Lowering of elevated tissue PCO₂ in a hemorrhagic shock rat model after reinfusion of a novel nanobiotechnological polyhemoglobin-superoxide dismutase-catalase-carbonic anhydrase that is an oxygen and a carbon dioxide carrier with enhanced antioxidant properties

Yuzhu Bian, Gao Wei, and Thomas M. S. Chang

Artificial Cells and Organs Research Center, Departments of Physiology, Medicine and Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, QC, Canada

Abstract

Even though erythrocytes transport both oxygen and carbon dioxide, research on blood substitutes has concentrated on the transport of oxygen and its vasoactivity and oxidative effects. Recent study in a hemorrhagic shock animal model shows that the degree of tissue PCO₂ elevation is directly related to mortality rates. We therefore prepared a novel nanobiotechnological carrier for both O₂ and CO₂ with enhanced antioxidant properties. This is based on the use of glutaraldehyde to crosslink stroma free hemoglobin (SFHb), superoxide dismutase (SOD), catalase (CAT) and carbonic anhydrase(CA)to form a soluble PolySFHb-SOD-CAT-CA. It was compared to blood and different resuscitation fluids on the ability to lower elevated tissue PCO₂ in a 2/3 blood volume loss rat hemorrhagic shock model. Sixty minutes of sustained hemorrhagic shock at 30 mm Hg resulted in the increase of tissue PCO₂ to 95 mm ± 3 mmHg from the control level of 55 mm Hg. Reinfusion of whole blood (Hb 15 g/dL with its RBC enzymes) lowered the tissue PCO₂ to 72 ± 4.5 mmHg 60 minutes after reinfusion. PolySFHb-SOD-CAT-CA (SFHb 10 g/dL plus additional enzymes) was more effective than whole blood in lowering PCO₂ lowering this to 66.2 ± 3.5 mmHg. Ringer's Lactated solution or polyhemoglobin lowered the elevated PCO₂ only slightly to 87 ± 4.5 mmHg and 84.8 ± 1.5 mmHg, respectively. Moreover, ST-elevation for whole blood (Hb 15 g/dL) and PolySFHb-SOD-CAT-CA (Hb 10 g/dL) was respectively 12.8% ± 4% and 13.0% ± 2% of the control 60 minutes after reinfusion. Both are significantly better than those in the Ringer's lactated group and the PolyHb group. In conclusion, this novel approach for blood substitute design has resulted in a novel nanobiotechnological carrier for both O₂ and CO₂ with enhanced antioxidant properties.

Correspondence: Professor Thomas M. S. Chang, Director, Artificial Cells & Organs Research Centre, Faculty of Medicine, McGill University, 3655 Drummond Street, Room 1004, Montreal, QC H3G 1Y6, Canada. artcell.med@mcgill.ca.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

Keywords

antioxidant; blood substitute; carbonic anhydrase; catalase; hemoglobin based oxygen carrier; nanobiotechnology; nanomedicine; oxygen and carbon dioxide transport; oxygen therapeutic; polyhemoglobin; superoxide dismutase; tissue PCO₂

Introduction

The increased demand of donor blood for transfusion, the lack of supply and the 1989 HIV crisis has resulted in intensified efforts to develop blood substitutes. (Chang 1964, 2007, 2009, 2012, Winslow 2006) Red blood cell (RBC) functions include the transport of O₂ and CO₂ in addition to antioxidant functions. However, the initial urgent need during the HIV crisis led to the development of simple oxygen carriers. This urgent rush has resulted in a number of failures. Two of the more successful ones are Polyhemoglobin (Jahr et al. 2008, Moore et al. 2009) and pegylated hemoglobin (Winslow 2006, Liu and Xiu 2008).

