Low Oxygen-Affinity Hemoglobin Solution Increases Oxygenation of Partially Ischemic Tissue During Acute Anemia

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BACKGROUND: Maintenance of postsurgical tissue oxygenation depends on the ability of the specific tissue to recruit perfusion and oxygen (O_2) supply. When native O_2-carrying capacity is lacking, fluids to improve O_2-carrying capacity based in hemoglobin (Hb) could prevent partially ischemic tissue hypoxia by increasing O_2 release from the remaining red blood cells.

STUDY DESIGN: Responses to facilitated O_2 transport after exchange transfusion with polymerized bovine Hb (PBH) were studied in a chronic partially ischemic tissue model, induced by large feeding arteriole ligation during hamster window chamber model implantation. PBH effects in microvascular perfusion and tissue oxygenation were studied after exchange transfusion of 40% of animal's blood volume. Experimental groups were defined by the concentration of PBH used, ie, PBH at 13 g/dL (PBH13); PBH at 4 g/dL in albumin solution to matching colloidal osmotic pressure (COP) (PBH4); and no PBH, only albumin solution at matching COP (PBH0).

RESULTS: Restitution of O_2-carrying capacity with PBH13 increased blood pressure and produced vasoconstriction compared with PBH4 and PBH0. On the other hand, PBH4 maintained blood pressure without substantial vasoconstriction, increased tissue partial pressure of O_2, arteriolar O_2 supply, and extraction to the partially ischemic tissue compared with PBH0 and PBH13.

CONCLUSIONS: Results suggest the existence of an optimal concentration of low O_2-affinity acellular Hb to increase oxygenation of partially ischemic tissue during anemic conditions. (J Am Coll Surg 2010;210:271–279. © 2010 by the American College of Surgeons)

Partial ischemia is defined as moderated restriction of blood supply to an organ or tissue. This ischemia induces moderated accumulation of metabolic products and changes in inflammation and permeability, mostly because the oxygenation and energy supply are still provided by the high O_2-carrying capacity in the blood, and blood perfusion is reduced. Although many partially ischemic conditions occur in conjunction with moderated changes in blood O_2-carrying capacity (eg, operation, bleeding, trauma), impeding maintenance of homeostasis and cell function and, if untreated, will result in local tissue necrosis.[1] During surgery, reasons for ischemia are manifold. Soft-tissue trauma, burn injuries, and prolonged immobilization can result in partial-ischemia–related complications. Almost any surgical dissection implies direct vascular trauma, compromising microvascular perfusion.[2] Reduction of the anatomical blood supply secondary to surgery results in areas of tissue with microhemodynamic disturbances, which are prone to ischemic complications and consequently can cause considerable morbidity.

Maintenance of postsurgical tissue oxygenation during acute isovolemic anemia depends on physiologic adjustments occurring at both the systemic and microcirculatory level. Undisturbed tissues compensate for the anemia by increasing perfusion and O_2 extraction. However, the relative contribution of these mechanisms depends on the ability of the specific tissue to recruit each of them. Tolerance to partially ischemic tissue to acute isovolemic anemia will also depend on the level of O_2 demand. The inspired O_2 fraction could also influence tolerance to anemia, but the contribution of O_2 dissolved in the plasma is very limited compared with lung free radical formation and subsequent tissue damage.[3]

Hb-based O_2-carrier (HBOC) blood substitutes are being developed to overcome limitations inherent in blood

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Abbreviations and Acronyms

COP = colloidal osmotic pressure
FCD = functional capillary density
HBOC = Hb-based O_2 carrier
MAP = mean arterial blood pressure
PBH = polymerized bovine Hb
PBH0 = no PBH, only albumin solution at matching COP
PBH4 = PBH at 4 g/dL in albumin solution to matching COP
PBH13 = PBH at 13 g/dL
pO_2 = partial pressure of O_2
PQM = phosphorescence-quenching microscopy

transfusion.[4] It has been amply demonstrated that unmodified Hb cannot be used as HBOC because of its nephrotoxicity, which is partially overcome by Hb polymerization.[5] But polymerized Hb remains vasoactive, an effect attributed to nitric oxide scavenging, extravasation, and vasculature hyperoxygenation.[6] An increase in blood pressure is the most frequent clinical manifestation of vasoconstriction and remains the primary unwanted side effect. HBOCs have been formulated to reproduce O_2-carrying capacity similar to blood, but they tend to increase O_2 release from RBCs to tissues through the process of facilitated oxygen diffusion.

