Acta Med. Okayama, 2008
Vol. 62, No. 4, pp. 235–239
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Acta Medica Okayama
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Original Article

Experimental Study of a Novel Method of Cardiopulmonary Resuscitation Using a Combination of Percutaneous Cardiopulmonary Support and Liposome-encapsulated Hemoglobin (TRM645)

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Percutaneous cardiopulmonary support (PCPS) has been applied for cardiopulmonary arrest (CPA). We have developed a novel method of cardiopulmonary resuscitation using PCPS combined with liposome-encapsulated hemoglobin (TRM645) to improve oxygen delivery to vital organs. Ventricular fibrillation was electrically induced to an adult goat for 10 min. Next, PCPS (30 ml/kg/min, V/Q: 1) was performed for 20 min. Then, external defibrillation was attempted and observed for 120 min. The TRM group (n=5) was filled with 300 mL of TRM645 for the PCPS circuit. The control group (n=5) was filled with the same volume of saline. The delivery of oxygen (DO_2) and oxygen consumption (VO_2) decreased markedly by PCPS after CPA, compared to the preoperative values. DO_2 was kept at a constant level during PCPS in both groups, but VO_2 slowly decreased at 5, 10, and 15 min of PCPS in the control groups, demonstrating that systemic oxygen metabolism decreased with time. In contrast, the decreases in VO_2 were small in the TRM group at 5, 10, and 15 min of PCPS, demonstrating that TRM645 continuously maintained systemic oxygen consumption even at a low flow rate. AST and LDH in the TRM group were lower than the control. There were significant differences at 120 min after the restoration of spontaneous circulation ($p<0.05$).

Key words: percutaneous cardiopulmonary support, liposome-encapsulated hemoglobin, cardiopulmonary resuscitation

Resuscitation using percutaneous cardiopulmonary support (PCPS), which is capable of rapid and strong circulatory and respiratory support, has been investigated for out-of-hospital cardiopulmonary arrest (CPA) patients admitted to emergency rooms [1–3]. However, protraction of ischemia in the brain, heart and other vital organs, even after initiation of support, is a problem with PCPS because the flow rate of circulatory support is low. In this study, to improve the therapeutic outcome of patients with CPA treated with PCPS, liposome-encapsulated hemoglobin (TRM645), which exhibits superior ability of oxygen transport from red blood cells to peripheral tissues, was administered simultaneously with resus-

Received December 26, 2007; accepted February 20, 2008.
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citation by PCPS to ensure oxygen metabolism in peripheral tissues under support at a low flow rate. The efficacy of this method for improving hypoxic conditions in the vital organs was investigated. TRM645 was developed and investigated by Terumo Corporation in cooperation with several laboratories [4–9]. Stroma-free hemoglobin solution was obtained from outdated human red blood cells [10–12]. The liposome particle size was about 200 nm, which is 1/30～40 th the size of human red blood cells and is capable of supplying sufficient oxygen to peripheral tissues with circulatory insufficiency [10–12]. Thus, TRM645 can reach peripheral tissues that can not easily be reached by red blood cells, and it is anticipated that these liposomes can be applied to various fields, such as the treatment of cerebral infarction [13, 14].

In addition, TRM645 has drawn attention as a substitute for blood transfusion because blood compatibility testing is not necessary, long-term storage for 6 months is possible, and infection with hepatitis B and C viruses or human immunodeficiency virus does not occur [15]. However, some risks of another infection and uncertainty of stable blood supply still exist because of use of donated human blood as raw material [16–18]. Recombinant human hemoglobin and albumin-heme have already been developed as a totally synthetic artificial oxygen carrier. At present, the feasibility of using this synthetic oxygen carrier is being tested in small animals, and preparations have been underway to establish large-scale production facilities [17].

This study was performed based on a new concept that liposome-encapsulated hemoglobin as an artificial oxygen transporter can be used to support circulation and respiration at a low flow rate, increasing the resuscitation rate and compensating for the disadvantages of PCPS. Since only a few research facilities are capable of simultaneously performing these 2 treatments, this procedure is rarely performed. The results of this study may contribute significantly toward the treatment of CPA patients.

