = 34 Torr), or polymeric Hb-Zerolink (P_{50} =4 Torr) than it was after albumin transfusion. The delivered oxygen reduced the increase in CBF, and oxygen transport by blood was equalized and restored to normal in all cases. Neither the stabilized non-dissociable tetramers nor the polymers extravasate in the brain because of the tight junctions of brain capillary endothelium that constitutes the blood-brain barrier. Thus, the regulation produced by the infused Hbs was presumably due to oxygen delivery and not to NO scavenging. Indeed, differences in CBF and in pial arteriolar responses between albumin and Hb transfusion persisted after NO synthesis was inhibited [11,15,21], and pial arterioles retained intact dilator responses to agonists known to require endothelial synthesis of NO [20,26].

Under similar experimental conditions, exchange transfusion of either low-affinity sebacyl crosslinked Hb or high-affinity Hb-Zerolink produced similar pial arteriolar constriction despite different oxygen affinities. Although the 12% concentration of the Hb-Zerolink solution used in the cat transfusion experiments (Fig. 5C) was higher than the 7% concentration of the sebacyl crosslinked Hb solution (Fig. 5A), the magnitude of the pial arteriolar constriction with either solution was similar to that observed with a 6% concentration of the Hb-Zerolink solution used in the rat transfusion experiments (Fig. 5D). Hence, the higher concentration of Hb-Zerolink used in the cat transfusion experiments is an unlikely explanation for the similar constriction seen with Hbs possessing low and high oxygen affinity. Thus, the constrictor response seen after exchange transfusion of either cell-free Hb was distinctly different from the dilation seen with exchange transfusion of the non-oxygen carrying albumin solution, thereby implying oxygen delivery with both low- and high oxygen affinity Hbs.

Part of the vasoconstriction seen with sebacyl crosslinked Hb may have been due to a myogenic response secondary to the arterial hypertension that arises from peripheral extravasation of this tetramer. However, in the case of Hb-Zerolink, peripheral extravasation and arterial hypertension are absent. Because the constriction of pial arterioles after exchange transfusion was not eliminated by inhibiting NO synthesis, the constrictor response was dependent on another mechanism, which appears to rely on synthesis of 20-HETE from arachidonic acid by cytochrome P450s known to reside in cerebral vascular smooth muscle [27-30]. Constriction is thought to be mediated by PKC-dependent inhibition of calcium-activated potassium channels. Interestingly, synthesis of 20-HETE by cytochrome P450 is oxygen-dependent over the physiologic range seen in blood [22]. The observation that inhibitors of 20-HETE synthesis and a 20-HETE antagonist block the pial arteriolar constrictor response to exchange transfusion of Hb-Zerolink supports the concept that this high-affinity HBOC is evoking constriction in response to excess oxygenation. The excess oxygenation would occur passively from the decrease in blood viscosity secondary to the decrease in hematocrit without a decrease in oxygen-carrying capacity. Thus, the constrictor response can be viewed as a homeostatic response to limit oxygen delivery. Therefore, the constriction offsets the decrease in viscosity and results in no change in total vascular resistance, blood flow, or bulk oxygen transport to the brain. Further support for the concept that this constrictor response of pial arterioles

represents an autoregulatory response to decreased viscosity is attained from the results that show that increasing plasma viscosity with PVP converted the constrictor response to a dilator response that helped to preserve CBF and oxygen transport.

The colloid osmotic pressure of the transfused solutions in the cat, which had a normal plasma colloid osmotic pressure of 17 Torr, varied from approximately 10 Torr with a 12% solution of Hb-Zerolink to 24 Torr with 7% sebacyl crosslinked Hb. Pial arteriolar constriction occurred immediately after the isovolemic exchange transfusion before major shifts in blood volume would occur with these solutions possessing different oncotic pressure. In addition, slow continuous infusions were performed after the initial exchange transfusion to keep hematocrit, and presumably blood volume, constant. Pial arterioles are not under adrenergic control. For these reasons, the constrictor response is unlikely to be attributed to hypovolemia.

Both high- and low-oxygen-affinity Hb maintained normoxic delivery of oxygen by constricting pial arterioles. This constriction occurred with plasma concentration of HBOCs in the range of 20-50 mg/ml, which was less than the concentration of the red cell-based Hb replaced by HBOCs. The plasma-based Hb may increase the efficiency of oxygen delivery compared to Hb segregated in red cells. This proposition is supported by experiments of superfusion of intestinal membranes [31]. Under conditions of hypoxia, a 30 mg/ml solution of cell-free sebacyl crosslinked Hb was able to support the function of the perfused intestinal membranes, whereas an equivalent amount of Hb inside red cell suspensions failed the test.

