Effects of Diaspirin Cross-Linked Hemoglobin (DCLHb) during and Post-CPR in Swine

MOSES S. S. CHOW, CHENGDE FAN, HIEU TRAN, HONG ZHAO, and LI ZHOU

University of Connecticut, School of Pharmacy and Hartford Hospital, Hartford, Connecticut (C.F., H.T., H.Z., L.Z.); and The Chinese University of Hong Kong, School of Pharmacy, Faculty of Medicine, Hong Kong, China (M.S.S.C.)

Received October 11, 2000; accepted December 20, 2000 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

The purpose of the study was to test the hypothesis that diaspirin cross-linked hemoglobin (DCLHb) can produce improved resuscitation during cardiac arrest. DCLHb, a derivative of human hemoglobin, has previously been demonstrated to produce a vasopressor response that is associated with increased blood flow to vital organs. In addition, it is an oxygen carrier. These effects may be beneficial to extreme low flow states, such as that during cardiac arrest and cardiopulmonary resuscitation (CPR). Experimental cardiac arrest and CPR were carried out in 32 anesthetized immature pigs. In each animal, ventricular fibrillation was induced for 5 min, followed by 10 min of standard CPR with a pneumatic device and room air ventilation. High (15 ml/kg) and low (5 ml/kg) doses of DCLHb or equivalent volume of normal saline were infused at the beginning of CPR in a random and blind manner. Cardiac output, organ blood flow, aortic pressure, coronary perfusion pressure, blood gases, and lactate concentrations were obtained before and during CPR. Following the 10-min CPR, the animals were defibrillated and the return of spontaneous circulation (ROSC) determined. DCLHb treatment achieved 75% ROSC compared with 25% in the saline group (p < 0.05). In addition, a better (p < 0.05) myocardial O₂ delivery, venous blood O₂ content, and myocardial and cerebral perfusion pressure were observed in the DCLHb group. DCLHb treatment during cardiac arrest and CPR significantly improves ROSC. This is most likely related to its improvement in coronary perfusion and myocardial oxygen delivery.

Cardiac arrest results in cessation of circulation to vital organs and is an emergency event requiring immediate intervention. Thus, the immediate objective in the management of cardiac arrest is the restoration of spontaneous circulation (ROSC). Standard cardiopulmonary resuscitation (CPR) with external chest compression partially restores the circulation and improves the chance of ROSC (Emergency Cardiac Care Committee and Subcommittees, American Heart Association, 1992).

It has been well documented that successful ROSC is correlated with a minimum coronary perfusion pressure of 15 to 20 mm Hg during CPR (Niaman et al., 1985; Kern et al., 1988; Paradis et al., 1990). This perfusion pressure provides sufficient coronary perfusion, i.e., delivers sufficient O₂ to the myocardium and thus increases the probability of successful ROSC.

Diaspirin cross-linked hemoglobin (DCLHb) is a stable, modified, and purified derivative of human hemoglobin (Baxter Healthcare Corporation, Round Lake, IL) that has been demonstrated to improve resuscitation in hemorrhagic shock (Przybelski et al., 1991; Cohn and Farrell, 1994; Schultz et al., 1994; Dunlap et al., 1995; Marchand et al., 1996; DeAngeles et al., 1997; Kumar et al., 1997; Fischer et al., 1999; Habler et al., 2000). DCLHb has been demonstrated to produce a pressor-type response in hemorrhagic animal model studies (Cohn and Farrell, 1994; Schultz et al., 1994; Dunlap et al., 1995; Marchand et al., 1996; DeAngeles et al., 1997; Kumar et al., 1997; Habler et al., 2000), as well as in normal human volunteers (Przybelski et al., 1994), patients undergoing hemodialysis (Swan et al., 1995), and in critically ill septic patients (Rhea et al., 1997). Blood flow studies in animal models have demonstrated an improvement in blood flow to major vital organs in hemorrhagic and septic shock states (Mourelatos et al., 1996; DeAngeles et al., 1997; Kumar et al., 1997). These effects suggest that DCLHb may potentially improve coronary perfusion during cardiac arrest and CPR.

