The Evaluation of the Efficacy of Oxygent® as an Oxygen-Carrying Substitute on Cerebral Blood Flow

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Abstract: Cerebral blood flow (CBF) is tightly regulated to meet metabolic demands, and it increases during hemodilution as arterial oxygen content (CaO_2) falls. Oxygent® is a perfluorocarbon (PFC) emulsion that has a high capacity to dissolve oxygen and can thus increase CaO_2 in patients breathing supplemental oxygen. PFCs have shown excellent oxygen therapeutic value in both phase II and phase III clinical trials. However, the effects of Oxygent® on CBF in hemodilution are unknown. We performed this study to investigate how Oxygent® alters CBF in rats undergoing stepwise isovolumetric hemodilution under isoflurane anesthesia with 100% oxygen ventilation. *In vivo* Laser Doppler Flow (LDF) probes measured CBF as blood was gradually replaced with equal volumes of either 5%Albumin (controls) or (1:1) PFC and Albumin. Hematocrit and blood gases were measured and CaO_2 calculated after each dilution. Target values were to achieve a hematocrit value of 10% and fluorocrit between 7.5-10%. We compared CBF in the Oxygent® vs. control group at decreasing levels of hematocrit and CaO_2 with repeated t-tests. At hematocrits less than 15%, CBF rate approached baseline in the Oxygent® group, and was lower than the control group ($p = 0.004$). At maximal hemodilution, Oxygent® treated rats also showed higher PaO_2 ($p < 0.001$) and required lower phenylephrine infusion rates to maintain blood pressure ($p = 0.002$). These data support that administration of Oxygent® improves tissue oxygenation during hemodilution.

INTRODUCTION

Oxygent® is an emulsion of perfluorocarbon (PFC) particles that has the ability to dissolve large amounts of oxygen at high levels of inspired oxygen (50 to 100%). Because of the small diameter of its particles and the dissolved state of associated oxygen, it efficiently carries it to tissues perfused at critically low flow rates. Several applications for PFCs are currently being studied as Phase II and III clinical trials, with over 800 humans treated to date (Spahn Vox Sang 2002) [1].

Generally, treatment with PFCs has been well tolerated, however, one Phase III clinical trial of patients undergoing cardiopulmonary bypass (CPB) was voluntarily suspended because patients in the PFC group had an increased incidence of strokes compared to the control group, who had a lower than normal incidence (Hill Can J Anaesth 2002) [2]. The specific methods of that study involved profound hemodilution in the PFC group, and after review of the data the strokes were thought to be the result of inappropriate study protocol (Riess Artif Cells Blood Substit and Immobil Biotechnol 2006) [3]. Two smaller studies have since compared the effects of PFC (Perflubron®, Alliance Pharmaceutical Corp, San Diego, CA) versus placebo in acute hemodilution and CPB. PFC decreased the need for allogenic blood transfusion in one study (Hill J Cardiothorac Vasc Anesth 2002) [4], while it increased cerebral blood flow (CBF) in the second study when administered in both low and high doses (Hill Ann Thorac Surg 2005) [5].

CBF is actively regulated to maintain sufficient tissue oxygen tension, and it increases when metabolic demand increases or when arterial oxygen content (CaO_2) decreases (Rosenblum Circ Res 197) [6] (Todd. Am J Physiol 1994) [7] (Muizelaar Am J Physiol Heart Circ Physiol 1992) [8] (Rebel Am J Physiol Heart Circ Physiol 2003) [9]. The finding of increased CBF with Perflubron® is concerning since it indicates a need to increase cerebral oxygen delivery. Yet, the effects of Oxygent® on CBF in hemodilution are unknown.

MATERIAL AND METHODS

Fourteen male Sprague-Dawley rats (Taconic farm, Germantown, NY), weighting 300 - 400 grams, were used in the study. The rats were housed at the U the University of South Florida, College of Medicine vivarium. The rats were kept in a 12 h light-dark cycle and had unrestricted access to standard rat chow and tap water prior to surgery. On the day of the experiment, rats were randomly assigned to either the PFC-albumin or albumin alone group.

The rats where anesthetized by intramuscular injection of 1 ml/kg of a mixture of 100 mg/ml Ketamine and 10 mg/ml Xylazine (Lidocaine). The rats were then weighed and the neck, head and both groin regions shaved and disinfected using a 5% povidone-iodine solution (Betadine aerosol spray, Purdue Frederick Co., Stamford, CT). Rectal temperature was measured (Mon-a-therm 6500, Mallinckrodt Medical, St. Louis, MO) and maintained at 37 +/- 0.5°C.