Materials and Methods

The experiment was performed using 10 adult goats (body weight ± standard deviations: 59 ± 6.5 kg). The goats were sedated with an intramuscular injection of ketamine and inhalation of isoflurane by a mask, followed by tracheotomy and tracheal intubation under general anesthesia with isoflurane (1%) and oxygen. The settings for mechanical ventilation were: fraction of inspired oxygen concentration (F_IO_2): 0.21, tidal volume: 10 mL/kg, respiratory rate: 12/min. After attachment to an electrocardiograph, the insertion of aortic and central venous pressure measurement lines, and posterolateral thoracotomy, the goats underwent ventricular fibrillation (VF) with direct current electric stimulation and ventilation was suspended for 10 min (CPA phase). After the CPA phase, circulation and respiration were supported for 20 min by right atrial blood withdrawal and right carotid arterial blood supply (30 mL/kg/min, V/Q: 1.0) using a PCPS instrument (Capiox EBS, Terumo Corporation, Kanagawa, Japan) (Fig. 1). After 20 min of circulatory and respiratory support, spontaneous circulation was resumed using an external defibrillator, and followed for 120 min (Fig. 2). The animal groups were established for circuits primed with 300 mL of TRM645 (TRM group, 5 animals) and the same volume of saline (control group, 5 animals), and changes in systemic oxygen metabolism induced by TRM645 were investigated. TRM645 was adjusted to 6 g/dl as the concentration of hemoglobin. Thus 300 mL of TRM645 contained 18 g of hemoglobin. Regarding the parameter measurement, the hemodynamic variables were measured over time immediately

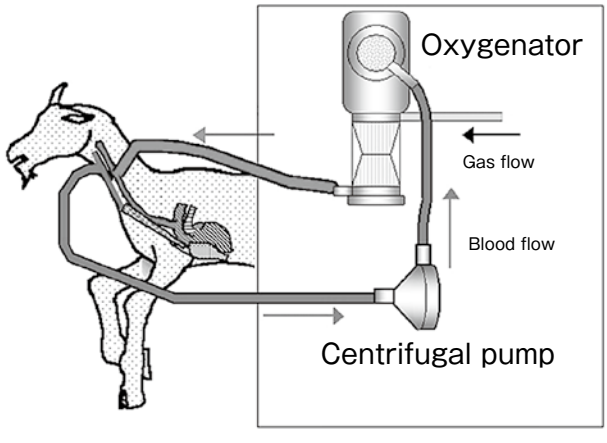

Fig. 1 Schematic illustration of the experimental percutaneous cardiopulmonary support circuit (Capiox EBS, Terumo Corporation, Kanagawa, Japan). The circuit was placed between the superior vena cava via the right jugular vein for withdrawal and right carotid artery for supply.

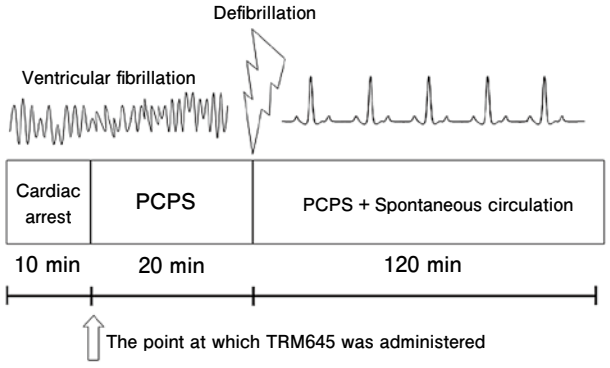

Fig. 2 Experimental procedure. Ventricular fibrillation was induced to a goat for 10 min. Next, PCPS for 20 min. Then, defibrillation was attempted and observed for 120 min. The arrow indicates the point at which TRM645 was administered by PCPS. PCPS, percutaneous cardiopulmonary support.

after the initiation of PCPS, with the values before cardiopulmonary arrest as preoperative values. The delivery of oxygen (DO_2) and oxygen consumption (VO_2) were calculated concurrently (5, 10, and 15 min of PCPS, and 60 and 120 min after restoration of spontaneous circulation: ROSC) as indices of oxygen metabolism in peripheral tissues. DO_2 and VO_2 were calculated using the following equation employing bypass flow (BF) and arterial and mixed venous oxygen contents (CaO_2 and CvO_2):

$$DO_2\ [mL/min] = BF\ [L/min] \times CaO_2\ [mL/dL] \times 10$$

$$VO_2\ [mL/min] = BF\ [L/min] \times (CaO_2 - CvO_2)\ [mL/dL] \times 10$$

$$CaO_2\ [mL/dL] = SaO_2\ [\%] \times Hb\ [g/dL] \times 1.34 + PaO_2\ [mm\ Hg] \times 0.003$$

$$CvO_2\ [mL/dL] = SvO_2\ [\%] \times Hb\ [g/dL] \times 1.34 + PvO_2\ [mm\ Hg] \times 0.003$$

SaO_2, arterial oxygen saturation; SvO_2, mixed venous oxygen saturation; PaO_2, arterial partial oxygen pressure; PvO_2, venous partial oxygen pressure.