DCLHb may possess additional beneficial effect during cardiac arrest and CPR. In our unpublished in vitro oxygen “saturation” studies of various solutions, DCLHb has been found to retain a much higher PO₂ than crystalloids when the solutions are bubbled, (i.e., “saturated”) with oxygen (Chow and Fan, 2000). These in vitro data suggest that DCLHb...
solution may provide better oxygenation than other colloids in the human body and may be advantageous during CPR.

Based on the previous observed hemodynamic effects as well as our in vitro oxygen “saturation” data, we hypothesize that DCLHb administration may provide improved oxygenation in the extremely ischemic state such as during cardiac arrest and CPR. Thus, we designed the present study to test the hypothesis that DCLHb administration during CPR improves resuscitation in an animal model of cardiac arrest.

Materials and Methods

DCLHb and Control Preparation

This study was approved by the Institutional Animal Care and Use Committees of the University of Connecticut and Hartford Hospital. All experiments were conducted at the Hartford Hospital Animal Laboratory (approved by Association for Assessment and Accreditation of Laboratory Animal Care, International) in compliance with National Institutes of Health guidelines and Food and Drug Administration Regulations for Good Laboratory Practice (21 CFR, Part 58) and United States Department of Agriculture Animal and Plant Health Inspection Service (Animal Welfare Act, 9 CFR, Parts 1, 2, and 3).

A 10% diaspirin cross-linked hemoglobin solution was prepared according to the method of Chatterjee et al. (1986) and Nelson et al. (1992) by Baxter Healthcare Corporation. Test animals were evaluated at two doses of DCLHb: 5 ml/kg (0.5 g/kg) or 15 ml/kg (1.5 g/kg). Control animals received normal saline treatment in identical volume as DCLHb.

Animal Preparation

Both male and female immature Yorkshire pigs (weight 18–22 kg) were randomized to receive either normal saline solution or DCLHb. The animals were acclimatized in a temperature- and humidity-controlled room for at least 24 h before study. Following an overnight fast, Telazol (tiletamine HCl and zolazepam HCl, a combination of a rapid-acting dissociative anesthetic and tranquilizer), 2 ml i.m., and 3% halothane inhalation were given for anesthesia induction and intubation. For maintenance of anesthesia 30-mg/kg bolus of pentobarbital followed by 12 to 15 mg/kg/h infusion was administered. Additional anesthesia (isoflurane) was used as needed. The adequacy of anesthesia was monitored by respiratory rate, heart rate, absence of voluntary movement, and toe clench. Body temperature was maintained at 37–38°C with a thermal blanket and monitored with a rectal thermometer. Ventilation was maintained with an Ohio control and displayed continuously. An arterial double-lumen pig-tail catheter (Boston Scientific Corporation, Watertown, MA) was inserted through the left femoral artery for recording left ventricular and aortic blood pressures and also used for colored microsphere infusion and blood sampling. A 4F Cook catheter (Cook Inc., Bloomington, IN) and a 5F bipolar balloon pacing-catheter (Bard Critical Care Division, C.B. Bard Inc., Tewksbury, MA) were inserted into the right internal jugular vein and right ventricle, respectively, for blood sampling and induction of ventricular fibrillation. A 7F pig-tail catheter (Schneider Inc., Minneapolis, MN) and a standard infusion line were placed into the left femoral artery and vein, respectively, for collection of colored microspheres, blood samples, and for infusion of test and control solutions. In addition, a 5F double-lumen catheter (American Edwards Laboratories, Santa Ana, CA) was advanced to the pulmonary artery for pressure recording and mixed venous blood sampling.