Polyhemoglobin (polyHb) is prepared by polymerizing hemoglobin using the basic glutaraldehyde method. (Chang 1971, Chang 2007) The commercial products are prepared using hemoglobin containing no RBC enzymes like superoxide dismutase, catalase and carbonic anhydrase. (Jahr et al. 2008, Moore et al. 2009) PolyHb has advantages of more than 1 year storage at room temperature, free from infective agents, no blood group antigens and unlimited supply. It has been tested extensively in clinical trials. (Jahr et al. 2008, Moore et al. 2009) The latest clinical trial on PolyHeme in the U.S. involves more than 600 ambulance patients. (Moore et al. 2009) This clinical trial shows that whereas patients in the control group needs donor blood transfusion on arrival at the hospital, those given PolyHb right in the ambulance can continue for more than 12 hours. (Moore et al. 2009) However, there is a 3% complication in the PolyHb group as compared to 0.6% in the control group. Another PolyHb prepared from bovine hemoglobin has been tested extensively in surgical patients. (Moore et al. 2009) South Africa, with its problem of HIV in donor blood has already approved the routine use of this bovine PolyHb in patients a number of years ago. (Moore et al. 2009) Recently, this bovine PolyHb has also been approved for routine clinical use in patients in Russia (OPK Biotech LLC 2011). In the meantime, research and developments have been carried out to avoid vasoconstriction due to nitric oxide scavenging in those with endothelial dysfunction. (Yu et al. 2010, Zapol 2012) Another area is study aim at preventing oxidative stress. (D'Agnillo and Chang 2008). Thus, PolySFHb-SOD-CAT, which contains hemoglobin and superoxide dismutase (SOD) and catalase (CAT) has been prepared and shown to protect against ischemic reperfusion injury in cerebral ischemia and in liver and kidney transplantation in rats (Chang et al. 2004, D'Agnillo and Chang 2008, Powanda and Chang 2002).

One of the major functions of RBC is the transport of carbon dioxide (Geers and Gros 2000, Kvarstein et al. 2004, Walter 2010). Large amount of CO₂ produced in the body depends on RBC to transport them to the lung. CO₂ in the blood plays an important role in acid-balance (Geers and Gros 2000). Excessive accumulation of CO₂ in the body leads to increased plasma proton (H⁺) concentrations which can result in acidosis, malfunction of the central nervous system, coma, and even death (Geers and Gros 2000). CO₂ is not too soluble in

water and only 5% of total body fluid, is present in the form of dissolved CO_2 , while the majority of CO_2 in all compartments is present in the soluble form of HCO_3^- . The conversion of CO_2 to H^+ and HCO_3^- is so slow that it cannot allow for the transport of enough CO_2 to be excreted from the lung. A zinc-containing enzyme, carbonic anhydrase (CA), in the RBCs is the most important enzyme responsible for the conversion of CO_2 to H^+ and HCO_3^- to allow for the proper transport of CO_2 (Geers and Gros 2000, Walter 2010). Approximately 75% of carbon dioxide is transported in the red blood cell and 25% in the plasma. The relatively small amount in plasma is due to the lack of carbonic anhydrase in plasma. (Geers and Gros 2000).

Sims et al. (2001) carried out studies in animal using tissue CO_2 microelectrodes. They show that tissue CO_2 is not reflected by blood PCO_2 . Furthermore, tissue CO_2 increases with severity of hemorrhagic shock and is correlated with survival (Sims et al. 2001). Besides, the degree of tissue PCO_2 elevation is directly related to mortality rates (Sims et al. 2001). In perfusion failure due to hemorrhagic shock, CO_2 produced as the metabolic function of the cells is not properly transported to the alveoli for excretion. Carbonic anhydrase in red blood cells plays the major role in the transport of tissue CO_2 to the lung for excretion. (Geers and Gros 2000) PolyHb prepared from ultrapure hemoglobin has no carbonic anhydrase to help in carbon dioxide transport. We therefore constructed a novel blood substitute based on nanobiotechnology to crosslink Hb, SOD, CAT and CA to form PolySFHb-SOD-CAT-CA (Bian et al. 2011). We have now carried out study in a hemorrhagic shock rat model to analyze the lowering of the elevated tissue PCO_2 using whole blood, 3 volume Lactated Ringer, PolyHb, PolySFHb and PolySFHb-SOD-CAT-CA.

