Design of Preservation Solutions for Universal Tissue Preservation In Vivo: Demonstration of Efficacy in Preclinical Models of Profound Hypothermic Cardiac Arrest

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ABSTRACT

The design of new solutions for the universal preservation of tissues is a quest that would facilitate multiple-organ harvesting from organ donors since current preservation solutions do not provide optimum preservation for all organs. In contrast, a new approach to bloodless surgery using hypothermic blood substitution (HBS) to protect the whole body during profound hypothermic circulatory arrest (clinical suspended animation) has focused on the development of a hybrid solution design with the objective of providing universal tissue preservation. In this study, a porcine model of uncontrolled lethal hemorrhage was employed. A combination of two new solutions, maintenance and purge, was used in a cardiopulmonary bypass (CPB) technique to affect profound hypothermia and prolonged cardiac arrest (60 min), with resuscitation after surgical repair of the vascular deficit induced to affect exsanguination. After rewarming and recovery, pigs were monitored for 6 weeks for neurological deficits, cognitive function (learning new skills), and organ dysfunction. All the normothermic control animals died (n = 10), whereas 90% (9 of 10) in the HBS group survived ($P < .05$). Moreover, all of the survivors were neurologically intact, displayed normal learning and memory capability, and had no long-term organ dysfunction. Histology of brains after 6 weeks revealed no ischemic damage in marked contrast to control animals, which all showed diffuse ischemic damage. The demonstrated efficacy of these synthetic, acellular HBS solutions for protection of all the tissues in the body during clinical suspended animation justifies their consideration for multiple-organ harvesting from cadaveric and living donors.

MULTIPLE-ORGAN HARVESTING from donors has increased with the increasing demand for organs. However, standard methods of preservation are not optimized for all transplanted tissues and organs. Currently, the formulation of solutions differs depending on the type of organ and whether the excised organs are stored statically on ice or perfused during machine preservation.

Historically, a variety of organ preservation solutions have been developed and, while there are undisputed industry standards for certain organs and applications, the concept of a universal preservation solution for all tissues and organs has

yet to be realized in practice. Generally, the solutions adopted for abdominal visceral organs (kidney, liver, and pancreas)

have not proved optimal for thoracic organs (heart and lungs) and vice versa. In contrast, a new approach to bloodless surgery using hypothermic blood substitution (HBS) to protect the whole body during profound hypothermic circulatory arrest (clinical suspended animation or corporoplegia, meaning body paralysis[1]) has focused on the development of a hybrid solution design with the objective of providing universal tissue preservation during whole-body hypothermic vascular flushing.[1–4]

The studies outlined here used this approach based on the Unisol concept,[5] in which two new solutions (maintenance and purge) formulated for separate roles in the procedure have been tested.[6,7] The principal solution is a hyperkalemic, intracellular-type solution designed to maintain cellular integrity during hypothermic exposure at the nadir temperature ($<10°C$). The companion solution is an extracellular-type purge solution designed to interface between blood and the maintenance solution during both cooling and warming. This novel approach to clinical suspended animation has been established in several large animal models[3,4] and more recently explored for resuscitation after traumatic hemorrhagic shock in preclinical models relevant to both civilian and military applications.[6,8–10] In this study, we demonstrate the efficacy of HBS with the hybrid Unisol solutions for whole-body protection in a porcine model of uncontrolled lethal hemorrhage (ULH).

METHODS
Design of New Hypothermic Blood Substitutes

The formulations of the Unisol solutions used in this study are listed in Table 1 and the rationale for their design has been described elsewhere.[2,3,5]

Preclinical Model of Profound Hypothermic Cardiac Arrest

ULH was induced in female pigs (80–120 lbs) by creating complex vascular injuries that simulated clinical trauma using the techniques and model we have previously established and published.[6,8,11] Following the induction of ULH, corrective intervention was implemented through a thoracotomy approach. A catheter was placed in the aorta and hyperkalemic organ preservation solutions (Unisol, Organ Recovery Systems, Des Plaines, Ill, United States) were infused via cardiopulmonary bypass (CPB) to induce profound (10°C) hypothermia rapidly (2°C/min), as described below.

