Effect of oxygen affinity and molecular weight of HBOCs on cerebral oxygenation and blood pressure in rats

[L'effet de l'affinité pour l'oxygène et du poids moléculaire des TOBH sur l'oxygénation et la tension artérielle chez les rats]

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Purpose: This study assessed the effect of oxygen affinity and molecular weight (MW) of o-raffinose cross-linked hemoglobin based oxygen carriers (HBOCs) on cerebral oxygen delivery and mean arterial blood pressure (MAP) following hemorrhage and resuscitation in rats.

Methods: Isoflurane anesthetized rats (n = 6–7 per group) underwent 30% hemorrhage and resuscitation with an equivalent volume of one of three different HBOCs: 1) High P_{50} Poly o-raffinose hemoglobin (Poly OR-Hb, P_{50} = 70 mmHg); 2) High P_{50} > 128 Poly OR-Hb (MW > 128 kDa, P_{50} = 70 mmHg) and 3) Low P_{50} > 128 Poly OR-Hb (MW >128 kDa, P_{50} = 11 mmHg). Hippocampal cerebral tissue oxygen tension, regional cerebral blood flow (rCBF), MAP, total hemoglobin concentration and arterial blood gases were measured. Data analysis by two-way ANOVA and *post hoc* Tukey tests determined significance ($P < 0.05$, mean ± SD).

Results: Hippocampal tissue oxygen tension increased in all HBOC groups following resuscitation. The rCBF remained unchanged after HBOC resuscitation in all groups. Following resuscitation, the peak MAP was higher in the High P_{50} Poly OR-Hb group (152 ± 13 mmHg) when compared to either the Low or High P_{50} large MW, (> 128 kDa) HBOC group (119 ± 15 mmHg or 127 ± 18 respectively, $P < 0.05$ for both).

Conclusions: O-raffinose polymerized HBOC, with or without lower MW components, maintained cerebral tissue oxygen delivery following hemorrhage and resuscitation in rats. The higher MW HBOCs showed a decrease in peak MAP, which did not alter oxygen delivery. No significant effect of oxygen affinity on cerebral tissue oxygen tension or blood flow was observed.

Objectif *: Évaluer l'effet de l'affinité pour l'oxygène et du poids moléculaire (PM) des transporteurs d'oxygène à base d'hémoglobine (TOBH) avec o-raffinose sur l'apport d'oxygène cérébral et la tension artérielle moyenne (TAM) après une hémorragie et une réanimation chez des rats.*

Méthode *: Des rats anesthésiés à l'isoflurane (n = 6–7 par groupe) ont subi une hémorragie à 30% et une réanimation avec un volume équivalent de l'un des trois différents TOBH suivants : 1) de l'hémoglobine Poly o-raffinose à P_{50} élevé (Poly OR-Hb, P_{50} = 70 mmHg) ; 2) 128 Poly OR-Hb à P_{50} élevé (PM > 128 kDa, P_{50} = 70 mmHg) et 3) 128 Poly OR-Hb à faible P_{50} (PM >128 kDa, P_{50} = 11 mmHg). La tension en oxygène du tissu cérébral hippocampique, le débit sanguin cérébral régional (DSCr), la TAM, la concentration d'hémoglobine totale et la gazométrie du sang artériel ont été mesurés. L'analyse de données, par double ANOVA et tests de Tukey ultérieur, ont permis de déterminer la valeur significative ($P < 0,05$, moyenne ± SD).*

Résultats *: La tension en oxygène du tissu hippocampique s'est accrue dans tous les groupes de TOBH après la réanimation. Le DSCr est resté le même dans tous les groupes après la réanimation avec les TOBH. Après la réanimation, la TAM était plus élevée dans le groupe Poly OR-Hb à P_{50} élevé (152 ± 13 mmHg) comparé au groupe de TOBH de P_{50} élevé et de grand PM, (> 128 kDa) (119 ± 15 mmHg ou 127 ± 18 respectivement, $P < 0,05$ pour les deux).*

Conclusion *: Les TOBH polymérisés avec o-raffinose, avec ou sans composants de faible PM, ont maintenu l'apport d'oxygène au tissu cérébral après une hémorragie et une réanimation chez des rats. Les TOBH de PM élevé ont montré une baisse de la TAM qui n'a*

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pas nui à l'apport d'oxygène. Aucun effet significatif de l'affinité pour l'oxygène sur la tension en oxygène du tissu cérébral ou sur le débit sanguin n'a été observé.