Material and methods

Materials

All the materials used in this experiment were purchased as below: From Sigma: Potassium Phosphate, Toluene, Sodium Phosphate, 99% Lysine Monohydrochloride, 25% Glutaraldehyde, Sephacryl S-300HR, 37% Hydrochloric Acid, Catalase, Superoxide dismutase, Carbonic anhydrase, 30% Brij 35 solution, Drabkin, 3%(w/w) Hydrogen Peroxide; From Fisher:

1.5 cm $\times$ 98 cm Chromatography Column; Millipore, 100 kDa Centrifugal Filter; From B. Braun medical Inc.: Ringer's Lactated Solution; From McGill University (MacDonald campus), fresh bovine blood; from Biopure Cooperation: ultrapure bovine hemoglobin.

SFHb preparation

This has been described in details elsewhere (Bian et al. 2011). Fresh bovine blood with heparin was centrifuged at 4000 g for 60 minutes at 4 °C to remove the plasma supernatant and upper layer of cell pellet. The red blood cells were washed four times with sterile, ice-cold saline and then suspended in twice the volume of potassium phosphate (12.5 mM, pH 7.4) for 30 minutes. Then 2 volumes of ice-cold reagent-grade toluene were used to remove stroma lipid. The sample was centrifuged at 16,000 g for 2 hours at 4 °C to remove cellular debris. The concentration of Hb was tested by Drabkin's Solution. This stroma free Hb

contains enzymes (SOD, CAT, CA) that are normally presented in red blood cells. This is different from commercial Polyhemoglobin that are prepared from ultrapure hemoglobin where all the enzymes have been removed.

Preparation of PolyHb, PolySFHb, and PolySFHb-SOD-CAT-CA

PolySFHb-SOD-CAT-CA (Figure 1)—This has been described in details elsewhere (Bian et al. 2011). SOD, CAT and CA were added to SFHb with a final concentration of SOD (1050 U/mL SFHb), CAT (21,000 U/mL SFHb), and CA (1070 U/mL SFHb). These were crosslinked by glutaraldehyde as follows to construct PolySFHb-SOD-CAT-CA. First, 1.3 M lysine was added at a molar ratio of 7:1 lysine/Hb before the cross-linking reaction. The reaction vessel was flushed with nitrogen gas and kept at 4 °C at all time to prevent the formation of methemoglobin. Twenty ml of the mixture was placed on a shaker at 4 °C for 1 hour at 140 rpm. Without stopping the shaker, 0.7 ml of 5% glutaraldehyde was added slowly at a molar ratio of 16:1 glutaraldehyde/Hb at a rate of 0.15–0.20 mL every 5–10 minutes. The reaction was allowed to crosslink on the shaker for 24 hours at 4 °C. The crosslinking was stopped by adding 2.0 M lysine at a molar ratio of 200:1 lysine/Hb.

PolySFHb—PolySFHb was prepared using the same method as above but using the content of red blood cells with the normal blood cell concentration of SOD, CAT and CA.

PolyHb—PolyHb was prepared by crosslinking ultrapure Hb that contains no red blood cell enzymes.

Purification and concentration of PolySFHb-SOD-CAT-CA, PolySFHb, and PolyHb

PolyHb-SOD-CAT-CA, PolySFHb, and PolyHb were each filtered using a sterile 0.45 µm filter and dialyzed against Ringer's lactated solution overnight using a dialysis membrane. A Sephacryl S-300 column was used to remove free tetrameric Hb from the PolySFHb-SOD-CAT-CA, PolySFHb, and PolyHb and collected only the fraction with molecular weight >300 Ka. The samples were placed in a dialysis membrane on a bed of Spectra/Gel absorbent at 4 °C and concentrated to a final Hb concentration of 5 g/dL or 10 g/dL.

Measurements of SOD, CAT and CA activities

Superoxide dismutase activity was determined by Superoxide Dismutase kit (R&D Systems), which follows the inhibition of nitroblue tetrazolium (NBT) reduction using xanthine-xanthine oxidase as a superoxide generator. Catalase measurement was based on the rate of disappearance of H₂O₂. For carbonic anhydrase, one Wilbur-Anderson (W-A) unit of CA activity is defined as the amount of enzyme that causes the pH of a 0.02 M Tris buffer to drop from pH 8.3 to 6.3 per minute. The reaction was initiated by the addition of substrate, and the time (T) needed for the pH of the reaction mixture to drop from pH 8.3 to 6.3 was recorded. A Fisher Accumet Basic pH meter (Fisher Scientific, Pittsburgh, PA) with MI-407 (P) Needle pH electrode (Microelectrodes Inc., Bedford, NH) was used to measure the change in pH caused by the hydration reaction of CO₂ catalyzed by CA. The control for the assay consisted of the same mixture without the test sample. The measurements in seconds were converted into W-A units according to the following formula: 1 W-A unit = [2

$\times (T_0 - T)/T$. The units were then plotted versus the Hb/CA concentration. (Bian et al. 2011).

