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Blood Substitution: An Experimental Study

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Abstract

Priming fluids for cardiopulmonary bypass have been extremely varied, with resultant hemodilution. Furthermore, major surgeries utilizing cardiopulmonary bypass require multiple postoperative transfusions of blood and blood products. The appeal of having a readily available blood substitute for major cardiovascular and neurosurgical operations could prove to be a life saver, while also eliminating the risk of diseases transmitted by transfusion. Blood substitutes could also lessen the reported complications resulting from blood damage due to prolonged circulation of the blood by the extracorporeal pump. A technique was examined in 15 dogs using hypothermia for maximum metabolic suppression, incorporating an aqueous blood substitute (Cryomedical Sciences, Inc., Rockville, MD). The anesthetized animals were cannulated for extracorporeal pump oxygenation. As temperature was lowered the dogs were exsanguinated and volume replaced with blood substitute to lower the hematocrit to <1%. After 3 hours of cardiac arrest and continuous perfusion at a core temperature <10°C, rewarming began. When temperature reached ≥10°C, the blood substitute was drained and the animals were autotransfused. The heart was started at 15°C and spontaneous respiration resumed at 29°C. Using the first generation blood substitute the survival rate was maximal (100%) at 2.5 hrs under 10°C and 3 hours of cardiac arrest. Research is underway on a new blood substitute, which is to serve as a universal hypothermic preservation solution (in situ organ preservation). When perfected, combining total blood substitution and cooling to ultraprofound (< 10°C) levels may prove beneficial in sustaining cerebral ischemia for prolonged time periods, without incurring major metabolic debt. This may provide significant benefits for neurovascular surgery by prolonging the safe limits of cardiac arrest for several hours, rendering currently inoperable tumors and aneurysms more approachable, as well as a multitude of cardiovascular applications. In addition, this technique could find application in other interventional techniques, including systemic trauma resuscitation and transplantation cases.

Introduction

The composition of the priming solution in cases requiring the use of cardiopulmonary bypass (CPB) and deep hypothermia has lately gained much consideration (1,2). The various reasons behind including or excluding any ingredient still leads to controversy (3). The extent of hemodilution is little debated albeit the effects of blood trauma are well established. (4,5). The concept of whole body blood substitution is a novel technique that may prove amenable to many cardiovascular and neurosurgical procedures (6). The focus of this study is to evaluate the potential application of this technique, using a first generation aqueous blood substitute, in cases using hypothermia. If perfected this method may provide an alternative

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to the current practice of hemodilution when employing CPB techniques.

Materials and Methods

This experimental study was conducted utilizing the guidelines and standards of the United States Public Health Services for the use and care of laboratory animals, and was approved by the institutional animal care and use committee of Allegheny-Singer Research Institute.

Basic preparation: The method used in this model was described by Bailes, et al (6). Fifteen adult mongrel dogs ranging in weight from 10 to 18 kg were used in the study. Preanesthetic medication consisted of .02 mg/kg atropine and a short acting barbiturate (pentothal), 20 mg/kg. The entire surgical preparation was carried out under aseptic technique. After cannulation of a cephalic vein, a plasmalyte drip was started at the rate of 60 ml/hr. Animals were endotrachealy intubated, and an azeotropic mixture of 68.3% halothane and 31.7% ether (flether) was administered at an initial concentration of 2% in 100% oxygen. Oxygen flow rate was at least 2 l/min. They were ventilated at a tidal volume of 250-300 ml at 10-16 breaths/min. ECG leads were placed and heart activity was monitored. An esophageal temperature probe, thermistor type T[a] was advanced into the esophagus to monitor body (core) temperature. A second sensor[b] was placed in the back to monitor the subcutaneous temperature. The right femoral artery was cannulated to monitor systemic arterial blood pressure and for arterial blood sampling. A 7 French Swan-Ganz catheter was advanced to the pulmonary artery through the right femoral vein to monitor the pulmonary artery wedge pressure. The right femoral vein was cannulated to monitor central venous pressure. The animal's blood was anticoagulated using heparin, 100 units/kg, achieving an activated clotting time greater than 300 seconds (438.6 ± 95.1, Mean ± SEM). Arterial blood samples were obtained for measurements of baseline blood gases and pH, and plasma Na^+ and K^+concentrations were determined by a KNA sodium potassium analyzer[c]. Hematocrit was measured by an IEC MB microhematocrit centrifuge[d]. A pre-hypothermia blood sample was collected and sent to the central laboratory for hematology, chemistry and enzymes analysis. A methylprednisolone dose of 10 mg/kg was administered.