BF was recorded continuously using a data acquisition system (PowerLab, ADInstruments, Castle Hill, NSW, Australia). BF was maintained with a Doppler flow meter (Transonic, Transonic Systems, Ithaca, NY, USA). Serum aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were measured periodically as parameters of organ injury by an automated chemical analyzer (DRI-CHEM3000, Fujifilm Medical Co., Ltd., Tokyo, Japan).

The animals were cared for by a veterinarian in accordance with the policies and guidelines for the care and use of laboratory animals by the National Cardiovascular Center Research Institute.

Results

All goats resumed spontaneous circulation after defibrillation. The hematocrit and hemoglobin values were not changed by the initiation of PCPS in either group. DO_2 and VO_2 decreased markedly during respiratory and circulatory support at a low flow rate by PCPS alone after CPA, compared to the preoperative values, and the systemic oxygen transport was about one-half of the preoperative value. DO_2 was kept at an almost constant level during PCPS in both the control and TRM groups, but VO_2 slowly decreased with time to 126, 101, and 90 mL/min at

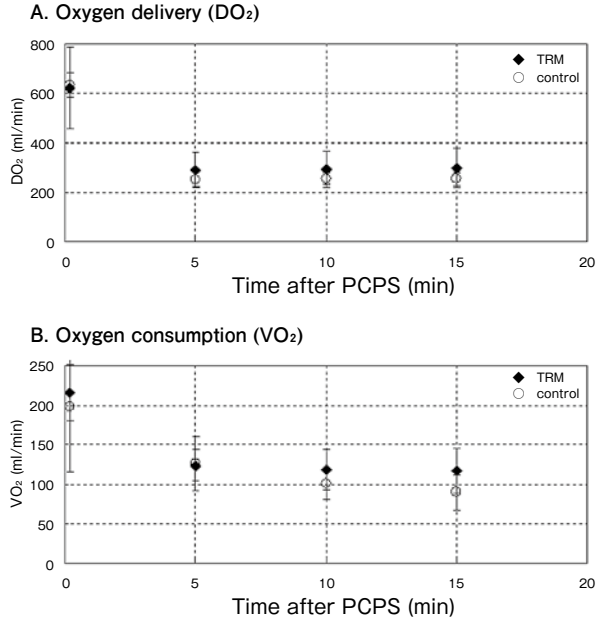

Fig. 3 The levels of DO_2 showed no difference before CPA. During PCPS bypass after 10 min CPA, DO_2 in both groups were decreased and stayed at low levels. VO_2 in both groups were decreased same as DO_2 after CPA. During PCPS bypass, VO_2 gradually decreased in the control group. In contrast VO_2 in the TRM group was kept stable. CPA, cardiopulmonary arrest; DO_2, oxygen delivery; VO_2, oxygen consumption; PCPS, percutaneous cardiopulmonary support.

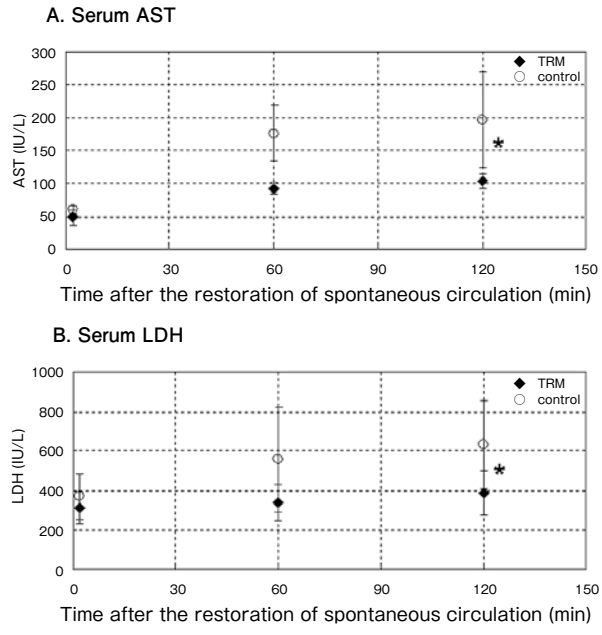

Fig. 4 Serum AST and LDH in the TRM group had the tendency to be maintained lower than the control. The levels of serum AST at 120 min after the restoration of spontaneous circulation in the TRM group were significantly lower than the control. Significance: $*p < 0.05$ versus control group. Serum LDH at 120 min after the restoration of spontaneous circulation was also significantly lower in the TRM group. Significance: $*p < 0.05$ versus control group. AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