Effects on Tissue PCO₂, MAP and ECG of hemorrhagic shock rat model

Sprague-Dawley rats, 220 ± 30 g, are randomly divided in groups of six and each group contains 4 rats. Different types of resuscitation fluids were randomly assigned to each group. Each of them receives intravenous heparin (150 IU/kg) and was anesthetized with pentobarbital solution 30 mg/g. Heparinized catheters were inserted into the right femoral vein and right femoral artery to measure MAP. MAP and ECG were followed using a Biopac Lab (BIOPAC Systems Inc., Goleta, CA). Tissue PCO₂ was measured using a PCO₂ microelectrode (Lazar Research Labs, USA) placed on the surface of vastus medialis muscle of the thigh at the incision site used for cannulation. MAP was reduced to 30 mmHg by withdrawing blood from the right femoral artery and maintained at 30 mmHg for 60 minutes. After this, each rat received one of the following intravenous resuscitation fluids at a rate of 0.5 ml/min

1. Reinfusion of rats' own shed whole blood;
2. Ringer's lactated solution, three volume of shed blood;
3. PolyHb in Ringer's lactate solution (5 g/dL) equivalent to the volume of shed blood.
4. PolyHb in Ringer's lactate solution (10 g/dL) equivalent to the volume of shed blood.
5. PolySFHb in Ringer's lactate solution (5 g/dL) equivalent to the volume of shed blood.
6. PolySFHb in Ringer's lactate solution (10 g/dL). The volume is equivalent to the volume of shed blood.
7. PolySFHb-SOD-CAT-CA in Ringer's lactate solution (5 g/dL) equivalent to the volume of shed blood;
8. PolySFHb-CAT-SOD-CA in Ringer's lactate solution (10 g/dL) equivalent to the volume of shed blood.

MAP, tissue PCO₂ and ECG were followed throughout the experiment and continued for 60 minutes after the completion of the above-mentioned reinfusions. After this, the rats were given IP Nembutal to terminate these acute studies.

(Animal research was carried out following McGill University's guidelines for animal studies)

Statistics

Data were expressed as mean + standard deviation (SD). Analysis of variance (ANOVA) and Student's t-test were applied to assess the difference between treatment groups. Statistical significance was defined as $p < 0.05$ to reject a null hypothesis.

Results

Characterization

Composition—Table I: shows the compositions of PolyHb, PolySFHb, and PolySFHb-SOD-CAT-CA.

1. PolyHb was prepared by crosslinking ultrapure Hb that contains no RBC enzymes. As a result, PolyHb contains polymerized Hb but no RBC enzymes.
2. PolySFHb was prepared by crosslinking stroma-free Hb (SFHb) extracted from the content of RBC. Thus it contains Hb, SOD, CAT and CA normally present in the red blood cells.
3. PolySFHb-SOD-CAT-CA was prepared by adding more SOD, CAT and CA to the SFHb before crosslinking. Thus, it contains higher concentrations of SOD, CAT and CA than those normally present in whole blood.

Absorbent gel at 4 °C was successful in adjusting the concentration of each of the solutions to a Hb concentration of either 5 g/dL or 10 g/dL. Only molecules larger than 300 K Da were collected from the passage through Sepactryl S-300 column as a result there was no small tetrameric Hb present.