ANEMIA and acute blood loss have been associated with increased morbidity and mortality in perioperative patients.[1–3] Increased mortality has also been associated with transfusion of allogeneic blood, one of the main therapies for resuscitating patients who have experienced severe blood loss.[2] Availability of a safe and efficacious red blood cell substitute could minimize morbidity and mortality associated with acute blood loss and alleviate adverse outcomes for which allogeneic blood transfusion is found to be an independent predictor.[2,4] Currently, no blood substitute has been approved for clinical use in North America.

Hemoglobin based oxygen carriers (HBOCs) are one form of red blood cell substitute currently under development. Hemoglobin based oxygen carriers are derived from chemically modified hemoglobins purified from human or animal blood. The predominant modifications are aimed at enlarging the molecular size, extending their intravascular half-life and preventing their extravasation. A number of different products have undergone clinical trials.[5–9] These products may have associated adverse effects, which include vasoconstriction and/or vasopressor effects. The increase in systemic vasoconstriction has been attributed to increased activity of vasoconstricting molecules including endothelin,[10,11] and catecholamines[12] and/or binding of vasodilatory molecules such as nitric oxide by HBOCs.[13,14] The direct vasoactive effect of different HBOCs appears to be related to the low molecular weight (MW) species in these products. This suggests that small HBOC molecules are more vasoconstricting.[15–17] Presumably, pronounced vasoconstriction would be disadvantageous because it could limit optimal delivery of oxygen to tissues.

In addition, debate exists as to the optimum oxygen affinity of HBOCs. A number of experimental studies have attempted to determine which oxygen affinity may provide superior tissue oxygen delivery. Some investigators argue that an HBOC possessing a higher oxygen affinity (Low P_{50}), would limit excessive tissue oxygen delivery and therefore, minimize hyperoxic vasoconstriction.[15,18–20] Conversely, a lower oxygen affinity (High P_{50}) might ensure optimal ease of oxygen release into the tissue.[21]

The current study was designed to assess the effect of oxygen affinity and MW of one HBOC on cerebral oxygen delivery in a 30% hemorrhage and resuscitation model in anesthetized rats. This model was designed to approximate moderate hemorrhage and resuscitation in the operating room. Changes in blood pressure and cerebral tissue oxygen tension ($P_{Br}O_2$) and regional cerebral blood flow (rCBF) were the primary endpoints of interest.

Methods

Animal model

All animal protocols were approved by the Animal Care and Use Committee at St. Michael's Hospital in accordance with the requirements of the Canadian Council on Animal Care. Anesthesia was induced in male Sprague-Dawley rats (Charles River, St. Constant, PQ, Canada), with ketamine/xylazine 100/7.5 $mg \cdot kg^{-1}$ intraperitoneally (Parke-Davis/Bayer, Toronto, ON, Canada) and maintained with 1%–2% isoflurane (Abbott, St. Laurent, PQ, Canada) delivered in 50% oxygen after intubation. Ventilation was maintained with a pressure-controlled ventilator (Kent Scientific, Litchfield, CT, USA) and was adjusted to achieve normocapnia and normoxia as determined by blood gas analysis (Radiometer ALB 500; London Scientific, London, ON, Canada). The tail artery and vein were cannulated using 24-G catheters to provide vascular access for direct measurement of mean arterial blood pressure (MAP) and blood gases, and to perform hemorrhage and resuscitation. Animals were then placed in a stereotaxic frame (ADI Instruments; Harvard Apparatus, St-Laurent, PQ, Canada), and the scalp incised sagittally. A 5-mm diameter burr hole was performed at the level of the bregma, 2–3 mm lateral to the sagital sinus, exposing the intact dura.

Combination oxygen sensing microelectrodes, temperature and laser Doppler flow probes (OxyLite and OxyFlow, Oxford Optronix, Oxford, UK) were placed 3–4 mm past the dura into the region of the hippocampus in order to measure both $P_{Br}O_2$ and rCBF. A period of 20 min was used to establish a stable baseline while a heating pad and lamp were used to maintain the brain temperature near 35°C. This degree of systemic hypothermia was maintained to approximate operating room conditions associated with acute blood loss and crystalloid resuscitation. Brain temperature, $P_{Br}O_2$, rCBF and MAP were recorded with a computerized data acquisition system (DASYLab 5.6; Kent Scientific).