Experimental Procedures

After animal preparation and catheterization, the animals were stabilized to maintain normal pH, blood gas, and blood pressure (pH 7.35–7.45, PCO2 34–45 mm Hg, O2 saturation >94%, aortic pressure >95 mm Hg). Ventricular fibrillation (VF) was then induced by direct current stimulation of the right ventricle. After inducing VF, ventilation was stopped for 5 min to simulate cardiac arrest. CPR was then started using a pneumatic chest compression device (cardiopulmonary resuscitator model 1003; Michigan Instruments Inc., Grand Rapids, MI). The compression device was set at approximately 80 compressions/min with a force sufficient to achieve a mean aortic blood pressure of 50 to 65 mm Hg (approximately 1.5-inch compression). After every five compressions, diastole was prolonged by 1.7 s and the lungs were inflated to an inspiratory pressure of approximately 20 cm of H2O via a synchronized pressure-limited ventilator with room air. (Based on an average lung compliance of 0.5 cm of H2O, the estimated ventilation volume was 1 liter/ventilation.) At 1 min of CPR, DCLHb or normal saline was administered in a random and blinded manner (see treatment below). CPR was stopped after a total of 15 min of fibrillation (10 min of CPR) and external defibrillation shock at 200 J was initiated (Fig. 1). If needed, the shock was repeated at 300 J and then at 360 J until sinus rhythm was restored. If the animals could not achieve or maintain a mean blood pressure >60 mm Hg with an organized sinus rhythm, ventilation was changed to 100% oxygen and epinephrine, lidocaine, or atropine was administered according to the American Heart Association and Advanced Cardiac Life Support guidelines. Successful resuscitation was defined as a ROSC postdefibrillation with a mean aortic pressure >60 mm Hg sustained for at least 2 min with, or without drugs (see experimental design in Fig. 1). Additionally, in eight animals that achieved ROSC, the ability to maintain the pressure for 2 h post-CPR was evaluated. At the end of the experiment,
animals were sacrificed and organ samples were collected for measurement.

Drug Treatment
During CPR, DCLHb or normal saline was infused intravenously (into the femoral vein) over 5 min in a random and blinded manner using an infusion pump. To evaluate any potential dose-response relationships, the pigs in the experimental group received either 5 ml/kg (0.5 g/kg) or 15 ml/kg (1.5 g/kg) of DCLHb, and the control pigs received either 5 ml/kg or 15 ml/kg of normal saline. All animals also received sodium bicarbonate infusion at 0.1 mg/kg/min at the beginning of CPR to decrease development of acidosis.

Measurements

Blood Gases, Lactate, and Hemoglobin. Blood samples were obtained at baseline, 11 min, and 14 min postinduction of ventricular fibrillation (corresponding to 6 and 9 min after the initiation of CPR) from the femoral artery, internal jugular vein, and pulmonary artery for measurement of blood gases (238 pH blood gas analyzer; Ciba Corning, Medfield, MA), lactate concentration (ultraviolet method; Sigma Chemical Co., St. Louis, MO), hemoglobin concentration (Coulter-Stacker counter for concentration greater than 0.6 g/dl; Hialeah, FL; and Sysmex 2500 for concentration less than 0.6 g/dl; TAO Medical Electronics, Kobe, Japan), and hematocrit (Coulter-Stacker counter). Oxygen content was calculated from the standard equation as $[\text{PO}_2 \times 0.003] + (1.34 \times \text{O}_2 \text{saturation} \times \text{hemoglobin}) \times 1/100$. O$_2$ saturation (SO$_2$) was derived specifically for pigs, based on a corrected derived equation (Kowallik et al., 1991): $\text{SO}_2 = 10^{0.15 + 0.07n}$, where $D = n(\text{logPO}_2 - \text{log}(35.7) + 0.441(\text{pH} - 7.4) - 0.0016(T_B - 37))$, $n = 2.86$ for body temperature ($T_B < 34$, $n = 9.60 - 0.377 \times T_B + 0.00575 \times T_B^2$ for $T_B > 34$).

Total and Regional Flow. Total and regional blood flow were determined by a colored microsphere technique (Kowallik et al., 1991). Yellow, blue, and red Dye-Trak microspheres (15 μm, 1.09 g/ml; Triton Technology Inc., San Diego, CA) were used. Reference blood was withdrawn from an arterial catheter continuously for 2 min at 7.64 ml/min. After 20 s of reference blood withdrawal, 1.5 ml of red- or yellow-colored microspheres (3 million spheres/ml) was infused into the left ventricle through the pig-tail catheter over 20 s (one color was used during normal sinus rhythm and another color during cardiac arrest). At the end of the study, the animals were sacrificed and tissues (e.g., heart, kidney, and brain) were collected. The fat attached to the tissue was dissected. The tissues were weighed and homogenized and 1 to 2 grams of tissue was collected for tissue and tissue samples. The accuracy and precision of the colored microspheres technique for measuring blood flow were verified by consecutive injection of microspheres of different colors and by injection of combinations of different colored microspheres. The evenness of distribution of microspheres in the circulation was verified by comparing the microspheres trapped in the left versus the right kidneys.

