BOVINE HEMOGLOBIN-BASED OXYGEN-CARRYING SOLUTION (HBOC-201) IMPROVES FLAP SURVIVAL IN A RAT MODEL OF EPIGASTRIC FLAP FAILURE

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Despite continued improvements in surgical technique and postoperative management of pedicled flaps, partial flap necrosis continues to be a substantial problem. Several researchers sought interventions that would decrease the incidence of this complication. The hypothesis of this study is that a bovine hemoglobin-based, oxygen-carrying solution (HBOC-201) will increase oxygen delivery, thus decreasing the area of necrosis of the marginally perfused portions of a pedicled flap. Eighty male Sprague-Dawley rats were randomly assigned to one of four groups (20 animals in each group): group 1, controls (surgical creation of flap only); group 2, HBOC-201, 2 g i.v., administered preoperatively and on days 3 and 5; group 3, HBOC-201, 4 g i.v., administered preoperatively and on days 3 and 5; and group 4, hemodilution (lactated Ringer's solution) administered preoperatively and on days 3 and 5. A ventral fasciocutaneous flap (5 × 7 cm) was elevated, based on unilateral superficial inferior epigastric vessels, and the flap was replaced and sutured. Animals were examined daily and euthanized on day 7. Prior to euthanasia, digital photographs were taken of each subject, and the images were analyzed for total area of the flap and area of necrosis, using ImagePro® software. Using the calculated percentage of necrosis for each animal, a mean value of percent necrosis was obtained for each animal group and used for statistical analysis. Animals in group 2 demonstrated a decreased area of necrosis when compared with the control group (9.14% vs. 22.24%, $P = 0.014$). In conclusion, the oxygen therapeutic HBOC-201, at a dose of 2 g, administered preoperatively and on days 3 and 5, decreased the area of necrosis in a rat model of epigastric skin-flap failure. Further investigation of this drug and its effects on flap survival is warranted. © 2006 Wiley-Liss, Inc. Microsurgery 26:203–206, 2006.

Despite continued improvements in surgical technique and postoperative management of pedicled flaps, partial flap necrosis continues to be a substantial problem in plastic and reconstructive surgery. Partial flap loss has been reported in as many as 10–31% of pedicled musculocutaneous flaps used for head and neck reconstructions.[1,2]

Pharmaceutical interventions have been sought to reduce the degree of reperfusion injury, increase vasoactivity, and alter the rheology of blood.[3–8] However, few studies to date have been conducted to determine the effects of oxygen therapeutic drugs on flap survival. In the 1980s, Ramasastry et al. studied the effects of the perfluorocarbon Fluosol-DA (20%), and found that it did not alter flap survival in a model of epigatric skin-flap failure; they attributed its failure as a treatment regimen to increased blood viscosity in animals treated with Fluosol-DA.[9]

A new class of oxygen therapeutic has been developed, based on polymerized hemoglobin. HBOC-201 (Hemopure®, Biopure Corp., Cambridge, MA) is an ultrapurified polymerized bovine hemoglobin-based oxygen-carrier in modified lactated Ringer's solution (Table 1). This drug has undergone phase III clinical trials for the treatment of perioperative anemia, and currently has a biological license application under review by the FDA.

HBOC-201 can be stored at room temperature, and has a shelf life of 2 years. This product requires no cross-match, and does not appear to elicit an immune response.[10] This product has some physiologic properties that could benefit patients who have had surgical flaps. Specifically, it has a lower half-saturation point P_{50} than native hemoglobin, thus delivering oxygen at a more efficient rate than native hemoglobin. Additionally, it has a decreased viscosity which could positively alter blood rheology to improve flap survival. Finally, HBOC-201 may act as an oxygen bridge, delivering oxygen without the need for red blood cells. These properties led us to hypothesize that HBOC-201 would improve flap survival in our rat model of epigastric pedicled flap failure. Previous studies demonstrated that the common causes for flap failure are venous congestion and arterial inflow obstruction. Both of these causes have similar pathways of decreased tissue oxygenation, resulting in cell death.

HBOC-201 has proved capable of delivering oxygen to critically ischemic tissue. Recent studies showed that the bovine hemoglobin-based, oxygen-carrying solution Hemopure® (HBOC-201) increased skeletal muscle oxygenation in a model of critical arterial stenosis.[11] It was also shown to be an effective low-volume resuscitation fluid in a porcine model of controlled hemorrhage/resuscitation.[12] We believe that HBOC-201 increases tissue oxygenation to areas of the flap with marginal perfusion, thus leading to decreased cell death and necrosis.

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Table 1. Properties of Oxygen Therapeutic HBOC-201*

Characteristic	Value
Hemoglobin concentration (g/dL)	13
Size distribution	
<65 kD	<5%
128 kD	17%
>128 kD	80%
P_{50} (mm Hg)	43
Colloid oncotic pressure (mm Hg)	25
$t^{1/2}$	19 h
Osmolarity (mOsm/L)	300
Electrolytes (mmol/L)	
NaCl	115
KCl	4
$CaCl_2$	1.4
NaOH	13
Sodium lactate	27
N-acetyl-L-cysteine (mg/dL)	200
Free glutaraldehyde (μg/dL)	<3.5
Endotoxin (EU/mL)	<0.05

*Supplied by Biopure Corp. (Cambridge, MA). $t^{1/2}$, half life.