All rats where tracheotomized and intubated with a 14G IV catheter (Acuvance Plus, Ethicon Inc., Cincinnati, OH). Controlled ventilation with 100% oxygen at a frequency of 68/min and a tidal volume of 3 ml was initiated using a rodent ventilator (Harvard Apparatus, South Natick, MA), and Isoflurane (Minrad inc., Bethlehem, PA) at an inspiratory concentration of 1.2 % to maintain anesthesia. Both femoral arteries and veins where cannulated with polyethylene tubing (ID 0.58mm, Becton-Dickinson Co., Parsippany, NJ).

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1874-2769/08

One artery was used to monitor pressure invasively, and the other to withdraw blood. One venous line was used to infuse phenylephrine, and the other line to replace collected blood volume either by Albumin 5% (Baxter Healthcare Co., Glendale, CA) or Albumin 5% and PFC solution (Oxygent (60% (w/v) F-t-butylcyclohexane perfluorocarbon emulsion (Alliance Pharmaceuticals, Inc.). The PFC emulsion used is slightly phosphate buffered and made isotonic with glycerol. The density of the fluorocarbon particles is 1.97. Arterial pressure was recorded and 1 ml of blood was collected to determine blood gasses and measure hematocrits. The ventilatory parameters where adjusted according to the results of the blood gas analysis to maintain a $PaCO_2$ between 35 and 40 mmHg.

After skin closure, each rat was turned to prone position and its head fixed in a stereotactic frame (David Kopf Instruments, Tujunga, CA). A midline skin incision was made to expose the skull. A small burr hole was drilled 3 mm right of bregma, and a 26 G single fibre LDF probe (DP4, Moor Instruments Inc, Wilmington, DE) connected to a monitor (Moorlab server, Moor Instruments Inc.) was inserted with the tip in the striatum at a depth between 4 and 5 mm, to a point where a good pulsatile flow-signal was detectable. The rat was then allowed to stabilize for 20 minutes. CBF measurement was continued until the end of the experiment. The data was stored and retrieved with standard software in a Notebook PC. The instrument uses absolute flow units to determine blood flow by a Laser Doppler method. The device was calibrated using a standard calibration solution provided by the manufacturer. Once monitoring was established, acute normovolemic hemodilution was performed by removing 4 ml of blood at a rate of 0.5 - 1 ml/min and then replacing t with the same amount of 5% albumin or 2 ml albumin 5% and 2 ml of PFC-solution. After the first hemodilution step, hematocrit was determined again and blood gases were analyzed.

The volumes to be replaced in the next steps of hemodilution were then calculated for each rat to sequentially decrease hematocrit to 25%, 20%, 15% and 12%. Hemodilution proceeded stepwise every 20 minutes with measurement of PaO_2, hematocrit and fluorocrit after each step. Fluorocrit was measured manually by determining the percentage of whole blood it comprised after centrifuging blood in vials to separate red cells, fluorocarbon solution and serum. In the PFC group, a target concentration between 7-10% volume % PFC was calculated and volume substitution was performed using the desired amount of PFC in 5% albumin. Mean arterial blood pressure (MAP) was maintained between 60 and 90 mmHg by continuous infusion of phenylephrine (Gensia Sicor Inc. Irvine, CA) at a rate of up to 15 µg/h. The experiment was terminated when either hematocrit values of less than 5% were measured, or when hemodynamic instability developed that could not be treated by infusion of 15 mcg/h of phenylephrine or less.

Arterial Oxygen Content

Oxygent is a 60% w/v PFC with an oxygen dissolving capacity of 43 volume % (43 ml O_2 / 100 ml pure PFC) at 37°C and 760 mmHg oxygen. The arterial CaO_2 can thus be calculated using the following equation:

$$CaO_2 = \{1.36 \times [Hb] \times SaO_2\} + \{0.003 \times PaO_2\} + \{(\text{PFC } O_2 \text{ solubility}) \times PaO_2 \times \text{fluorocrit}\}$$

Hemoglobin concentration was calculated from hematocrit as follows:

$$Hb\ (g/dl) = Hct \times 0.34$$

We assumed that mean cell hemoglobin concentration (MCHC) was comparable in all subjects.