Pressure. Aortic, left ventricular, and pulmonary artery pressures were monitored during the study and recorded at 0, 6, 9, 12, and 14 min from the start of VP using disposable transducers that were referenced to the level of the right atrium. Coronary perfusion pressure was calculated as aortic diastolic pressure minus mean right ventricular pressure. Cerebral perfusion pressure was calculated as mean aortic pressure minus pulmonary artery diastolic pressure.

Statistical Analysis
Since the main purpose of this study was to compare the effect of DCLHb to saline treatment, statistical analysis for all quantitative data was performed using a $t$ test to determine the degree of statistical significance between these two treatment groups. For primary analysis, the data obtained at 14 min (last observation point) were used. For all parametric data, the normality test was first performed and transformation of data (e.g., log transformation) was carried out if needed for subsequent analysis. For ROSC, chi square analysis was used. A $p = 0.05$ was considered statistically significant for all tests.

Results
A total of 43 pigs was initially entered into this study. However, 11 pigs were excluded from subsequent analysis for the following reasons: three pigs were used for testing the protocol during the initial model development phase of these studies; one additional pig received a very high dose (45 ml/kg) as an initial dose-response observation, which showed central organ edema. Of the remaining seven pigs excluded, two were excluded due to protocol violation (one due to improper CPR and another due to incorrect drug infusion rate), two had left ventricular failure (high left ventricular end-diastolic pressure), and three had other anatomical or infectious disease-related problems (cardiac valve vegetation, fever, and abnormal coronary sinus blood gas data).

Four of the seven pigs excluded were in the DCLHb group and three in the saline group; all were excluded before breaking the blinded results. A total of 32 pigs completed the protocol (eight in each group: high-dose and low-dose DCLHb; high-dose and low-dose normal saline) and were included for analysis.

The overall outcome of treatments comparing DCLHb and the saline (control) are shown in Table 1. The results showed that DCLHb achieved ROSC significantly better than saline treatment ($p < 0.05$). The animals in the DCLHb groups required fewer direct current shocks as well as epinephrine doses. Of the eight animals that achieved ROSC initially and were continuously monitored for an additional 2 h, seven were able to achieve and maintain a systolic blood pressure ≥60 mm Hg for the entire duration (all happened to be in the DCLHb group).

The mean blood gas values, O$_2$ content, hemoglobin, hematocrit, and lactate concentrations obtained from the arterial (aorta), venous (pulmonary artery), and internal jugular venous sites are summarized in Table 2. Although no significant differences in any of the baseline values were found, significantly higher mixed venous O$_2$ content and lower systemic O$_2$ extraction and arterial lactate were found in the

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Outcome of DCLHb or saline treatment</th>
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<tbody>
<tr>
<td></td>
<td>DCLHb</td>
</tr>
<tr>
<td>No.</td>
<td>Dose</td>
</tr>
<tr>
<td>ml/kg</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5</td>
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<td>8</td>
<td>15</td>
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</table>
DCLHb compared with the control group at 14 min of VF post-treatment. The cell-free plasma hemoglobin concentration in the DCLHb group was 1.3 ± 0.3 g/dl.

The mean systolic and diastolic pressures in the left ventricle, aorta, and pulmonary artery, as well as the calculated coronary and cerebral perfusion pressures are summarized in Table 3. No significant difference in any of the baseline values were found; however, significantly higher cerebral perfusion and coronary perfusion pressures were obtained in the DCLHb group compared with the control post-treatment.

The mean cardiac output, myocardial blood flow, and cerebral O2 delivery during normal sinus rhythm (baseline) and CPR are shown in Table 4. DCLHb treatment produced a significantly higher myocardial blood flow and O2 delivery during CPR than that seen in the control group.

When comparing the high dose of DCLHb to the low dose, no statistically significant differences were observed in any measured parameters. A trend toward a more beneficial effect was observed for the following: ROSC (seven of eight high dose, versus five of eight low dose); hemoglobin concentration; cardiac output; myocardial blood flow; cerebral blood flow; myocardial O2 delivery; lactate concentration; and left ventricular, aortic, cerebral perfusion, and coronary perfusion pressures. On the other hand, the high dose appeared to result in a less favorable trend for the following: PO2, O2 content, pH, and pulmonary artery pressure.