The objective of this study was to identify the role of circulating concentration of low O_2-affinity Hb in oxygenation of partially ischemic tissue during anemic conditions. Partially ischemic tissue was induced by ligation of major feeding arterioles during hamster chamber window model preparation. The partially ischemic hamster chamber window model tissue had a substantial reduction in blood perfusion. Acute anemic conditions were induced by isovolemic hemodilution of 40% of the blood volume, decreasing systemic Hct from 49% to 29% (RBC Hb from 14.8 g/dL to 9.7 g/dL). To achieve this objective, the window model was exchange-transfused with different concentrations of polymerized bovine Hb (PBH, Oxyglobin; Biopure Corporation; approved in the United States and European Union for veterinary use). PBH concentrations tested were 0, 4, and 13 g_{PBH}/dL at matching colloidal osmotic pressure (COP). COP was adjusted with human serum albumin diluted in normal saline.

METHODS

Animal preparation

Investigations were performed in 55- to 65-g male Golden Syrian hamsters (Charles River Laboratories). The hamster window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere.[7,8] Partial ischemia was induced by ligation of major feeding arterioles during dorsal window implantation (Fig. 1A). Arterial and venous catheters filled with a heparinized saline solution (30 IU/mL) were implanted in the carotid and jugular vessels. Catheters were tunneled under the skin, exteriorized at the dorsal side of the neck, and securely attached to the window frame. Animal handling and care followed the *Guide for the Care and Use of Laboratory Animals*.[9] The experimental protocol was approved by the local animal care committee.

Inclusion criteria

The microvasculature was examined 3 to 4 days after the window implantation operation, and only animals passing an established systemic and microcirculatory inclusion criteria were included. Animals were suitable for the experiments if systemic parameters were within normal range, ie, heart rate $>$ 340 beats per minute; mean arterial blood pressure (MAP) $>$ 80 mmHg; systemic Hct $>$ 45%; partial pressure of arterial O_2 $>$ 50 mmHg; and microscopic examination of the tissue in the chamber observed under a $\times$650 magnification did not reveal signs of low perfusion, inflammation, edema, or bleeding. Hamsters are a fossorial species with a lower arterial pressure of O_2 (pO_2), but their microvascular pO_2 distribution in the chamber window model is similar to other rodents.[10]

Acellular Hb solution

PBH is commercially available as Oxyglobin (Biopure Corporation). PBH is produced by nonspecific polymerization with glutaraldehyde (mean molecular weight 200 kDa) in a modified Lactated Ringer's solution. PBH (Oxyglobin) has a concentration of 13 g_{Hb}/dL (COP 38 mmHg, viscosity 1.9 cp). Additional PBH concentrations evaluated were 0 and 4 g_{Hb}/dL. COP of all solution was balanced using human serum albumin diluted in normal saline to 38 mmHg. PBH solution viscosities for 0 and 4 g_{Hb}/dL were 1.2 cp and 1.3 cp, respectively.

Acute isovolemic exchange transfusion (hemodilution) protocol

Before the exchange transfusion, animals were randomly divided into 3 experimental groups.[11] Briefly, the volume of the exchange transfusion was calculated as 40% of blood volume, estimated as 7% of body weight. The acute anemic state was induced by exchanging 40% of the animal blood volume with the test solution. Blood was withdrawn from the carotid artery catheter, and simultaneously the test solution was infused using the jugular vein catheter at the same rate (Fig. 2).