Statistical Analysis

Cerebral blood flow was found to stabilize quickly and remain stable during the second half of each 20 minute interval between dilutions. We calculated the mean LDF values after each hemodilution by averaging the values obtained in the 5 minutes preceding the next step of hemodilution. The data were analyzed by comparing CBF in both groups with Mann-Whitney rank sum tests or repeated t-tests for every hematocrit or CaO_2 step (Table **2**, Table **3**, Fig. **1**, Fig. **2**). The software used was MS Excel (Microsoft Co., Redmond, WA) and Sigma Stat 2.3 / SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). p values of less than 0.05 were considered statistically significant in all tests.

RESULTS

Hemodynamics

Table **1** shows the blood gas and hemodynamic values in both experimental and control groups after the maximal hemodilution achieved. PaO_2 values differed significantly between the two groups with a PaO_2 of 502.1 ± 10.46 in the PFC group versus 419.93 ± 15.92 in the albumin group ($p < 0.001$). There was no differences in MAP, $PaCO_2$, pH or weight of the rats during the experiment, however, a greater rate of phenylephrine infusion was required to maintain

Table 1. Systemic Blood Gas and Hemodynamic Values (mean ± SEM). *p<0.05

Variable	PFC	Albumin	p
$PaCO_2$ (mmHg)	37.19 ± 0.50	38.01 ± 0.74	0.351
PaO_2 (mmHg)	502.1 ± 10.46	419.93 ± 15.92	<0.001*
pH	7.369 ± 0.006	7.376 ± 0.006	0.418
MAP (mmHg)	74.32 ± 0.87	73.55 ± 0.89	0.538
Phenylephrine (mcg/h)	1.98 ± 0.20	3.06 ± 0.34	0.002*
Weight (g)	349.14 ± 12.85	347.57 ± 11.04	0.928

Table 2. Cerebral Blood Flow Values (± SEM) for Different Hematocrit Ranges in Albumin and Perfluorocarbon Groups. † t-test, ‡ Mann-Whitney Rank Sum Test. *p<0.05

Hematocrit ranges (%)	Laser Doppler Flow (% of baseline)		
	PFC	Albumin	p
39-45	101.06 ± 1.06	100 ± 0	0.694 ‡
29 – 38.9	112.57 ± 4.86	121.44 ± 6.75	0.308 †
23 – 28.9	134.03 ± 10.61	156.73 ± 24.48	0.358 †
20 – 22.9	140.68 ± 20.72	146.87 ± 13.68	0.803 †
15 – 19.9	139.89 ± 13.97	175.65 ± 9.55	0.056 †
10 – 14.9	145.26 ± 10.32	198.86 ± 12.17	0.004 † *
5.0 – 9.9	124.95 ± 9.65	226.47 ± 10.53	<0.001 † *
< 5.0	107.76 ± 4.58	166.24 ± 4.84	<0.001 † *

MAP in the albumin controls (3.06± 0.34 mcg/h vs. 1.98± 0.20 mcg/h, p = 0.002). This difference was not observed at lesser hemodilutions.

LASER DOPPLER FLOW DYNAMICS

We grouped several LDF values and expressed them as percent of baseline blood flow, according to their respective hematocrit ranges. Mean CBF values for each hematocrit range were then compared between treatment groups and statistically analyzed (Table **2**, Fig. **1**).

In both groups, blood flow in the striatum initially increased almost linearly with lowering hematocrits and then decreased towards baseline at hematocrits of less than 10%. In the group receiving PFC, CBF approached baseline in states of severe hemodilution after rats reach severely hemodiluted states (hematocrit < 15%), whereas in the albumin group, the blood flow remained significantly higher across three dilution ranges (Table **2**, Fig. **1**).

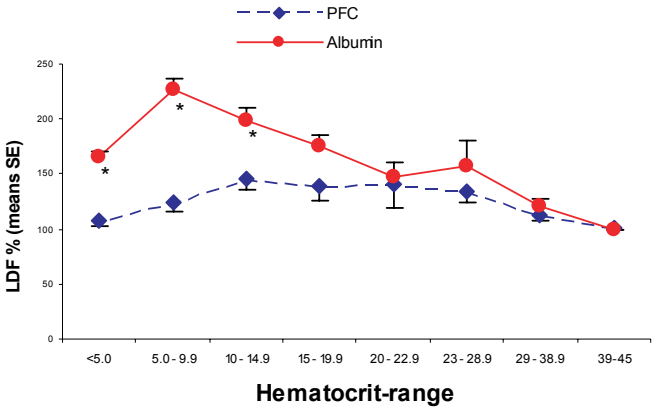

Fig. (1). Cerebral Laser Doppler Flow (LDF) at different hematocrit ranges for perfluorocarbon (PFC) and albumin groups. *p<0.05.