Cannulation and cardiopulmonary bypass: The right external jugular vein was cannulated with a 16 gauge cannula and the carotid artery was cannulated with a 14 gauge cannula to establish the extracorporeal cardiac bypass circuit. The circuit as described by Bailes, et al (6) consisted of a heat exchanger[e], a Sarns roller pump[f] and a pediatric bubble oxygenator[g].

The oxygenator had an additional built-in heat exchanger and also acted as a venous reservoir. This circuit was modified with two additions. The first modification was a drain line connected to the venous side of the circuit facilitating exsanguination. The other was a port connecting the oxygenator to a funnel to allow adding the blood substitute to the circuit. Once connection to the bypass circuit was complete, the plasmalyte drip was stopped and surface cooling began by lowering the animal into an ice water bath. As cooling progressed, several blood samples were drawn for blood gases and electrolytes analysis, and pH was managed using alpha-stat strategy. Once the esophageal (core) temperature reached 23°C, or the heart rate slowed to a rate below 45 beats/minutes, exsanguination was started. The blood was collected in sterile containers and placed in refrigeration. Extracorporeal circulation was initiated to wash out the remaining blood and the entire blood volume was exchanged with the blood substitute. A cardioplegic form of the blood substitute solution was employed to stop the heart. Immediately following cardiac arrest the respirator was turned off. The PEEP was kept at approximately 3 mmHg. The blood substitute (K15)[h] was continuously circulated by the roller pump for up to 3 hours under 10°C. The K15 was drained and volume replaced after each hour in order to keep the hematocrit less than 1% and to avert acidosis.

The blood substitute and physiologic parameters: The K15 composition in mmoles is: 117 Na^+, 15 K^+, 118 Cl^-, 1.5 Ca^{++}, 10 Mg^{++}, 10 glucose, 25 HEPES (n-[2-hydroxyethyl] piperazine - n' - [2 - ethanesulfonic acid]), 6% dextran 40, osmolality and pH are 308 and 7.80 respectively. Recirculation of the K15 solution continued for approximately 180 minutes at a core body temperature of 1.5°±.6°C. The mean pump flow rate was 605.0±51.0 ml/min. The mean blood pressure maintained by the pump was 35 mmHg and the central venous pressure was 5 mmHg throughout the continuous circulation phase. The wedge pressure was kept below 5 mmHg, PO_2 above 200 mmHg and the pH around 7.4.

At the end of the procedure, dogs were removed from ice and placed in water to maintain a temperature gradient between body temperature and the surrounding environment of no more than five degrees. The blood substitute was drained and 3 liters of a reduced K^+ version of the substitute were released into the circuit.

Rewarming and resuscitation: External and internal warming continued until esophageal temperature reached 10°C. The animals' own blood was added to the circuit and removal of the blood substitute continued until the whole blood volume was reintroduced into the circulation. The heart either resumed

a Respiratory Support Products Inc., Santa Ana, CA
b Sensortec Inc., Clifton, NJ
c Radiometer, Copenhagen, Denmark
d Needham Heights, MA
e Electromedics, Inc., Engelwood, CO
f Sarns, 3M, Ann Arbor, MI
g William Harvey, Santa Ana, CA.
h Cryomedical Sciences Inc., Rockville, MD

(mM/liter)	Theoretical composition	T0 (Mean value and SEM)	T5	T55	*P* value
NA+	117	122 1.0	123 1.0	127 1.2	.02
K+	15	15.3 .1	14.2 .8	12.1 .4	.00002
Cl-	118	118.3 1.1	117.7 1.2	114.7 1.2	.09
CA++	1.5	2.3 .1	2.2 .1	2.3 .1	N.S.
Mg++	10	10.6 .7	9.8 .6	7.9 .8	.03
Glucose	10	10.3 .04	10.1 .1	9.7 .2	.02
pH	7.80	7.86 .05	7.72 .05	7.61 .05	.003
Osmolality*	308	309.1 .1	310.7 2.2	310.9 1.8	N.S.

* mOsm/liter

Table 1
Changes in the blood substitute K15 composition when circulated in the body for 5 min. and 55 min.