1. Ringer's Lactate solution contains no Hb or enzymes
2. Shed blood: contains 15 g/dL of Hb and also contain RBC enzymes.
3. PolyHb contains either 10 g/dL or 5 g/dL of Hb but no RBC enzymes
4. PolySFHb contains either 10 g/dL or 5 g/dL of Hb with the enzymes normally present in RBC. This is because whole blood contains a higher concentration of Hb and enzymes than PolySFHb with Hb of 10 g/dL or 5 g/dL where the amounts of Hb and enzymes per 100 ml of the solution are correspondingly lower. Furthermore, depending on the type of enzymes, there is some decrease in enzyme activities after crosslinking. Thus, the total carbonic anhydrase activity in the Hb 10 g/dL PolySFHb is only about half that of the whole blood. The 5 g/dL PolySFHb is only about ¼ of that of whole blood.
5. PolySFHb-SOD-CAT-CA was therefore prepared to have total carbonic anhydrase activity closer to that of whole blood. Thus, the carbonic anhydrase activity in the Hb 10 g/dL preparation (1,360,000 U/dl) is closer to 1,875,000 U/dl of whole blood.

Effects on tissue PCO₂

The control baseline tissue CO₂ tension is 55.8 ± 3 mm Hg when measured with the CO₂ microelectrode. Tissue PCO₂ values rose quickly and significantly during hemorrhagic shock, so that after 60 minutes of hemorrhagic shock at 30 mm Hg, tissue PCO₂ increased to 92 mm ± 3 mmHg (Figures 2–4). The effects of reinfusion using different fluids are shown in Figures 2 and 3.

Figure 2 shows the results obtained with Ringer's lactated solution, whole blood, polyhemoglobin (Hb 10 g/dL with no enzyme) and PolySFHb-SOD-CAT-CA (Hb 10 g/dL). In the group reinfused with Ringer's lactated solution equivalent to 3 volumes of lost blood, the PCO₂ only dropped slightly to 87 ± 4.5 mmHg from $92 \text{ mm} \pm 3$ mmHg in 60 minutes after reinfusion. After reinfusion with blood (Hb 15 g/dL with RBC enzyme including CA at 1,875,000 U/dL), the tissue CO₂ tension decreased to 72 ± 4.5 mmHg. When reinfused with PolyHb (10 g/dL) that contains no red blood cell enzymes, the tissue PCO₂ only decreased to 82.4 ± 3.5 mmHg. With PolySFHb-SOD-CAT-CA that contains Hb 10 g/dL with enzymes including CA at 1,360,000 U/dL tissue CO₂ decreased to 66.2 ± 3.5 mmHg that is significantly more effective than blood (Figure 4).

We next investigate the effects of infusion of different concentrations of the different solutions (Figure 3). When reinfused with PolySFHb-SOD-CAT-CA at half the concentration of Hb 5 g/dL and enzyme including CA at 680,000U/dL, PCO₂ decreased to 71 ± 2 mmHg similar to that of whole blood of 72 ± 4.5 mmHg. When reinfused with PolySFHb that contains the original red blood cell enzymes (Tables I and II) but at the concentration of Hb 10 g/dL with CA of 950,000 U/dl, the tissue CO₂ decreased to 74 ± 5 mm Hg as compared to 72 ± 4.5 mmHg for whole blood at Hb of 15 g/dL(Figure 4). With half the concentration (Hb 5 g/dL and CA of 475,000 U/dl) PolySFHb only lowered the tissue PCO₂ to 77 ± 3.5 mm Hg.

Figure 4 is a summary of the results for all the solution tested. The effectiveness in lowering the elevated PCO₂ is related to the amount of carbonic anhydrase in the infusion fluid (Table II). Ringer's lactated solution and PolyHb contain no carbonic anhydrase and are the least effective in lowering the elevated tissue PCO₂. The other solutions contain variable amounts of carbonic anhydrase (Table II). The effectiveness is related to the amount of the enzyme in the solution infused.

Effects on blood pressure

The shock model was obtained by withdrawing two third of the blood volume and maintaining the blood pressure at 30 mmHg for 60 minutes. After that, the rats were resuscitated with different types of fluids (Figures 5 –7).