When comparing the animals that successfully achieved ROSC with those that did not, statistically significant differences were observed for the following: dose of epinephrine required (0.18 ± 0.21 versus 1.08 ± 0.89 mg); number of electric shocks (2 ± 1 versus 3 ± 0); arterial lactate concentration (5.20 ± 1.59 versus 6.50 ± 1.34 mM); aortic systolic pressure (78 ± 19 versus 62 ± 19 mm Hg); aortic diastolic pressure (27 ± 10 versus 17 ± 9 mm Hg); cerebral perfusion pressure (18 ± 6 versus 8 ± 9 mm Hg); coronary perfusion pressure (17 ± 7 versus 7 ± 8 mm Hg); myocardial blood flow (18.7 ± 12.1 versus 5.6 ± 4.8 ml/min/); cerebral blood flow (16.6 ± 7.3 versus 12.1 ± 5.9 ml/min/); and myocardial O2 delivery (1.8 ± 1.3 versus 0.7 ± 0.5 ml/min).

**TABLE 2**
Comparison of mean blood gases, O2 content, hemoglobin, and hematocrit.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DCLHb Group (n = 16)</th>
<th>Control Group (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (A)</td>
<td>7.40 ± 0.03</td>
<td>7.41 ± 0.03</td>
</tr>
<tr>
<td>pH (V)</td>
<td>7.37 ± 0.04</td>
<td>7.40 ± 0.04</td>
</tr>
<tr>
<td>pH (IJ)</td>
<td>7.37 ± 0.03</td>
<td>7.38 ± 0.04</td>
</tr>
<tr>
<td>pCO2 (A)</td>
<td>41.56 ± 2.94</td>
<td>41.31 ± 2.80</td>
</tr>
<tr>
<td>pCO2 (V)</td>
<td>45.44 ± 4.99</td>
<td>44.88 ± 6.64</td>
</tr>
<tr>
<td>pCO2 (IJ)</td>
<td>47.00 ± 5.14</td>
<td>47.06 ± 6.72</td>
</tr>
<tr>
<td>O2 sat (A)</td>
<td>94.39 ± 2.69</td>
<td>93.22 ± 3.34</td>
</tr>
<tr>
<td>O2 sat (V)</td>
<td>76.56 ± 1.90</td>
<td>72.54 ± 16.94</td>
</tr>
<tr>
<td>O2 sat (IJ)</td>
<td>76.58 ± 15.21</td>
<td>73.81 ± 15.71</td>
</tr>
<tr>
<td>lactate</td>
<td>2.29 ± 0.65</td>
<td>0.02 ± 0.30</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.18</td>
<td>7.41 ± 0.15</td>
</tr>
<tr>
<td>pCO2</td>
<td>41.31 ± 2.80</td>
<td>39.75 ± 13.67</td>
</tr>
<tr>
<td>pCO2</td>
<td>44.88 ± 6.64</td>
<td>74.86 ± 28.50</td>
</tr>
<tr>
<td>pCO2</td>
<td>47.06 ± 6.72</td>
<td>72.79 ± 32.55</td>
</tr>
<tr>
<td>O2 sat</td>
<td>93.22 ± 3.34</td>
<td>80.94 ± 13.60</td>
</tr>
<tr>
<td>O2 sat</td>
<td>72.54 ± 16.94</td>
<td>21.52 ± 10.97</td>
</tr>
<tr>
<td>O2 sat</td>
<td>73.81 ± 15.71</td>
<td>30.36 ± 16.43</td>
</tr>
<tr>
<td>lactate</td>
<td>2.29 ± 0.65</td>
<td>0.02 ± 0.30</td>
</tr>
</tbody>
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A, aortic; V, ventricular; IJ, internal jugular; ESs O2, systemic oxygen extraction is defined by (aortic – venous) × oxygen content/aortic oxygen content; EXc O2, cerebral oxygen extraction is defined by (aortic – internal jugular) × oxygen content/aortic oxygen content; sat, saturation.

*p < 0.05 at same time points (DCLHb group vs. control group).