	Survivors Mean Time in minutes (± SEM)	Non-Survivors	*P* value
< 10°C	159.7 (6.7)	192.2 (6.9)	.009
< 20°C	181.1 (7.6)	228.0 (7.4)	.002
< 30°C	261.8 (6.3)	310.0 (11.4)	.001
CPB	219.9 (8.6)	255.8 (4.9)	.01
Cardiac arrest	172.1 (7.0)	224.2 (5.5)	.0005

Table 2
Duration of hypothermia, cardiac arrest and cardiopulmonary bypass time by outcome.

normal sinus rhythm spontaneously when temperature reached 10.4°-28.1°C (15.0°±1.3°C), or was converted by an external shock of 150-200 W/second (joules). Respiration was resumed at 24.3°-34.1°C (29.1°±0.9°C), and mechanical ventilatory support was reinstated. The animals were removed from the bath and placed on an electrically heated water pad. As rewarming progressed, anesthetic was given to smooth the transitional phase from cold narcosis to recovery. In addition, several blood samples were drawn for blood gases and electrolytes analysis. Base deficit was corrected by $NaHCO_3$ administration and PCO_2 was maintained near 35 mmHg. When temperature was above 30°C, animals were weaned from the pump, decannulated, and anesthesia and sedation discontinued. They were then ventilated with 100% O_2. The ECG was continuously monitored and animals were allowed to recover without any restrictions and observed for neurological functions. Several post-hypothermia blood parameters were analyzed on samples collected at one, two and three days and one, two and three weeks following the procedure.

Results

During the procedure, three K15 samples were collected and analyzed. The first, a baseline measurement (T0) before the solution is introduced in the circuit, the second 5 minutes after being circulated in the animal's body (T5), and the third after 55 minutes of continuous circulation (T55). All three samples were collected from a representative sample of six animals subjected to this protocol. All parameters were measured, except for HEPES and Dextran 40. A statistical analysis of variance (ANOVA) was conducted to determine the significance of the changes in the composition of the blood substitute. A p value of ≤ 0.05 was considered significant. The results of these analyses are shown in Table 1.

Two animals' hearts were not successfully resuscitated due to technical errors in using insufficient energy for conversion. The remaining thirteen animals survived the actual procedure. Two animals died and one was sacrificed in less than 24 hours postoperatively.These animals had pulmonary edema, as revealed on autopsy. Of the remaining ten animals one died at four days of neurological complications (seizures) and one died ten days postoperatively of a severe blood transfusion reaction. This reaction was due to the post-operative use of non-matched donor blood. The remaining eight animals survived long term and were behaviorally normal when sacrificed at 30, 32, 40, 51, 54, 55, 64 and 86 days post-procedure. There were differences between survivors (n=10) and non-survivors (n=5) in terms of duration of hypothermia and time on cardiopulmonary bypass (see Table 2). Of the long term survivors two animals were entirely free from complications and six had transient neurological deficits including hind limb weakness. These transient deficits subsequently resolved and all animals at the time of sacrifice had no neurological complications. Representative histopathological examination of the central nervous system of these animals revealed no abnormalities (6).

Discussion

This experimental paradigm builds on a previous pilot

study to explore the possibility of total blood removal and the continuous circulation of an aqueous blood substitute while lowering the body temperature to ultra-profound levels (6). The results suggested that it is not only possible to achieve a completely bloodless state but also to extend hypothermic cardiac arrest to a period approaching three hours while circulating the blood substitute. Parallel studies using a higher nadir of between 8°-10°C are showing improved neurological functions and faster return to normal in hematological and biochemical parameters (7). The problem of pulmonary edema was resolved by monitoring the pulmonary artery wedge pressure during the continuous perfusion phase, especially during fluid exchanges. The transient neurological deficits encountered in the six animals could be due to low blood flow in the spinal cord area due to perfusion via the common carotid artery or to low PCO_2. Concurrent investigation using the femoral artery as the inflow port are showing tentative improved results. Post operative bleeding tendencies were not observed in this group in contrast to findings by other researchers (8). However, we should mention that prothrombin time increased by 3 seconds to be 11.8 seconds on average for the immediate postoperative value returning to normal 24 hours later, at which time the platelet count fell by 50% and then became normal by the third postoperative day.

Even though the K15 used in this study is an inceptive solution used to test the potential applications of this technique, there were no deaths in animals undergoing this procedure for periods of three hours of cardiac arrest, less than 2.5 hours under 10°C. The good outcome with the present technique is superior to that previously reported by other investigations (8). The K15 solution has inherent limitations as a multi-organ preservation solution and future development of new solutions may provide a superior blood substitute in terms of improved tissue protective function. These new solutions may lead to extensions of cardiac arrest times to near four hours (9). We are postulating that improved results in extending the time limits may be due to the complete removal of the blood plasma and formed elements, as compared to hemodilution.