METHODS

Study Groups

This protocol was approved by the Wilford Hall Medical Center Animal Care and Use Committee. All animals used in this study were handled according to the *Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Research, National Research Council*. All animals were housed in individual cages and given water and standard chow ad libitum. After a 7-day observation period, the animals underwent the surgical procedure and injections, as determined by group. Eighty male Sprague-Dawley rats were randomly assigned to one of four groups, all of which had surgically created epigastrc skin flaps. Group 1 ($n = 20$) served as control animals. These animals underwent the surgical creation of an epigastric skin flap, and had no further interventions. Group 2, HBOC-1 ($n = 20$), received 2 g of HBOC-201 (Hemopure®, Biopure Corp.) per tail vein injection preoperatively, and then on postoperative days 3 and 5. Group 3, HBOC-2 ($n = 20$), was administered 4 g of HBOC-201 per tail vein injection preoperatively and on postoperative days 3 and 5. Finally, group 4, LR ($n = 20$), received intravenous injections of 3.5 cc of lactated Ringer's solution per tail vein preoperatively and on postoperative days 3 and 5. This volume was chosen to correspond to the highest volume of HBOC-201 given, and served as a hemodilution control.

Surgical Procedure

All animals had the same operative procedure performed by one of two members of the research team. All animals were anesthetized with intraperitoneal injections of an acepromazine and ketamine mixture (ketamine 75 mg/kg, and acepromazine 2.5 mg/kg). The flap was raised in a cranial-to-caudad direction, with identification of bilateral superficial inferior epigastric arteries at the epigastric/femoral artery junction. The right superficial inferior epigastric bundle was ligated and divided. The left superficial inferior epigastric bundle was examined, and the left lateral branch of the superficial inferior epigastric artery was ligated and excluded from the flap, leaving only the medial braches of the left superficial inferior epigastric artery.[13] Flaps were not exposed to any period of ischemia. Finally, the flap was replaced and secured with running 6-0 nylon. Postoperatively, animals recovered and received buprinex as needed for pain.

Flap Evaluation

Upon completion of a 7-day observation period, all animals were euthanized via CO_2 asphyxiation, and digital images were taken of each animal. All digital images were analyzed by blinded observers using Image Pro® software (version 4.1 for Windows NT). The software was used to calculate total flap area (cm^2) and area of flap necrosis. Using these data, the percentage of flap necrosis for each animal was then calculated, using the following formula:

$$\text{Percent necrosis} = \frac{\text{Area of necrosis}}{\text{Total area of flap}} \times 100.$$

Using the calculated percentage of flap necrosis for each animal, a mean value for percent necrosis was obtained for each animal group and used for statistical analysis.

Statistical Analysis

Statistical analysis of the percent necrosis data was initially completed by one-way analysis of variance (ANOVA), and followed by Tukey's test for multiple comparisons of groups, using GraphPad InStat version 3.05 for Windows 95/NT (GraphPad Software, San Diego, CA). These data are presented as mean values ± standard error of the mean (SEM). Significance was defined as $P < 0.05$.

RESULTS

Despite adequate analgesia and individual housing, several subjects were excluded from analysis secondary to autocanibalism of the surgical flap. One subject from the control group and two animals from group 2 were excluded. Several animals demonstrated varying degrees of autocanibalization of nonflap areas, and thus remained in the study.

Animals in group 2 demonstrated less necrosis of flaps when compared to the control and LR groups. Animals in the control group had a mean percent flap necrosis of 22.24%, while the area of necrosis for the group receiving the 2 g injection of HBOC-201 was significantly decreased

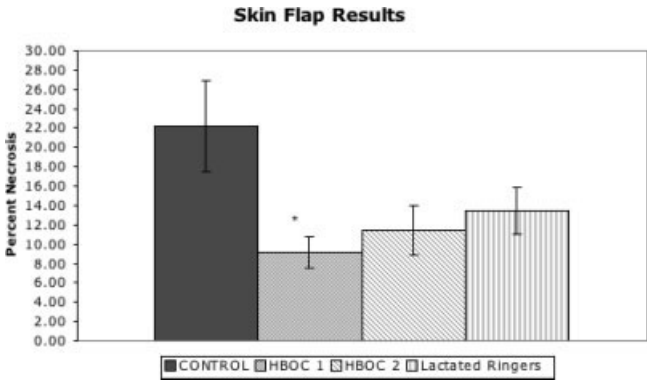

Figure 1. Total area of flap necrosis for each group. Animals in both HBOC-201 groups demonstrated less percent necrosis than control and LR groups. Animals in control group had mean percent flap necrosis of 22.24%, while area of necrosis for HBOC-1 (group 2) and HBOC-2 (group 3) was 9.14% (P = 0.014 vs. control) and 12.22%. While animals in HBOC-2 (group 3) and LR (group 4) demonstrated decreased percent necrosis vs. controls, differences were not statistically significant (P = 0.061 and P = 0.096, respectively).