**TABLE 3**
Mean blood pressures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DCLHb Group (n = 16)</th>
<th>Control Group (n = 16)</th>
</tr>
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<tbody>
<tr>
<td>Aortic Systolic</td>
<td>110.9 ± 16.08</td>
<td>96.35 ± 12.42</td>
</tr>
<tr>
<td>(PA) Systolic</td>
<td>17.43 ± 8.52</td>
<td>18.00 ± 11.35</td>
</tr>
<tr>
<td>Aortic Diastolic</td>
<td>87.00 ± 16.24</td>
<td>72.00 ± 14.24</td>
</tr>
<tr>
<td>(PA) Diastolic</td>
<td>8.00 ± 6.37</td>
<td>8.50 ± 7.37</td>
</tr>
<tr>
<td>CoPP</td>
<td>93.63 ± 12.96</td>
<td>89.38 ± 13.74</td>
</tr>
<tr>
<td>CePP</td>
<td>90.63 ± 15.92</td>
<td>81.13 ± 17.84</td>
</tr>
</tbody>
</table>

PA, pulmonary artery; CoPP, coronary perfusion pressure (calculated as aortic diastolic pressure – LV diastolic pressure); CePP, cerebral perfusion pressure (calculated as aortic diastolic pressure – pulmonary artery diastolic pressure).

*p < 0.05 (DCLHb vs. control group at same time points, two-sample t test).
In our present study in pigs that suffered 5 min of ventricular fibrillation followed by 10 min of CPR, DCLHb treatment significantly improved resuscitation (i.e., successful ROSC at the end of CPR) compared with saline treatment. This improvement in resuscitation can be most likely attributed to the higher coronary perfusion pressure following DCLHb. In the DCLHb group the coronary perfusion pressure was 64% higher than that in the saline group. Also in animals with successful ROSC, the coronary perfusion pressure was more than double compared with nonsuccessful ones. These observations on coronary perfusion as a key factor to success of ROSC are consistent with the work of others in CPR research (Nienaber et al., 1985; Kern et al., 1988; Paradis et al., 1990).

Another factor relating to the improved ROSC following DCLHb administration is probably its better systemic “oxygenation” effect compared with saline. In the DCLHb group, the systemic oxygen is carried by both the red cell hemoglobin plus the plasma DCLHb. This results in a small but significant increase in the total hemoglobin (red cell plus plasma) over baseline following administration of DCLHb compared with saline (1.2 ± 1.0 versus 0.4 ± 0.8 g/dl, p < 0.5). However, an important contribution of DCLHb toward the systemic “oxygenation” effect is probably related to its cell-free plasma hemoglobin concentration. Following DCLHb administration, the cell-free plasma hemoglobin concentration is 1.3 ± 0.3 g/dl. This concentration of DCLHb solution can carry a significantly higher PO₂ compared with saline solution, as shown by our in vitro oxygen “saturation” studies (Chow and Fan, 2000). Furthermore, the plasma hemoglobin can circulate to a much greater body space, i.e., the plasma water, than the red cell hemoglobin and thus may provide better oxygenation to the ischemic tissues. This postulated superior oxygenation effect following DCLHb is consistent with the significantly higher venous O₂ content and a trend for higher arterial O₂ compared with control (Table 2).

The precise biochemical mechanism of the pressor effect leading to improved coronary and cerebral perfusion pressure following DCLHb cannot be determined in the present study. In previous studies in hemorrhaged rats, DCLHb was shown to remove nitric oxide and increase plasma endothelin (Gulati et al., 1996). Furthermore, DCLHb was found to enhance contraction of isolated pig vessels and this contraction was inversely related to nitric oxide present (Muldoon et al., 1996). Thus, the pressor effect and improved coronary perfusion in the present CPR study are probably related to the removal of nitric oxide in the circulation by DCLHb.

The optimum dose of DCLHb in CPR is unknown and requires further study. Although there was a trend toward better ROSC and myocardial flow in the high-dose group, there was no consistent trend in other parameters and no statistically significant differences were observed in any measured parameters between the high and low doses. The low dose causes less of a volume load. In addition, in our study, the “low” dose (5 ml/kg), when administered during CPR, is already able to achieve a plasma concentration of 1.3 ± 0.3 g/dl, which is 3-fold higher than that expected at normal hemodynamic conditions (Chow et al., 1996). This is due to altered pharmacokinetics of drugs during CPR (Chow et al., 1983; Zhao et al., 1987, 1989). Such an alteration in pharmacokinetics can be a distinct advantage for a drug such as DCLHb, so that a low or normal dose can achieve “high” plasma concentrations to provide better oxygenation.