TABLE I Characteristics of hemoglobin based oxygen carriers (HBOC)

Sample	*P_{50} (mmHg)*	*Viscosity (cSt)*	*pH*	*P_aCO_2 (mmHg)*	*P_aO_2 (mmHg)*	*Hemoglobin ($g \cdot L^{-1}$)*	*% Oxygen saturation*	*MetHb (%)*	*O_2 Content ($mmol \cdot L^{-1}$)*
High P_{50} Poly OR-Hb	70	1.2	7.31 ± 0.05	1.3 ± 0.8	209.8 ± 84.4†	102 ± 2†‡	79.4 ± 6.1‡	5.2 ± 0.6†‡	4.7 ± 0.4†‡
High P_{50} > 128 Poly OR-Hb	70	2.7	7.25 ± 0.01‡	1.8 ± 0.4	346.8 ± 91.49*	143.3 ± 1.4*	82.7 ± 2.1‡	10.9 ± 0.7*‡	6.3 ± 0.3*
Low P_{50} > 128 Poly OR-Hb	11	2.4	7.40 ± 0.09†	3.9 ± 5.8	297.79 ± 31.50	141.6 ± 2.9*	91.2 ± 0.4*†	7.2 ± 0.6*†	7.2 ± 0.2*

*$P < 0.05$ *vs* High P_{50} Poly OR-Hb; †$P < 0.05$ *vs* High P_{50} > 128 Poly OR-Hb; ‡$P < 0.05$ *vs* Low P_{50} > 128 Poly OR-Hb. For definitions, please refer to text.

TABLE II Arterial blood gas and co-oximetry data following hemorrhage and HBOC resuscitation

Time	*Sample*	*pH*	*P_aCO_2 (mmHg)*	*P_aO_2 (mmHg)*	*Hemoglobin ($g \cdot L^{-1}$)*	*% Saturation*	*MetHb (%)*	*O_2 Content ($mmol \cdot L^{-1}$)*
High P_{50} Poly OR-Hb								
10 min	Baseline	7.41 ± 0.06	35.5 ± 6.5	145.6 ± 16.5	127.3 ± 10.4	99.9 ± 2.7	0.8 ± 0.2	7.6 ± 0.8
30 min	Hemorrhage	7.36 ± 0.06	32.7 ± 7.9	146.8 ± 32.9	106.5 ± 9.6*	99.4 ± 0.9	0.9 ± 0.3	6.4 ± 0.6*
60 min	Resuscitation	7.40 ± 0.06	39.8 ± 5.3	194.7 ± 47.3*	121.2 ± 11.5	94.7 ± 3.8*	2.1 ± 0.5*	6.9 ± 0.9*
90 min	Resuscitation	7.44 ± 0.05	36.9 ± 7.8	189.5 ± 41.6*	118.5 ± 5.1	94.9 ± 4.3*	2.4 ± 0.9*	6.6 ± 0.4*
High P_{50} > 128 Poly OR-Hb								
10 min	Baseline	7.36 ± 0.02	44.3 ± 4.34	166.2 ± 12.6	126.5 ± 2.3	100.0 ± 0.0	1.1 ± 0.1	7.6 ± 0.1
30 min	Hemorrhage	7.36 ± 0.03	35.4 ± 3.1	183.7 ± 25.4	101.0 ± 13.0*	98.8 ± 3.0	0.9 ± 0.4	5.9 ± 1.0*
60 min	Resuscitation	7.37 ± 0.04	42.0 ± 4.6	254.2 ± 50.8*†	125.5 ± 10.2	95.2 ± 1.3*	4.5 ± 0.6*†§	6.9 ± 0.6
90 min	Resuscitation	7.40 ± 0.04	37.7 ± 5.1	243.8 ± 59.2*	121.8 ± 19.5	95.3 ± 1.8*	4.8 ± 1.3*†§	6.7 ± 1.2
Low P_{50} > 128 Poly OR-Hb								
10 min	Baseline	7.42 ± 0.04	37.2 ± 7.2	196.6 ± 38.0	125.5 ± 9.5	100.0 ± 0.0	1.1 ± 0.1	7.5 ± 0.6
30 min	Hemorrhage	7.39 ± 0.06	33.9 ± 11.3	185.5 ± 31.9	109.0 ± 6.9*	100.0 ± 0.0	1.0 ± 0.2	6.5 ± 0.4*
60 min	Resuscitation	7.39 ± 0.05	40.0 ± 8.1	234.7 ± 41.7	122.6 ± 7.7	98.5 ± 0.7*†‡	3.3 ± 0.6*†‡	7.1 ± 0.4
90 min	Resuscitation	7.43 ± 0.04	35.6 ± 6.8	230.6 ± 48.6	118.6 ± 13.5	98.8 ± 0.7*†‡	3.6 ± 0.9*†‡	6.8 ± 0.8*