Similar results were obtained when blood flow was compared at different CaO_2 levels (Table **3**, Fig. **2**). There is a highly significant negative linear correlation between CBF and CaO_2 in the albumin group ($p < 0.001$) and a non-significant negative linear correlation in the PFC group ($p < 0.076$). When CaO_2 was lowered to less than 10 ml O_2 /dl, CBF of controls was significantly higher than that of their PFC counterparts, whose CBF again approached baseline. Accordingly, even at low levels of CaO_2 (which includes oxygen associated with PFCs), subjects with PFC-dissolved oxygen did not experience the reflex to increase cerebrovascular blood flow.

DISCUSSION

We demonstrated that Oxygent® paired with 100% oxygen ventilation was capable of maintaining CBF similar to baseline values after isovolemic hemodilution to hematocrits below 15%. In contrast, controls had significantly higher CBF which peaked at approximately 225% of baseline values (Fig. **1**, Table **2**). The same relationship between CBF and CaO_2 in the PFC group was observed below CaO_2 of 10 ml O_2 / dl blood (Fig. **2**, Table **3**), compared to controls. At a minimal value of less than 7.5 ml O_2/dl, the difference in CBF between groups was approximately 100%.

Because a coupling mechanism between CBF and tissue hypoxia has been shown to increase CBF in order to meet oxygen demand (Sokoloff Fed Proc 1981) [10], our findings suggest enhanced tissue oxygen delivery in rats whose hematocrit was supplemented by Oxygent®. Furthermore, the finding that CBF was significantly different between groups at the same CaO_2 levels in agreement with prior findings that oxygen associated with PFCs is unloaded and metabolized more efficiently (>90%) than that bound to hemoglobin in red blood cells (≈25%) (Keipert. Adv Exp Med Biol 1994) [11]. This is likely due to their small size of PFC (<.19 micron diameter) compared to erythrocytes (6-8 micron diameter) and the fact that associated oxygen is dissolved and not bound. Our findings support the growing body of evidence that PFCs such as Oxygent® may be neuroprotective in special circumstances, ranging from acute hemorrhage to permanent MCA occlusion in animal experimentations (Waschke Anesth Analg 1994) [12] (Kolluri Stroke 1986) [13] (Kolluri Surgical Neurology 1986) [14] (Stern Am J Emerg Med 1995) [15] (Woitzik Neurol Res 2005) [16]. The efficacy of Oxygent® in maintaining CBF at baseline values provides further evidence that PFCs can protect against

Table 3. Cerebral Blood Flow Values (± SEM) for Different CaO_2 Ranges in Albumin and Perfluorocarbon Groups.† t-test,‡ Mann-Whitney Rank Sum Test. *p<0.05

CaO_2 ranges (mlO_2/dl blood)	Laser Doppler Flow (% of baseline)		
	Perfluorocabon	Albumin	p
>20	102.81 ± 2.81	100 ± 0	0.629 ‡
17.5 – 19.99	106.69 ± 3.64	104.69 ± 2.92	0.717 †
15 – 17.49	129.97 ± 20.2	144.43 ± 31.77	0.453 †
12.5 – 14.99	135.52 ± 13.1	118.76 ± 18.6	0.255 †
10 – 12.49	141.94 ± 11.17	156.3 ± 13.29	0.417 †
7.5 – 9.99	142.18 ± 7.78	185.53 ± 10.12	0.002 † *
< 7.5	106.01 ± 6.02	203.05 ± 9.84	<0.001† *

ischemic stroke in animals that undergo cardiopulmonary bypass (CPB), even when massive air emboli are introduced (Yoshitani Anesth Analg 2006) [17] (Cochran, Ann Thorac Surg 1997) [18].