One potential concern in our study is the use of sodium bicarbonate infusion. Sodium bicarbonate administration is controversial and administration of bolus doses of sodium bicarbonate is no longer recommended by the American Heart Association for cardiac arrest and emergency care. The constant infusion (0.1 mg/kg/min used in our study) has been shown to counter the gradual development of acidosis, which invariably occurs during CPR (Bleske et al., 1992). In the present study all animals received this dosage and thus its use should not bias the study results.

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The present study has several limitations with respect to the effect of DCLHb. First, our study did not adequately evaluate the potential harmful effects of DCLHb, due to the short duration and acute nature of the study. Recently, DCLHb was found to result in an increased mortality (compared with saline treatment) in patients with severe traumatic hemorrhagic shock (Sloan et al., 1999). This is in contrast to an acute animal model showing decreased mortality over a 60-min observation period compared with albumin treatment (Habler et al., 2000). A postulated mechanism for the unfavorable clinical study is the pressor effect, which could have adversely influenced the outcome with DCLHb by accelerating hemorrhage in the hemorrhagic shock patients. Such an effect was not apparent in the acute animal study. The postulated undesirable mechanism for the hemorrhagic shock is unlikely to be relevant to the condition of cardiac arrest and CPR since the latter is different from hemorrhagic shock. The pressor effect is known to be desirable for cardiac arrest and CPR in achieving successful ROSC.

Our present study also differed from the acute hemorrhagic animal study in that we used normal saline rather than albumin as a comparative control, similar to the clinical study.

Second, the present animal study only evaluated the ROSC
after 5 min of ventricular fibrillation (cardiac arrest) followed by 10 min of external massage and ventilation. The effect of longer arrest and CPR is unknown. Furthermore, the influence by other interventions and drugs normally administered early during such a setting, e.g., epinephrine and antiarrhythmic agents, when used in combination with DCLHb is unknown.

Third, we did not adjust the effect of plasma DCLHb on O2 saturation. A previous study showed that at every pH, DCLHb had a higher P50 and lower Bohr effect (Vandegriff et al., 1988). Since the plasma concentration of DCLHb in the present study represented only about 10% of total hemoglobin, the error on O2 saturation contributed by the differences in P50 and Bohr effect between DCLHb versus regular red cell Hb) should be small. Although the contribution of the reduced Bohr effect of DCLHb on O2 saturation is small, the consequence of a reduced Bohr effect from plasma DCLHb may be physiologically significant in that it may result in better tissue acid-base balance, O2 delivery to tissues (e.g., muscle), and CO2 transport to the lungs (Vandegriff et al., 1989).

The finding of the present animal study may have potential useful implications in the management of CPR, especially in the out-of-hospital arrest situation where ventilation is inadequate (e.g., unable to intubate the patient) and transport time to the hospital is prolonged. Under such conditions, severe ischemia will invariably develop, leading to irreversible damage to vital organs. Intravenous infusion of DCLHb not only may lessen the deterioration of coronary perfusion pressure during the prolonged CPR, but also provide exogenous O2 (via the plasma) to the vital organs. Such combined action may allow preservation of the ischemic myocardium and other vital organs before irreversible damage occurs. However, future animal and clinical studies will be needed to verify such potential benefit.

In conclusion, the present animal study demonstrated that DCLHb administration during CPR improved ROSC post 15 min cardiac arrest and CPR. This beneficial effect of DCLHb is most likely related to its improvement in the coronary perfusion pressure and systemic and myocardial oxygenation. These results may have important implications in the future management of CPR.

Acknowledgments

We thank William Dyckman and Edward Hall (Hartford Hospital, Hartford, CT), and Dr. Ken Burhop, Ph.D. (Baxter Healthcare Corporation, Round Lake, IL), for technical support and advice, and Professor Brian Tomlinson (Chinese University of Hong Kong, Hong Kong, China) for review of the manuscript.

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Send reprint requests to: Dr. Moses S. S. Chow, School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, China. E-mail: msschow@cuhk.edu.hk