*$P < 0.05$ compared to baseline within each group; †$P < 0.05$ *vs* High P_{50} Poly OR-Hb, at the same sample time; ‡$P < 0.05$ *vs* High P_{50} > 128 Poly OR-Hb, at the same sample time; §$P < 0.05$ *vs* Low P_{50} > 128 PolyOR-Hb, at the same sample time.

Hemoglobin based oxygen carriers

Three different HBOCs were prepared by a process of pasteurization, chromatographic purification, viral filtration, and o-raffinose cross-linking of hemoglobin from out-dated human blood collected from Food and Drug Administration-approved collection sites (> 42 days from donation). Characteristics of these HBOCs are outlined in Table I. The first HBOC consisted of polymeric o-raffinose cross-linked hemoglobin raffimer (Poly OR-Hb) with a heterogeneous MW composition (~ 55% ≤ 128 kDa) and a High P_{50} (low oxygen affinity), (High P_{50} Poly OR-Hb, P_{50} = 70 mmHg).[22] The other two HBOCs consisted of Poly OR-Hb prepared essentially entirely with a MW of > 128 kDa (5–10% ≤ 128 kDa). Two different HBOCs were utilized, one which had a low oxygen affinity (High P_{50} > 128 Poly OR-Hb, MW > 128 kDa, P_{50} = 70 mmHg) and one which had a high oxygen affinity (Low P_{50} > 128 Poly OR-Hb, MW >128 kDa, P_{50} = 11 mmHg). The MW distributions of the > 128 kDa HBOC products were similar.

Hemorrhage and resuscitation protocol

Three different groups of animals were studied in which one of the three HBOC solutions was used for resuscitation (n = 6–7 rats per group). Each group underwent a controlled hemorrhage of 30% of the estimated blood volume at a constant rate over a period of ten minutes by using a programmable "push-pull" pump (PHD 2000; Harvard Apparatus). After 30 min of hypovolemia, each animal was resuscitated with an equal volume of High P_{50} Poly OR-Hb, High P_{50} > 128 Poly OR-Hb or Low P_{50} > 128 Poly OR-Hb infused over ten minutes. After completion of fluid resuscitation, all variables were recorded for an additional 90 min before the animal was sacrificed by anesthetic overdose (ketamine 100 mg *iv*; Parke-Davis). For each group, arterial blood gas analysis

(Radiometer ALB 500) and co-oximetry (Radiometer OSM 3, London Scientific) were measured at baseline (ten minutes), after hemorrhage (30 min) and after fluid resuscitation (60 and 90 min). Brain temperature, $P_{Br}O_2$, and rCBF measurements from the probe were recorded at 30-sec intervals for each experimental animal.

Data analysis

Individual laser Doppler flowmetry measurements were normalized to the baseline values derived from averaging the data over the first 20 min. All data were assessed with SAS (SAS Institute Inc., Cary, NC, USA). Data were analyzed using a two-way analysis of variance (ANOVA) at ten, 30, 60 and 90 min and post-hoc Tukey tests were performed when appropriate. A one-way ANOVA was utilized to compare the maximum blood pressure response to HBOC resuscitation between the three treatments. All data are expressed as mean ± SD and significance assigned at a value of $P < 0.05$.