Transfusion with blood (Hb 15 g/dL) resulted in a transient increase of the mean arterial pressure (MAP) to 109 ± 5 mmHg (Figures 5 and 7). This is most likely due to some hemolysis during bleeding in the collected shed blood. After the completion of infusion, the MAP returned to 90 ± 6 mmHg. Transfusion with 3 volume Ringer's lactated solution increases the MAP, but at the completion of infusion the MAP quickly dropped to 54 ± 4 mmHg. When transfused with PolySFHb-SOD-CAT-CA (Hb 10 g/dL), there was no transient increase in MAP and the MAP could be maintained at 80 ± 4 mmHg when followed for 1 hour after the completion of infusion (Figures 5 and 7). Similarly, transfusion with PolySFHb (Hb 10 g/dL), could also maintain the MAP at 78 ± 5 mmHg. When transfused with PolyHb (Hb 10 g/dL), MAP could be kept at 75.7 ± 4.5 mmHg. When polySFHb-SOD-CAT-CA, polySFHb, and PolyHb were given at a lower concentration (Hb 5 g/dL), the blood pressure could only be maintained at 65 ± 6 mmHg, 61 ± 5 mmHg, and 62 ± 4 , respectively (Figures 6 and 7).

Preliminary results on ST elevation

ST elevation could be due to (1) ischemia (2) ischemia-reperfusion injury (3) abnormal tissue metabolism due to elevated PCO_2 . ST elevation increased during shock and the recovery of the ST segment 60 minutes after reinfusion depends on the type of fluids infused (Figure 8).

When reperfused with whole blood (Hb 15 g/dL) the ST-wave recovered to an increase of $12.8\% \pm 4\%$ of the control level before shock. When reinfused with PolySFHb-SOD-CAT-CA at a concentration of Hb 10 g/dL with its enhanced SOD 195,000 U/dl and CAT 3,500,000 U/dl and CA at 1,360,000 U/dl the change in ST elevation was $13.0\% \pm 2\%$ of the control. This was not significantly different from that of whole blood, but was significantly better than PolyHb and PolySFHb. Thus for PolyHb (Hb 10 g/dL) the ST elevation was $16.0\% \pm 2.4\%$ and with PolySFHb (Hb 10 g/dL), the ST elevation was $18.7\% \pm 2\%$ of the control.

Discussion

Recent research using a new tissue PCO_2 microelectrode shows that elevation of tissue PCO_2 is not reflected by blood PCO_2 . (Kvarstein et al. 2004, Tronstad et al. 2010) Furthermore, increase in tissue PCO_2 is related to increased mortality in hemorrhagic shock. (Sims et al. 2001) Carbonic anhydrase in red blood cells plays the major role in the transport of tissue CO_2 to the lung for excretion. (Geers and Gros 2000, Walter 2010) The ongoing research and development on blood substitutes have not taken this into consideration. For example, Polyhemoglobin prepared from ultrapure hemoglobin has no carbonic anhydrase to help in carbon dioxide transport. PolyHb is useful when only oxygen supply is needed and it has been approved in South Africa and Russia. (Jahr et al. 2008, OPK Biotech LLC 2011). However, in conditions like severe sustained hemorrhagic shock there is much elevation of tissue PCO_2 . Mortality in such a condition is related to the degree of tissue PCO_2 elevation (Sims et al. 2001). We therefore carried out the present study to see if in addition to polyhemoglobin that is only an oxygen carrier, do we also need a carbon dioxide transport function. We prepared a novel blood substitute based on nanobiotechnology to crosslink hemoglobin and enhanced superoxide dismutase (SOD), catalase (CAT) and carbonic anhydrase (CA) to form PolyHb-SOD-CAT-CA. We evaluated the effects of different resuscitation fluids on tissue PCO_2 in a rat hemorrhagic shock model.

Tissue PCO_2

Sixty minutes after sustained hemorrhagic shock tissue PCO_2 increased to $92 \text{ mm} \pm 3 \text{ mmHg}$ from the control level of 55 mmHg . Reinfusion with 3 volumes Ringer's Lactated solution or Polyhemoglobin only lowered the elevated PCO_2 slightly to $87 \pm 4.5 \text{ mmHg}$ and $82.4 \pm 3.5 \text{ mmHg}$, respectively. On the other hand, reinfusion of