(9.14%; $P = 0.014$). Animals in groups 3 and 4 demonstrated decrease percent necrosis when compared to controls. However, the differences were not statistically significant (group 3, 11.43%, $P = 0.061$; group 4, 13.45%; $P = 0.096$) (Fig. 1). When comparisons were made between group 2 and groups 3 and 4, no statistical differences were noted (9.14% vs. 11.43% and 13.45%, $P = 0.446$ and $P = 0.197$, respectively).

DISCUSSION

This preliminary study of the oxygen therapeutic HBOC-201 (Hemopure®) sought to determine whether the administration of this agent could alter flap survival in a model of planned epigastric pedicled-flap failure. This product consists of polymerized bovine hemoglobin in a modified lactated Ringer's solution (Table 1). Previous studies showed that this product more efficiently delivered oxygen than native hemoglobin. Hughes et al.[14] found that subjects given HBOC-201 after isovolemic hemodilution had increased diffusion capacity (up to 34% greater than controls). This increased oxygen diffusion was attributed to the properties of this bovine hemoglobin preparation. Specifically, the P_{50} of HBOC-201 is greater than that of human hemoglobin (32 vs. 26), which promotes the unloading of oxygen. Additionally, the bovine hemoglobin molecule has more pronounced Haldane (CO_2) and Bohr (pH) effects, which also contribute to more efficient offloading of oxygen.[14] Similarly, Page et al., in an in vitro model, demonstrated that HBOC-201 had a greater ability to extract oxygen than erythrocyte hemoglobin.[15]

HBOC-201 also has a viscosity less than that of whole blood (1.3 vs. 3.6 centipose at 37°C), which may change the rheology of blood in a manner beneficial to the flow dynamics of flaps. In addition, studies showed that subjects resuscitated with HBOC-201 reversed markers of anaerobic metabolism at significantly smaller volumes than traditional resuscitation fluids.[12] These enhanced oxygen transport properties and ability to reverse the markers of anaerobic metabolism led us to hypothesize that this compound could improve flap survival by augmenting tissue oxygenation.

A possible mechanism for our demonstrated improvement in flap survival is suggested by the work of Horn et al.[11] Horn demonstrated increased skeletal muscle oxygenation in animals with $> 95\%$ arterial stenosis, and attributed this to increased oxygen extraction in poststenotic tissues. Our model did not contain arterial stenosis, but did have areas of interrupted flow, at the capillary level. These areas were found in the most distal portions of flaps. We hypothesize that HBOC-201 improves oxygen delivery at areas of capillary disruption and decreased blood flow by delivering oxygen via a much smaller vehicle than erythrocytes. This vehicle is without the three-dimensional constraints of erythrocyte, and is thus able to travel to more distal areas of the flap and deliver oxygen. However, we did not investigate peripheral flap-tissue oxygenation in this preliminary study.

This product could potentially have many uses in plastic and reconstructive surgery. For example, its properties of increased oxygen extraction/delivery could be advantageous in treating threatened flap failures. It could decrease the rate of transfusions and the deleterious effects of transfusion. Clinical trials in the use of these products for perioperative anemia demonstrated decreased allogeneic transfusion requirements in subjects treated with HBOCs, and thus may greatly impact the care of patients in many specialties.

This study demonstrated that the administration of oxygen therapeutic HBOC-201 decreased areas of necrosis at a dose of 2 g i.v., administered preoperatively and on days 3 and 5, in a rat model of epigastric flap failure. It appears that higher doses of HBOC-201 are not necessary to achieve this effect. The group exposed to the higher dosage of HBOC-201 demonstrated a decreased area of necrosis when compared to controls, though this was not statistically significant. This could represent a potentially toxic effect of HBOC-201 at higher doses. In a recent review of the toxicities of hemoglobin solutions, Buehler and Alayash discussed the various toxicities of these compounds.[16] The deleterious effects of early HBOCs are well-documented. The early use of diaspirin cross-linked hemoglobin was associated with increased systemic and pulmonary vasoconstriction, oxidative stress, and renal impairment.[16] However, the product used in this study represents several generations of HBOC improvement. As

stated earlier, it is highly purified and was shown to be a rather effective resuscitation fluid in the treatment of shock in swine.[12] Additionally, concerns about the effects of hemoglobin toxicities on neural tissue were investigated in our laboratory and deemed to be unfounded.[17] As elucidated by Buehler and Alayash,[16] it is quite difficult to accurately determine the effects and toxicity of this group of drugs, as HBOCs represent a variable class of therapeutic agents. Our study was limited by the size of the groups and an endpoint which was macroscopic, thus limiting us to determining relatively large variations between groups. This study is clearly a primer for additional studies. For example, more investigation into the optimal dosage of this product in regard to flap augmentation must be done. Similarly, the use of this product in cases of threatened or partial flap loss, and its potential as an adjunct in the scenario of flap salvage, should be elucidated. Further investigation of this drug and its effects on flap survival is warranted.

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