Results

Poly-hemoglobin raffimer solutions

Prior to infusion into the animals, all HBOC solutions were analyzed and found to be within the acceptable specifications. Hemoglobin based oxygen carriers were iso-oncotic with plasma. The hemoglobin concentration and calculated oxygen content of both high MW hemoglobin solutions were comparable, but significantly higher than the HBOC containing low MW components ((High P_{50} Poly OR-Hb) $P < 0.05$, Table I). The oxygen saturation of the Low P_{50} HBOC (91.2 ± 0.4%) was higher than that of both High P_{50} solutions (82.7 ± 2.1 and 79.4 ± 6.1%, $P < 0.05$ for both, Table I). The initial methemoglobin concentration for each HBOC solution ranged from 5.2 ± 0.6 to 10.9 ± 0.7% (Table I). The pH of High P_{50} > 128 Poly OR-Hb was slightly lower that of the Low P_{50} > 128 Poly OR-Hb solution (7.25 ± 0.01 *vs* 7.40 ± 0.09, $P < 0.05$, Table I). The P_aO_2 of the High P_{50} > 128 Poly OR-Hb was significantly higher than that of HBOC containing low MW components. The Low and High P_{50} > 128 Poly OR-Hb solutions had higher viscosity than High P_{50} Poly OR-Hb (Table I). All other initial measured values did not differ between groups.

Hemorrhage resuscitation experiments

No significant differences were found between baseline blood gas or co-oximetry values for any of the three experimental groups at baseline (Table II). Arterial blood gas analysis demonstrated no difference within or between groups with respect to pH and P_aCO_2 at any time (Table II). A significantly higher P_aO_2 was observed at 60 min in the group resuscitated with High P_{50} > 128 Poly OR-Hb, relative to the High P_{50} Poly OR-Hb (Table II, $P < 0.05$). Following hemorrhage, there was a comparable reduction in hemoglobin concentration in all groups, relative to baseline (Table II, $P < 0.05$). Following resuscitation, the hemoglobin concentration returned to baseline in all groups without any significant differences between groups (Table II). Arterial blood oxygen content paralleled the hemoglobin concentration values. There were no differences in oxygen content between groups at any time. Oxygen saturation decreased significantly in all groups following HBOC resuscitation. The post-resuscitation oxygen saturation values were higher in the Low P_{50} > 128 Poly OR-Hb group relative to both High P_{50} HBOC groups ($P < 0.05$ for both, Table II). Methemoglobin concentrations increased significantly in all groups following resuscitation. The methemoglobin levels were highest in the High P_{50} > 128 Poly OR-Hb group (4.8 ± 1.3%), next highest in the Low P_{50} > 128 Poly OR-Hb group (3.6 ± 0.9%) and lowest in the High P_{50} Poly OR-Hb group (2.4 ± 0.9 %), (Table II). These relative values corresponded to the differences in starting methemoglobin concentrations in the different HBOC solutions (Table I).

No differences in brain temperatures were recorded between groups at any time. After HBOC resuscitation, brain temperatures were 33.3 ± 1.2, 34.3 ± 0.6 and 34.1 ± 0.7°C in the low MW, and either high MW HBOC groups, respectively. Baseline MAP values were not different between groups (81.5 ± 5.0, 76.2 ± 9.5 and 70.4 ± 12.9 mmHg). The peak MAP was significantly higher in the High P_{50} Poly OR-Hb (152 ± 13 mmHg) when compared to either high MW HBOC groups (119 ± 15 and 127 ± 18 mmHg, Low and High P_{50} > 128 Poly OR-Hb respectively; $P < 0.05$, Figure). After the peak, MAP values remained elevated in all groups relative to baseline ($P < 0.05$, Figure). Following 30% hemorrhage, there was a transient drop in tissue oxygen tension and rCBF for all groups. These values all recovered to baseline levels prior to fluid resuscitation with HBOC. In all groups, the hippocampal tissue oxygen tension increased above baseline values after resuscitation, but no significant differences were observed between groups. Following resuscitation, the rCBF remained near baseline in all groups without any significant differences between groups.