PolySFHb-SOD-CAT-CA (Hb 10 g/dL plus enzymes) or whole blood (Hb 15 g/dL with its RBC enzymes) lowered the tissue PCO_2 to $66.2 \pm 3.5 \text{ mmHg}$ and $72 \pm 4.5 \text{ mmHg}$, respectively. The result also shows that by changing the amount of carbonic anhydrase, it is possible to adjust the effectiveness to be comparable or higher than that for red blood cells

depending on the clinical requirements. This is shown by the result obtained for PolySFHb-SOD-CAT-CA (Hb 10 g/dL) that contains 1,360,000 U/dl of CA. It lowered tissue CO_2 to 66.2 ± 3.5 mmHg as compared to 72 ± 4.5 mmHg for whole blood with CA of 1,875,000 U/dl. On the other hand, PolySFHb-SOD-CAT-CA at half the concentration (Hb 5 g/dL) with CA at 680,000 U/dl lowered PCO_2 to 71 ± 2 mmHg similar to 72 ± 4.5 mmHg of whole blood with CA of 1,875,000 U/dl. The lower CA required for PolySFHb-SOD-CAT-CA as compared to blood is most likely because it is a solution while red blood cells are particles of 7 micron diameter. A solution can more efficiently perfuse the microcirculation in conditions like hemorrhagic shock with vasoconstriction and decreased perfusion of the microcirculation in most tissues.

Conclusion

Our present results show that oxygen carrier alone cannot effectively lower the elevated tissue PCO_2 in hemorrhagic shock. On the other hand a novel combined oxygen and carbon dioxide carrier can effectively lower the elevated tissue PCO_2 level. Another group recently shows that the degree of tissue PCO_2 is related to mortality in an animal hemorrhagic shock model (Sims et al. 2001). Thus it would be important to carry out further research regarding the addition of a CO_2 carrier to the oxygen carrier for future blood substitutes in certain clinical conditions where there are elevations of PCO_2 as in severe hemorrhagic shock or other ischemic conditions.

Acknowledgments

Professor TMS Chang acknowledges the support of the operating term grant (MOP13745) from the Canadian Institutes of Health Research and also the Chinese Scholarship Council graduate student scholarship award to Gao Wei Xi'an, Jiaotong University, Xi'an, P.R. China for her to come here for her PhD training.

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Figure 1.

Upper: Free molecules of hemoglobin, superoxide dismutase, catalase and carbonic anhydrase in solution. Lower: Glutaraldehyde is used to crosslink the free molecules into a soluble nanobiotechnological complex of Polyhemoglobin-Superoxide dismutase-catalase-carbonic anhydrase.

Figure 2.

Tissue PCO_2 in hemorrhagic shock (rats). Three time periods: (1). Hemorrhagic shock by removing 67% of total blood volume and maintaining the MAP at 30 mm Hg for 1 hour. (2) Reinfusion of one of the resuscitation fluids. (3) Followed for 1 hour after reinfusion. Resuscitation fluids are (A) Shed blood; (B) Ringer's lactated solution 3 volumes of shed blood; (C) Polyhemoglobin (PolyHb) prepared with pure Hb containing no RBC enzymes and given at the concentration of Hb 10 g/dL, same volume as shed blood; (D) PolySFHb-SOD-CAT-CA prepared from RBC content (SFHb) enriched with additional superoxide dismutase (SOD), catalase (CA) and carbonic anhydrase (CA) given at the concentration of Hb10 g/dL in the same volume as shed blood.

Figure 3.

Tissue PCO₂ in hemorrhagic shock (rats). As above but with the following resuscitation fluids given in the same volume as shed blood. (A) PolyHb with no RBC enzymes as above but at a lower concentration of Hb 5 g/dL; (B) PolySFHb prepared from RBC content (SFHb) with RBC enzymes at the concentration of Hb 5 g/dL; (C) PolySFHb prepared with SFHb as B above but at a higher concentration of Hb 10 g/dL; (D) PolySFHb-SOD-CAT-CA prepared with SFHb but with further enrichment of additional SOD, CAT and CA given at the concentration of Hb 5 g/dL.

Figure 4.

This figure compares the effects on tissue PCO₂ of all the groups. As shown in graph: Ringer's Lactated solution, PolyHb (10 g/dL), PolySFHb 10 g/dL, Blood 15 g/dL and PolySFHb-SOD-CAT-CA 10 g/dL). Those solutions with hemoglobin concentration of 5 g/dL are: 2 (PolyHb), 4(PolySfHb), 6(PolySFHb-SOD-CAT-CA).

Figure 5.