Ordinarily, increases in the P_{50} of red cell Hb (as occurs naturally during development as fetal Hb is replaced by adult Hb) act to decrease CBF and oxygen transport to the brain, presumably as a homeostatic mechanism to prevent overoxygenation of the brain as a consequence of increased oxygen unloading from low-affinity Hb [16,17]. Furthermore, during the extreme hypoxic conditions of cerebral ischemia, others have shown that increasing the P_{50} of red cell Hb with an allosteric inhibitor or using liposome-encapsulated Hb with a P_{50} of 40 Torr reduces infarct volume from experimental stroke [32-34]. On the other hand, the experiments that used recombinant HBOCs with P_{50} ranging from 3 to 18 Torr also demonstrated substantial reductions in infarct volume [8,9]. These observations suggest that increasing the P_{50} of segregated Hb is beneficial for unloading oxygen in ischemic tissue, whereas the unloading of oxygen is relatively independent of the P_{50} of cell-free Hb. Even in nonischemic tissue, the observation that pial arteriolar constriction to exchange transfusion of HBOC was not attenuated by decreasing the P_{50} from 34 Torr to 4 Torr implies that oxygen unloading in nonischemic tissue is relatively independent of the P_{50} of cell-free Hb.

These observations present a paradox and indicate that classical views based on the functional characteristics of blood do not apply to cell-free oxygen delivery. Plasma normally represents a major site of resistance to oxygen diffusion from the red cell to the tissue [35]. Increasing the P_{50} of red cell Hb increases the PO_2 gradient for oxygen diffusion across the plasma. In contrast, cell-free Hb increases the solubility of oxygen in the plasma and decreases the resistance for oxygen diffusion as long as exchange of oxygen between the HBOC and dissolved oxygen is constantly occurring [23,25,36]. Thus, the movement of HBOCs within plasma facilitates the diffusion of oxygen from the red blood cell to the endothelium [6,37, 38]. Although HBOCs with a P_{50} of 3 Torr (and low off-rate constant kinetics) exchange less oxygen per unit time with plasma than do those with high P_{50} , the amount of Hb in the plasma necessary to facilitate oxygen transport from the red cell to the endothelium may be relatively small, especially if transit times across the microcirculatory exchange site are of long duration, as expected during ischemia. Of interest is the observation is that additional reduction in infarct volume can be attained by increasing the concentration of Hb Polytaur from 3% to 6% in the solution that is exchange-transfused [9]. Thus, an optimal ratio of red cell-based Hb to plasma-based Hb presumably exists, and the optimal concentration ratio may depend on the nature of

the pathophysiologic condition and possibly on the ratio of the oxygen affinity in the two compartments.

In the absence of a new paradigm for the characteristics of cell-free oxygen delivery, it is difficult to suggest which HBOC would be more appropriate for clinical trials. The data presented here suggest only that, in the absence of extravasation, all HBOCs obtained by either a chemical or recombinant procedure enhance the delivery of oxygen to the tissue *in vivo*. That cell-free oxygen delivery may be excessive at reduced hematocrit and blood viscosity is also clearly indicated. A prudent use of these substances in clinical trials is recommended. Further work is needed on the long-term consequences of transfusion of HBOCs in pathophysiologic conditions.

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Abbreviations

20-HETE, 20-hydroxyeicosatetraenoic acid; CBF, cerebral blood flow; CMRO₂, cerebral metabolic rate of oxygen; Hb, hemoglobin; HBOC, hemoglobin-based oxygen carriers; NO, nitric oxide; *P*₅₀, partial pressure of oxygen at 50% oxyhemoglobin saturation; PVP, polyvinylpyrrolidone.

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Fig. 1.

Cerebral blood flow (A) and cerebral oxygen transport (B) in anesthetized cats ($\pm$ SE; $N=8$) in a time control group and after isovolemic exchange transfusion with a 7% solution of fumaryl crosslinked tetrameric hemoglobin (FMHb; $P_{50}=18$ Torr) or a 6% albumin solution. Plasma FMHb concentration was 2.5 g/dl. The corresponding arterial oxygen contents in the 3 groups with different hematocrits (Hct) were 15.0 ± 0.6 , 11.8 ± 0.3 , and 8.7 ± 0.3 ml O₂/dl. Blood flow was inversely related to arterial oxygen content, resulting in no differences in oxygen transport. Adapted from [11].

Fig. 2. Cerebral blood flow (A, C) and cerebral oxygen transport (B, D) in anesthetized cats after isovolemic exchange transfusion with a 6% solution of sebacyl crosslinked tetrameric hemoglobin (A, B) with a P_{50} of 34 Torr ($N=12$), which is close to cat blood (P_{50} of 37 Torr), or a 12% solution of zerolink polymeric hemoglobin (C, D) with a P_{50} of 4 Torr ($N=9$). Hct, hematocrit. Plasma Hb concentration was 2.5 g/dl for sebacyl crosslinked Hb and 4.8 g/dl for Hb-Zerolink. Values are means $\pm$ SE. Adapted from [14,15].

Fig. 3.