Discussion

This study demonstrated the maintenance of adequate

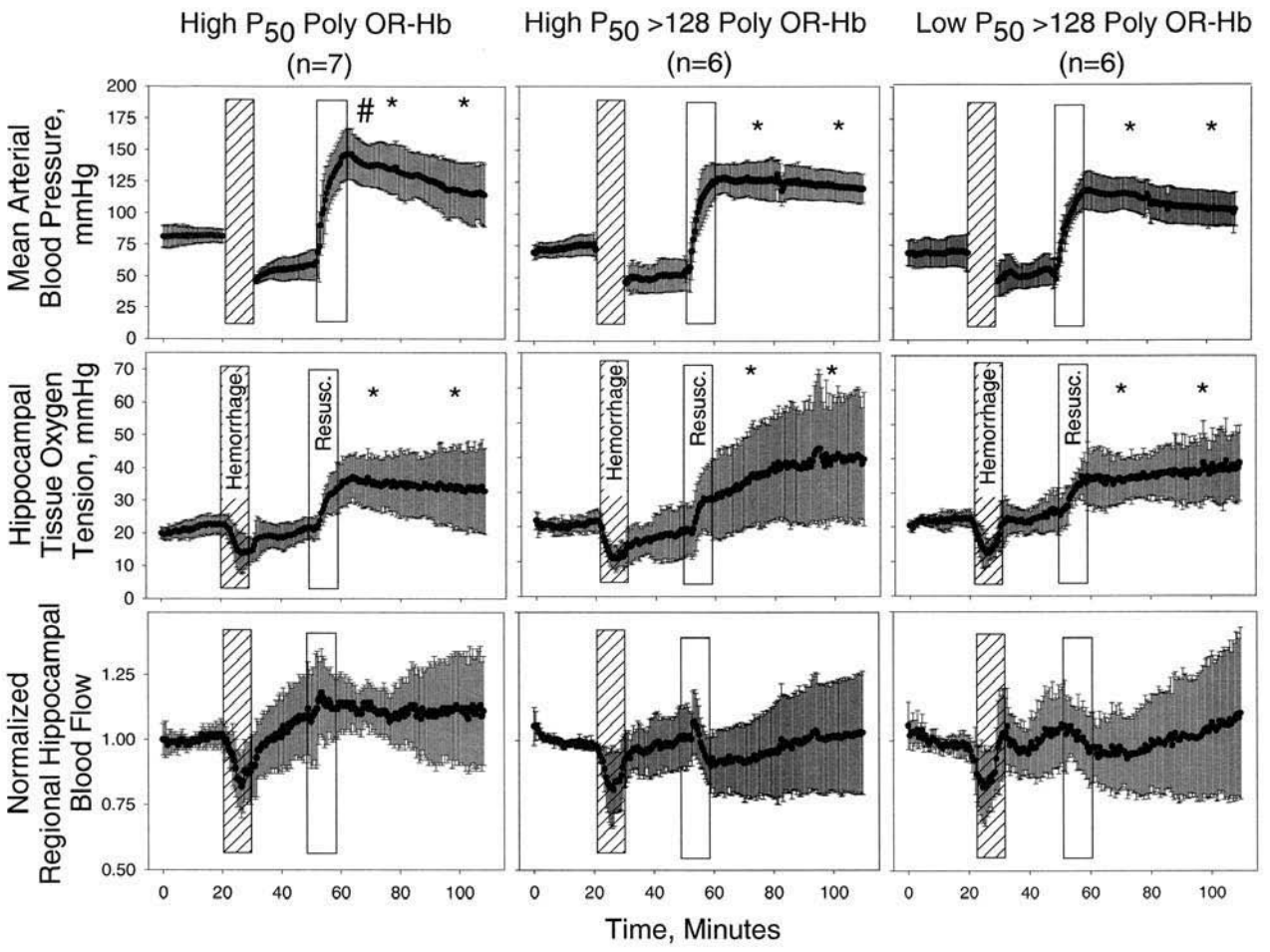

FIGURE Data from all animals for mean arterial blood pressure (MAP), cerebral tissue oxygen tension and regional cerebral blood flow, presented as mean ± SD, before during and after hemorrhage (hatched box) and hemoglobin based oxygen carrier (HBOC) resuscitation (open box). The peak MAP was greater in the High P_{50} Poly o-raffinose hemoglobin (Poly OR-Hb) group when compared to both High and Low P_{50} > 128 Poly OR-Hb groups. After HBOC resuscitation hippocampal tissue oxygen tension increased relative to baseline in all three HBOC groups. The regional hippocampal blood flow was not different from baseline after resuscitation with all three HBOCs. #$P < 0.05$ between groups; *$P < 0.05$ compared to baseline.