5, 10, and 15 min of PCPS in the control groups, demonstrating that systemic oxygen metabolism decreased with time. In contrast, the decreases in VO_2 were very small in the TRM group: 124, 118, and 117 mL/min at 5, 10, and 15 min of PCPS, demonstrating that TRM645 continuously maintained systemic oxygen consumption under circulatory support at a low flow rate (Fig. 3).

As for blood chemistry, AST (49, 93, and 104 vs. 61, 176, and 197 IU/L at ROSC, 60, and 120 min for the TRM and control groups, respectively) and LDH (315, 342 and 388 vs. 370, 560 and 633 IU/L at ROSC, 60, and 120 min for the TRM and control groups, respectively) were apparently lower in the TRM group 120 min after the restoration of spontaneous circulation. There were significant differences (Student's *t*-test) in the values of AST and LDH on 120 min after the return of spontaneous circulation ($p < 0.05$) (Fig. 4).

Discussion

Cardiopulmonary resuscitation by a PCPS system, using a combination of blood supply and withdrawal catheters that can be inserted percutaneously, an extracorporeal membrane oxygenator, and a centrifugal pump, is performed as an aggressive and strong circulatory and respiratory support method by tertiary emergency medical service facilities such as university hospitals or emergency and critical care centers. However, in 2004, only about 3,700 (about 4%) of about 95,000 patients with out-of-hospital cardiopulmonary arrest achieved restoration of spontaneous circulation and survival for 1 month in Japan [19]. Even though spontaneous circulation can be restored by the application of new resuscitation methods, including PCPS, complications, such as encephalopathy after resuscitation and multiple organ failure, can occur, and discharge without severe disability is difficult in many cases. Since the PCPS procedure is limited to the femoral arterial/venous approach in many cases, it is difficult to obtain an optimum flow rate for maintaining systemic circulation; this is one of the causes of the low rate of discharge without severe disability. In cardiopulmonary arrest patients who are in an extreme state of shock, peripheral circulatory insufficiency is marked, and hypoxic conditions in the brain and heart can be prolonged after PCPS. Thus, no marked improvement can be obtained with regard to social rehabilitation. Consequently, there is no standard treatment at present. Liposome-encapsulated hemoglobin was developed as a temporary substitute for transfusion following hemorrhage [4, 7, 8]. For cardiopulmonary arrest, improvement of metabolism by the administration of liposome-encapsulated hemoglobin alone is difficult because it is not possible to guarantee their systemic circulation and delivery to the whole body. Investigation of a clinical application method to sufficiently exploit the traits of liposome-encapsulated hemoglobin is underway. In this study, DO_2 was decreased to half because of the low circulatory flow rate during PCPS for experimental cardiopulmonary arrest. Subsequently, VO_2 was maintained early after the initiation of PCPS, but decreased with time even under PCPS, suggesting that this is one cause of the protraction of oxygen shortage in peripheral tissues due to the low flow rate of circulation, even with PCPS for cardio-

pulmonary arrest or after the restoration of spontaneous circulation, leading to secondary organ impairment. In the method we have proposed, liposome-encapsulated hemoglobin delivered oxygen to the peripheral tissues under low circulatory PCPS, to areas at which red blood cells could not transport oxygen, contributing to maintenance of systemic oxygen metabolism. This method may have reduced secondary organ injury after the restoration of spontaneous circulation. This study established a novel strong circulatory and respiratory support method that supplements the disadvantages of the 2 methods in current use: systemic circulation was maintained during cardiopulmonary arrest by PCPS although the flow rate was low, and the oxygen supply to peripheral tissues was ensured using TRM645.

We determined the feasibility of a novel resuscitation method which used PCPS and TRM645 using a cardiac arrest model of goats. PCPS combined with TRM645 showed potential to maintain the oxygen delivery and consumption during early stage of ROSC and to prevent organ injury after resuscitation.

Acknowledgments. This work was partially supported by a Ministry of Education, Science, Sports and Culture of Japan Grant-in-Aid for Scientific Research, 17659571 (to K.O.).

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