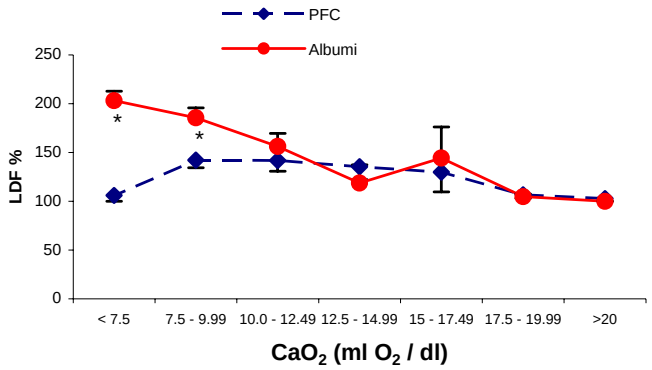

Fig. (2). Cerebral blood Laser Doppler Flow (LDF) at different ranges of arterial oxygen content for perfluorocarbon (PFC) and albumin groups. *p<0.05.

We also examined the effects of maximal hemodilution on hemodynamic and blood gas variables with and without treatment with Oxygent®. The results, displayed in Table **1**, revealed no difference in $PaCO_2$, pH, MAP, and weight of the rats between groups. The amount of phenylephrine infused to maintain the MAP between 60 and 90 mmHg was significantly greater at the highest hemodilution in the albumin control group, indicating a less stable physiological system compared to the Oxygent® group. This may have been due to improved cardiac oxygen delivery in the PFC group, leading to improved left ventricular contractile function, as was demonstrated by Habler *et al.* in hemoglobins of < 3g/dL (Habler Res Exp Med 1998) [19]. The PaO_2 was significantly higher in the Oxygent® group, in accordance with previous literature that the presence of a PFC emulsion is capable of increasing partial pressures of oxygen and therefore improving oxygen unloading capabilities.

Many surgical as well as medical applications exist for oxygen therapeutics such as Oxygent®. Our findings are particularly pertinent to clinical applications that involve acute hemodilution, such as during cardiopulmonary bypass (CPB), and they help alleviate concern that PFCs may increase incidence of stroke. Our conclusion that oxygen delivery to the brain is preserved by using Oxygent® with 100% oxygen ventilation in rats with critically low hematocrit values might explain why PFC administration has been shown to decrease mortality in near-fatal hemorrhage (Stern Am J Emerg Med 1995) [15]. Furthermore, it may protect against neurological injury in CPB, even when air emboli are introduced intravascularly (Yoshitani Anesth Analg 2006) [17] (Cochran, Ann Thorac Surg 1997) [18], and to decrease the need for allogenic blood transfusion when used in conjunction with acute normovolemic hemodilution in surgery patients (Hill J Cardiothorac Vasc Anesth 2002) [4] (Spahn Anesthesiology 2002 [20]. This study was limited in that it relied on calculated CaO_2 based on hematocrit, SaO_2, PaO_2, fluorocrit, and the specific properties of Oxygent® (oxygen dissolving capacity of 43 volume % (43 ml O_2 / 100 ml pure PFC) at 37°C and 760 mmHg oxygen).

Nonetheless, herein we report a significant effect. Also, we did not measure intracortical tissue oxygen tension. However, since cerebral oxygen balance and CBF have been shown to be strongly correlated (Sokoloff Fed Proc 1981) [10], CBF can be reliably used as surrogate marker for adequacy of tissue oxygen delivery. Another factor affecting flow rates is viscosity, and the relatively higher viscosity of PFC emulsion compared to albumin likely may have affected the observed differences in the two groups.

REFERENCES

[1] Spahn DR. Artificial oxygen carriers: status 2002. Vox Sang 2002; 83: Suppl 1: 281-5.

[2] Hill SE. Oxygen therapeutics: current concepts. Can J Anaesth 2002; 48: S32-S50.

[3] Riess JG. Perfluorocarbon-based Oxygen Delivery. Artif Cells Blood Substit and Immobil Biotechnol 2006; 34: 567-80.

[4] Hill SE, Leone BJ, Faithfull NS, Flaim KE, Keipert PE, Newman MF. Perflubron emulsion (AF0144) augments harvesting of autologous blood: a phase II study in cardiac surgery. J Cardiothorac Vasc Anesth 2002; 16: 555-60.

[5] Hill SE, Grocott HP, Leone BJ, White WD, Newman MF. Cerebral physiology of cardiac surgical patients treated with the perfluorocarbon emulsion, AF0144. Ann Thorac Surg 2005; 80: 1401-7.