local $P_{Br}O_2$ following hemorrhage and resuscitation with o-raffinose polymerized hemoglobin, in the presence of a systemic hypertensive response. Neither the MW nor oxygen affinity of Poly OR-Hb affected $P_{Br}O_2$. However, a reduction in peak MAP was associated with an increase in the HBOC MW. This reduction in vasopressor effect occurred despite a higher initial hemoglobin concentration and higher viscosity in the high MW HBOC groups. These data suggest that the lower MW components of Poly OR-Hb contribute to the vasopressor effect associated with HBOC infusion, but do not account for all vasoactivity of these HBOCs. Despite the effect on MAP, $P_{Br}O_2$ increased comparably following resuscitation with all HBOC compounds. Therefore, cerebral tissue oxygen delivery was maintained in all treatment groups.

In this study, the oxygen affinity of the HBOC solutions did not significantly affect cerebral tissue oxygen delivery. Therefore, no advantage of manipulating P_{50}, within this range, was identified. The increase in $P_{Br}O_2$ was achieved without any increase in rCBF for all HBOC solutions suggesting that the ability of each HBOC to deliver oxygen to the brain was not impaired. Similar results have previously been measured following normovolemic hemodilution with an HBOC.[23] In that study, hemodilution with pen-

tastarch resulted in a significant increase in rCBF with no increase in $P_{Br}O_2$, whereas with HBOC hemodilution, $P_{Br}O_2$ increased with no change in rCBF. This balance of maintained cerebral oxygen delivery without cerebral hyperemia could be beneficial in limiting reperfusion injury and in other clinical settings in which increases in cerebral blood flow may be potentially harmful.

The increase in systemic blood pressure after HBOC administration has been attributed to increased activity of vasoconstricting molecules including endothelin,[11,24] and catecholamines,[12] and/or binding of vasodilatory molecules such as nitric oxide by HBOCs.[13,14,25] The increase in vascular tone could also be a direct compensatory effect of increased oxygen delivery to tissues at the microvascular level. In addition to direct vasoconstriction effect, the increase in blood pressure could also be due to an increase in intravascular volume secondary to increased colloid oncotic pressure. However, this effect should have been uniform, as all tested HBOCs had similar colloid osmotic pressures. The direct vasoactive effect of different HBOCs appears to be inversely related to their MW which suggests that small molecules are more vasoconstricting.[15–17] Clinical and experimental studies suggest that the order of vasoreactivity is highest for the smaller MW HBOCs including diaspirin cross-linked hemoglobin, moderate for polymerized hemoglobins which contain a portion of small MW hemoglobins, and lowest for surface modified hemoglobins that have higher MWs.[26] The current study supports this hypothesis within a single group of chemically modified HBOCs.

This study was designed to compare the effects of three different HBOCs on cerebral oxygen delivery. A direct comparison of resuscitation with HBOCs to crystalloid or colloid was not performed. However, in previous studies using similar experimental techniques, hemodilution or hemorrhage and resuscitation with normal saline and pentastarch maintained $P_{Br}O_2$ and significantly increased rCBF.[23,27] In one study, resuscitation with 5% albumin resulted in an increase in both parameters.[27] The main differences between these results and those obtained with HBOC resuscitation are that MAP and $P_{Br}O_2$ increase to a larger degree while rCBF remains unchanged following HBOC resuscitation.

Hemodilution with HBOCs has also been utilized to determine if oxygen affinity affects the delivery of oxygen to tissue. The P_{50} of human blood is 28 mmHg. An HBOC with a higher oxygen affinity (P_{50} < 28 mmHg) would be expected to bind oxygen more tightly and therefore have improved oxygen uptake and provide a greater oxygen reservoir. A lower affinity HBOC (P_{50} > 28 mmHg) could be expected to have an increased capacity to unload oxygen at the tissue.[28] A number of experimental studies have compared the relative ability of HBOCs with different P_{50} values to deliver oxygen to tissue. Some of these studies have demonstrated that high affinity HBOCs maintained better tissue oxygen delivery.[15,20,29] Conversely, other experimental studies demonstrate that low oxygen affinity HBOCs were superior at delivering oxygen to tissues.[21] Other investigators were unable to demonstrate any difference in oxygen delivery when comparing HBOCs with different oxygen affinities.[18,30,31] Overall, the existing data do not provide conclusive evidence for the optimal HBOC oxygen affinity for tissue oxygen delivery. One limitation of most of these studies is that identical HBOC compounds with differing oxygen affinities have not been assessed. Therefore, the confounding effects of different HBOC structures and properties may have contributed. One study in which the same HBOC structure was modified to produce a similar HBOC with three different oxygen affinities (P_{50} of 9, 16 and 30 mmHg) demonstrated that the highest affinity HBOC (P_{50} 9 mmHg) had reduced oxygen delivery, while an HBOC with a slightly lower oxygen affinity (P_{50} 16 mmHg) may have exhibited more favourable oxygen delivery characteristics.[19] Our results do not provide evidence of superior oxygen delivery with either low or high oxygen affinity HBOCs. This may be due to the fact that the volume of hemorrhage and resuscitation was limited to 30%.