Cerebral metabolic rate of oxygen (CMRO₂) (A) and cerebral fractional oxygen extraction (B) in 6 fetal lambs undergoing an exchange transfusion with adult sheep blood to increase the P_{50} of red blood cell (RBC) hemoglobin (Hb) from 17 to 32 Torr (adapted from [17]), in 9 newborn lambs who underwent an exchange transfusion with adult sheep blood to increase the P_{50} of RBC Hb from 26 to 37 Torr (adapted from [16]), in 8 cats after exchange transfusion with a 7% solution of fumaryl crosslinked tetrameric hemoglobin with a P_{50} of 18 Torr (adapted from [11]), and in 12 cats after exchange transfusion with a 7% solution of sebacyl crosslinked tetrameric hemoglobin (adapted from [15]) with a P_{50} of 34 Torr, which is close to cat blood (P_{50} of 37 Torr). CMRO₂ was unaffected by the exchange transfusion in all groups. Fractional oxygen extraction, which is the ratio of CMRO₂ to O₂ transport, was sensitive to changes in RBC P_{50} but not to differences in the P_{50} of plasma-based HBOC. Values are means $\pm$ SE.

Fig. 4.

Cerebral blood flow (A) and cerebral fractional oxygen extraction (B) versus arterial oxygen content in anesthetized cats after exchange trans-fusion with a 6% solution of albumin ($N = 8$) or a 7% solution of sebacyl crosslinked tetrameric hemoglobin ($N = 10$) and graded reductions in arterial oxyhemoglobin saturation from ~98% to ~80%, ~65%, and ~50% produced by decreasing inspired oxygen concentration. Increased blood flow was similar at equivalent arterial oxygen content, resulting in no change in fractional oxygen extraction, the ratio of oxygen consumption to oxygen transport. Values are means $\pm$ SE. Adapted from [19].

Fig. 5.

Percent changes in pial arteriolar diameter measured by intravital microscopy after exchange transfusion with albumin or cell-free hemoglobin (Hb) solutions to reduce hematocrit by ~40%. (A) Exchange transfusion with a 5% albumin solution produced dilation ($N = 7$), whereas exchange transfusion with a 6% solution of sebacyl crosslinked tetrameric hemoglobin (HbSebacyl; $N = 8$) produced constriction in cats (adapted from [20]). (B) Differences in diameter responses between albumin and HbSebacyl transfusion persisted when the cat pial arterioles were bathed with the nitric oxide synthase inhibitor N^G -nitro-L-arginine (L-NNA; 300 μ M) in cerebrospinal fluid (adapted from [15]). (C) Exchange transfusion with albumin solution ($N = 10$) produced dilation, and exchange transfusion with Hb-Zerolink polymer (HbZL; $N = 9$) with a P_{50} of 4 Torr produced constriction comparable to that of the low-affinity HbSebacyl (P_{50} of 34 Torr). Increasing plasma viscosity by cotransfusion of polyvinylpyrrolidone (PVP) with HbZL ($N = 6$) converted the constrictor response to a dilator response in cats (adapted from [14]). (D) Exchange transfusion with albumin ($N = 6$) and HbZL ($N = 6$) in rats produced responses similar to those in cats. Constriction to HbZL exchange transfusion persisted when superfusing the cranial window with L-NNA ($N = 6$) but was abolished when superfusing with the 20-HETE synthesis inhibitor HET0016 (1 μ M; $N = 6$) in cerebrospinal fluid (adapted from [21]). Values are means $\pm$ SE.

Fig. 6.

Infarct volume after 2 h of middle cerebral artery occlusion and 22 h of reperfusion in mice exchange-transfused during ischemia with 5% albumin solution ($N=13$), 6% HbPolytaur solution ($P_{50}=18$ Torr; $N=5$), or 6% Hb (Polytaur)n ($P_{50}=3$ Torr; $n=5$). Both recombinant hemoglobin polymers decreased infarct volume despite their high oxygen affinity. Values are means $\pm$ SE. Adapted from [9].

Fig. 7.

Fractional delivery (ΔY) of bound oxygen for HBOCs with increasing P_{50} values exposed to a partial PO_2 gradient from 100 Torr (at the lung) to 2 Torr (at the peripheral tissue) and with a cooperativity of $n = 1$ and $n = 3$. The classic Hill equation was used for the simulation.

$$Y = \frac{(PO_2)^n}{(P_{50})^n + (PO_2)^n}$$

Table 1

Properties of hemoglobin solutions

	P_{50} (Torr)	Cooperativity n	Concentration (g/dL)	COP (Torr)	Viscosity (cp at 37 °C)	Plasma retention half-time (h)
Fumaryl crosslinked Hb	18	1.7	7	23	0.9	4.3 (rat) 6.1 (cat)
Sebacyl crosslinked Hb	34	2.1	7	24		3.5 (rat) 6.5 (cat)
Hb-Zerolink	4	1	6	2	1.7	7.5 (rat)
Hb Polytaur	18	1.8	12	10	3.1	10 (cat)
Hb (Polytaur)n	3	1	6			3 (mouse)

COP, colloid osmotic pressure (cat plasma=17 Torr).
Hb-Zerolink viscosity at 37 °C estimated from measurements at 23 °C.