[6] Rosenblum WI. Effects of reduced hematocrit on erythrocyte velocity and fluorescein transit time in the cerebral microcirculation of the mouse. Circ Res 1971; 29: 96-103.

[7] Todd MM, Wu B, Maktabi M, Hindman BJ, Warner DS. Cerebral blood flow and oxygen delivery during hypoxemia and hemodilu-

tion: role of arterial oxygen content. Am J Physiol 1994; 267(5 Pt 2): H2025-31.

[8] Muizelaar JP, Bouma GJ, Levasseur JE, Kontos HA. Effect of hematocrit variations on cerebral blood flow and basilar artery diameter *in vivo*. Am J Physiol Heart Circ Physiol 1992; 262: H949-54.

[9] Rebel AJ, Ulatowski A, Kwansa H, Bucci E, Koehler RC. Cerebrovascular response to decreased hematocrit: effect of cell-free hemoglobin, plasma viscosity, and CO_2. Am J Physiol Heart Circ Physiol 2003; 285: H1600-8.

[10] Sokoloff L. Relationships among local functional activity, energy metabolism, and blood flow in the central nervous system. Fed Proc 1981; 40: 2311-6.

[11] Keipert PE, Faithfull NS, Bradley JD, Hazard DY, Hogan J, Levisetti MS, Peters RM. Oxygen delivery augmentation by low-dose perfluorochemical emulsion during profound normovolemic hemodilution. Adv Exp Med Biol 1994; 345: 197-204.

[12] Waschke KF, Riedel M, Albrecht DM, van Ackern K, Kuschinsky W. Effects of a perfluorocarbon emulsion on regional cerebral blood flow and metabolism after fluid resuscitation from hemorrhage in conscious rats. Anesth Analg 1994; 79: 874-82.

[13] Kolluri S, Heros RC, Hedley-Whyte ET, Vonsattel JP, Miller D, Zervas NT. Effect of Fluosol on oxygen availability, regional cerebral blood flow, and infarct size in a model of temporary focal cerebral ischemia. Stroke 1986; 17: 976-80.

[14] Kolluri S, Heros RC, Hedley-Whyte ET, Vonsattel JP, Miller D, Zervas NT. Comparison of the effect of fluosol DA and dextran 40 on regional cerebral blood flow, infarction size, and mortality in cats with temporary occlusion of the middle cerebral artery. Surgical Neurology 1986; 26: 3-8.

[15] Stern SA, Dronen SC, and McGoron AJ, *et al*. Effect of supplemental perfluorocarbon administration on hypotensive resuscitation of severe uncontrolled hemorrhage. Am J Emerg Med 1995; 13: 269-75.

[16] Woitzik J, Weinzierl N, Schilling L. Early administration of a second-generation perfluorochemical decreases ischemic brain damage in a model of permanent middle cerebral artery occlusion in the rat. Neurol Res 2005;27: 509-15.

[17] Yoshitani K, de Lange F, Ma Q, Grocott HP, Mackensen GB. ReducWoitzik Jtion in air bubble size using perfluorocarbons during cardiopulmonary bypass in the rat. Anesth Analg 2006; 103: 1089-93.

[18] Cochran RP, Kunzelman KS, Vocelka CR, Akimoto H, Thomas R, Soltow LO, Spiess BD. Perfluorocarbon emulsion in the cardiopulmonary bypass prime reduces neurologic injury. Ann Thorac Surg 1997; 63: 1326-32.

[19] Habler O, Kleen M, Hutter J, Podtschaske A, Tiede M, Kemming G, Welte M, Corso C, Batra S, Keipert P, Faithfull S, Messmer K. IV perflubron emulsion versus autologous transfusion in severe normovolemic anemia: effects on left ventricular perfusion and function. Res Exp Med (Berl) 1998; 197: 301-18.

[20] Spahn DR, Waschke KF, Standl, T, Motsch J, Van Huynegem L, Welte M, Gombotz H, Coriat P, Verkh L, Faithfull S, Keipert P.Use of perflubron emulsion to decrease allogeneic blood transfusion in high-blood-loss non-cardiac surgery: results of a European phase 3 study. Anesthesiology 2002; 97: 1338-49.

Received: March 28, 2008 Revised: April 14, 2008 Accepted: April 21, 2008