A large number of experimental studies have demonstrated that resuscitation with different HBOCs has the capacity to restore MAP,[32,33] and improve tissue blood flow,[34] capillary density[33,35] and tissue oxygen tension.[26,35] These positive effects reverse the base deficit,[32,36] reduce lactate production[15] and decrease acute mortality.[15,37,38] Improved outcomes in one experimental study were attributed to an HBOC with lower vasoactive potential and a higher oxygen affinity.[15] Resuscitation with HBOCs has been demonstrated to restore cerebral blood flow and $P_{Br}O_2$ [34,39] However, these positive experimental results must be interpreted with caution because one clinical study was stopped prematurely due to higher mortality in the HBOC group.[40] Further experimental studies are required to assess the value of HBOC in hemorrhage resuscitation before they can safely be utilized to resuscitate patients from hypovolemic shock.

There are several limitations to this study. It was designed to model a hemorrhage that occurs in the operating room, so the results may not be directly

applicable to severe and sudden hemorrhage that occurs in conscious patients following trauma. The chemical properties of the starting HBOC solutions were different in some measurements. The lower MW HBOC had a necessarily lower hemoglobin concentration relative to both high MW solutions so that all three solutions were iso-oncotic with plasma. However, this would not account for the larger increase in MAP observed in the low MW group. The pH of the High P_{50} > 128 Poly OR-Hb (7.25 ± 0.01) was slightly lower than that of the High P_{50} Poly OR-Hb (7.31 ± 0.05). However, immediately after resuscitation the pH of the first blood sample was similar in both groups (7.37 ± 0.04 *vs* 7.40 ± 0.06). Therefore, no difference in the oxygen affinity would be expected *in vivo*. Similarly, differences in the oxygen content of the three HBOCs *in vitro* did not result in any significant difference in the post-resuscitation blood oxygen content which was similar in all groups (Table II). Although the methemoglobin concentrations of the three HBOC solutions were different, this did not affect oxygen delivery to the brain. Changes in blood viscosity are known to affect vascular tone. In the current study, one HBOC group had a viscosity of 1.2 cSt while the other two were closely matched at 2.7 and 2.4 cSt, and similar to whole blood viscosity (~3 cSt). At a 30% blood replacement ratio this would result in a maximum difference in viscosity of 0.5 fold. Cabrales *et al.* have demonstrated that a threefold increase in blood viscosity results in about a 30% increase in hamster skin fold blood flow.[41] If we assume a similar degree of response in our model then a 0.5 fold change in viscosity would be expected to produce a 5% change in blood flow. This small effect is not likely to be experimentally or clinically significant.

All baseline values demonstrated relatively small standard deviations. However, there was an increase in variability following hemorrhage and HBOC resuscitation. This variability is in keeping with other published reports in rat[23,42]and demonstrates significant individual variation in the response to resuscitation. Despite this variation, statistically significant increases in brain tissue oxygen tension were observed after resuscitation with each HBOC. This study did not assess cerebral effects at the cellular level.

In summary, adequate oxygen delivery to cerebral tissue was demonstrated for three different HBOC solutions following 30% hemorrhage and resuscitation. Resuscitation with higher MW HBOCs (> 128 kDa) was associated with a reduction in the peak increase in MAP. This did not impair the ability of the HBOCs to maintain $P_{Br}O_2$. No effect of oxygen affinity was observed, possibly due to the limited extent of hemorrhage and resuscitation (30%).

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