MAP in hemorrhagic shock (rats). Three time periods: (1). Hemorrhagic shock by removing 67% of total blood volume and maintaining the MAP at 30 mm Hg for 1 hour. (2) Reinfusion of one of the resuscitation fluids. (3) Followed for 1 hour after reinfusion. Resuscitation fluids are (A) Shed blood; (B) Ringer's lactated solution 3 volumes of shed blood; (C) Polyhemoglobin (PolyHb) prepared with pure Hb containing no RBC enzymes and given at the concentration of Hb 10 g/dL, same volume as shed blood; (D) PolySFHb-SOD-CAT-CA prepared from RBC content (SFHb) enriched with additional superoxide dismutase (SOD), catalase (CA) and carbonic anhydrase (CA) given at the concentration of Hb 10 g/dL in the same volume as shed blood.

Figure 6. MAP in hemorrhagic shock (rats). As in Figure 5 but with the following resuscitation fluids given in the same volume as shed blood. (A) PolyHb with no RBC enzymes as above but at a lower concentration of Hb 5 g/dL; (B) PolySFHb prepared from RBC content (SFHb) with RBC enzymes at the concentration of Hb 5 g/dL; (C) PolySFHb prepared with SFHb as B above but at a higher concentration of Hb 10 g/dL; (D) PolySFHb-SOD-CAT-CA prepared with SFHb but with further enrichment of additional SOD, CAT and CA given at the concentration of Hb 5 g/dL.

Figure 7.

This is a combined figure to compare the effects on MAP of all the groups. As shown in graph: Ringer's Lactated solution, PolyHb (10 g/dL), PolySFHb 10 g/dL, Blood 15 g/dL and PolySFHb-SOD-CAT-CA 10 g/dL). Those solutions with hemoglobin concentration of 5 g/dL are: 2 (PolyHb), 4(PolySfHb), 6(PolySFHb-SOD-CAT-CA).

Figure 8.
% of ST elevation 60 minutes after reinfusion compare to value before hemorrhagic shock : whole blood (Hb 15 g/dL); PolyHb (Hb 10 g/dL); PolySFHb (Hb 10 g/dL) and PolySFHb-SOD-CAT-CA (Hb 10 g/dL).

Table I

Hb concentration, molecular weight and enzyme activity/gm Hb.

	Hb (ultrapure)	SFHb (rbc content)	PolyHb (From Hb)	PolySFHb (From SFHb)	PolySFHb-SOD-CAT-CA (From SFHb + additional SOD, CAT and CA)
Source	Bovine	Bovine	Bovine	Bovine	Bovine
Molecular weight	64 kDa	64 kDa	>300 k Da	>300 k Da	>300 kDa
Tetramers%	100	100	0	0	0
Hb (g/dL)	7	7	5 or 10	5 or 10	5 or 10
CA(U/gHb)	0	125,000	0	95,000	136,000
CAT (U/gHb)	0	50,000	0	47,000	350,000
SOD (U/gHb)	0	7,500	0	7,000	19,500

Hb (Hemoglobin with no rbc enzymes), SFHb (Stroma-free hemoglobin from rbc contents with rbc enzymes), PolyHb (Polyhemoglobin prepared from Hb), PolySFHb (Prepared from SFHb) and PolySFHb-SOD-CAT-CA. (prepared from SFHb with more SOD, CAT and CA added).

Table II

Total Hemoglobin and enzymes in each 100 ml of solution.

	Solution or suspension	Hemoglobin (grams)	Superoxide dismutase (U)	Catalase (U)	Carbonic anhydrase (U)
Ringer lactate	Solution	0	0	0	0
Blood	Suspension (7 micron)	15	112,500	750,000	1,875,000
PolyHb 10 g/dL	Solution	10	0	0	0
PolyHb 5 g/dL	Solution	5	0	0	0
PolySFHb 10 g/dL	Solution	10	70,000	470,000	950,000
PolySFHb 5 g/dL	Solution	5	35,000	235,000	475,000
PolySFHb-SOD-CAT-CA 10 g/dL	Solution	10	195,000	3,500,000	1,360,000
PolySFHb-SOD-CAT-CA 5 g/dL	Solution	5	97,500	1,750,000	680,000