Early clinical profound hypothermia and bypass studies not using hemodilution showed a multitude of severe neurological damage (10). It was presumed that low-temperature viscosity may have caused the deficiencies in the brain microcirculation. Other studies have suggested that red cell aggregates may partially obstruct the capillary beds, leading to poor tissue perfusion (11,12). Initially, the use of stored blood as the priming fluid had many shortcomings ranging from blood pool syndrome to disease transmission. In addition, utilizing blood with CPD-A has been described to negatively affect neurological outcome due to increased levels of glucose and lactate (13). The current practice of crystalloid hemodilution made a noticeable improvement in the microcirculation over that achieved with blood priming (14). The value of adding a colloid such as albumin or hetastarch to increase plasma oncotic pressure has not been universally supported (15,16). In general, the composition of the priming fluid remains extremely varied from institution to institution, not to mention the extent of hemodilution employed. Different levels or percent hemodilution have been performed clinically, ranging from a hematocrit of 30 to 20 percent when using hypothermia as an adjunct in cardiac arrest procedures (17,18).

The degree of hemodilution is little explored even though hematological causes are blamed for much of the adverse effects of hypothermia (4,5). Efforts to extend the one hour time limit of such procedures have included lowering the temperature to profound levels to protect the central nervous system (8,19). These studies, however, did not indicate using a lower hematocrit. Moreover, blood derangements that occur due to stasis and sludging as a result of hypothermia have limited the broad applications of this technique (20-23). In a recent experimental study profound hemodilution (hematocrit <5%) was postulated to have prevented the coagulopathies occurring at profound temperatures (21). Other studies have suggested that excessive hemodilution is associated with loss of the diluent to the extravascular space, leading to progressive reduction of the intravascular volume (24). These observations were probably due to a decrease in osmotic or oncotic pressure when using saline or Ringer's solution. Most edema problems were more prominent in the lungs and monitoring of the pulmonary artery wedge pressure was recognized as an indicator for pulmonary complications (25).

The aspect of having red cells (hemoglobin) in the system during perfusion for oxygen carrying purposes could prove imprudent. It may also have little value in the profound hypothermic state, since the body's, and most importantly the central nervous system's, oxygen consumption is reduced, at a rate of 7 percent per °C; therefore, the demand for oxygen is greatly lowered (26). In addition, at very low temperatures the physical ability of solutions to carry dissolved oxygen is considerably enhanced. This dissolved oxygen may be adequate if the temperature is lowered sufficiently. Furthermore, when temperature is lowered, the hemoglobin saturation curves shift to the left and more oxygen remains bound to hemoglobin, the oxygen dissociation from hemoglobin stops below 12°C (27), rendering little value to having the red cells in the system at these ultraprofound temperatures. Thus, the reduced hemoglobin requirement for oxygen transport and the undesirable increase in viscosity and inherent coagulopathies (28) have made blood removal a very attractive research option. The bloodless state may ameliorate or prevent ischemic injury, by removing erythrocytes, leukocytes and platelets thus eliminating the formed elements of blood, which are implicated as mediators of ischemia and reperfusion injury. The result is the interruption of the cascade of events leading to ischemic

injury. It is possible that during hypothermia and cardiac arrest, as in normothermia, a bloodless state may be substantially advantageous to sustaining ischemia as compared to when blood is in contact with tissue (29). However, experimental trials of exsanguination under hypothermia have had limited success (30,31).

Anecdotal accounts of total blood removal and replacement with a balanced salt solution with and without colloid, under hypothermic condition, showed that it is possible to achieve a bloodless perfusion of between 20 and 90 minutes (32,33). The significance of the current study is the availability of a blood substitute that could offer several attractive features not realized by simply removing the blood (6). In addition to total vascular and capillary washout and the removal of catabolic products, blood substitution provides the opportunity to control the extracellular environment. Also, it could, through pharmacologic components currently being evaluated, provide a membrane stabilizing effect to protect cellular membrane integrity and preserve the intracellular medium. Protecting the intracellular milieu is vital for preserving the biochemical processes by maintaining transmembrane concentrations of solutes, which are essential to the function of cells. This becomes important when the regulatory membrane transport mechanisms are reduced from active processes to essentially passive diffusion in cold ischemia. The blood substitute can also provide substrates for regenerating high energy phosphate compounds upon rewarming. Since the blood substitute is an acellular solution a more effective and expeditious cooling may be attained. On the systemic level, this method of hypothermia, cardiac arrest, blood substitution and low flow perfusion may be advantageous over the classic methods of hypothermia procedures. The use of continuous extracorporeal circulation permits delivery of sufficient substrates and oxygen, apparently without incurring a major metabolic debt. In addition, the advantages of circulatory arrest could perhaps be intermittently employed to minimize the risk of catastrophic aneurysm rupture or to provide vascular collapse. The preliminary results suggest that colder temperatures can be safely achieved while circulating a blood substitute for a period of up three hours of cardiac arrest.

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