Induction of Asanguineous Hyperkalemic Hypothermic Metabolic Arrest

A specially designed 24 F double lumen catheter (Cardeon, Cupertino, Calif, United States) with a silastic balloon was inserted into the lacerated thoracic aorta. A standard CPB circuit, was used comprising pumps and heat exchanger (Sarns Inc., Ann Arbor, Mich, United States), membrane oxygenator, nonheparin-bonded circuit tubing, and reservoir (Gish Biomedical Inc., Santa Ana, Calif, United States). The reservoir was primed with 3 L of cold (2°C) high potassium (70 mEq/L) organ preservation maintenance solution (Table 1; Unisol-I intracellular type UHK, Organ Recovery Systems). Flow was started at 500 mL/min through the aortic catheter and resulted in cardioplegic arrest instantaneously. Mechanical ventilation was stopped and a 36 F venous cannula was

inserted into the right atrium to initiate full CPB at flow rates of 3 to 4 L/min. When the core temperature reached 20°C, 2 L of reservoir fluid were exchanged for low-potassium maintenance fluid (Table 1; Unisol-I intracellular type, 25 meq/L, ULK, Organ Recovery Systems). Once the core temperature reached 10°C, flow rates on the bypass machine were reduced to 10 to 20 mL/kg/min and the heat exchanger was adjusted to maintain the temperature. The reservoir fluid was exchanged (~1 L every 15 minutes, total of 4 L) with potassium-free extracellular purge perfusate (Table 1; Unisol-E-K-free, Organ Recovery Systems) to reverse hyperkalemia. During 60 minutes of hypothermia and low-flow bypass, all the vascular injuries were repaired.

At the start of the warming phase, the CPB reservoir was drained to 0.51 of fluid, and 0.5 L of pig whole blood in citrate dextrose solution was added. Flow rates on the CPB were increased to 3 to 4 L/min and temperature of the fluid adjusted to achieve a rewarming rate of 0.5°C/min. As the core temperature increased from 10°C to 32°C, the reservoir was periodically drained and whole blood (maximum of 4000 cc) was introduced to keep up with the increasing oxygen demands. Electrolyte and acid base abnormalities were corrected as needed. Spontaneous cardiac activity typically resumed with the reversal of hyperkalemia and hypothermia. Internal cardioversion was performed if required and mechanical ventilation was restarted.

After a brief period of stabilization, the animals were gradually taken off the CPB. A dose of cefazolin (1 g) and dexamethasone (0.25 mg/kg) was given intravenously, and infusion of autologus shed blood was started and slowly continued over the next 2 hours. Postoperative anesthesia was maintained with isoflurane, and the animals were allowed to breathe spontaneously when they were hemodynamically stable, lactic acidosis was clearing, and normal respiratory drive had returned. This typically took 3 hours. Before transferring the animals to a warm humidified observation cage, the pulmonary arterial and carotid catheters and pleural drains were removed. Intramuscular injections of buprenorphine hydrochloride (0.3 mg) were given every 6 hours for pain control. Endotracheal tubes were removed when the animals were awake, oral intake of water was encouraged on the day of surgery, and regular food was given the next day. Nonhypothermic control animals were treated in the same way except that a normal core temperature was maintained throughout.

POSTOPERATIVE TESTING
Biochemical Markers

In the surviving animals, blood was drawn weekly for measurement of complete blood count, electrolytes, liver enzymes (bilirubin, alkaline phosphatase, aminotransferases), renal function tests (creatinine, urea nitrogen), markers of cell damage (creatine kinase, lactate dehydrogenase, uric acid), pancreatic enzymes (amylase), and nutritional parameters (serum protein, albumin, globulin, triglyceride, cholesterol, lipoproteins).

Neurologic and Cognitive Function Testing

During the postoperative period, neurologic testing was done using a scoring system that has been published previously.[8] This score takes account of the level of consciousness, behavior, feeding, cranial nerves, motor and sensory functions, and coordination. For cognitive function (learning capacity), a method of training was used that is based on the concept of operant conditioning. The detailed description, rationale, and

Table 1. Composition of New Hypothermic Blood Substitutes Compared With Gold standard Organ Preservation Solutions

Components (mmol/L 1^{-1})	BMPS	UW	Unisol-I ULK	Unisol-I UHK	Unisol-E [K+-free]
Ionic					
Na+	100.0	30.0	100.0	62.5	147.2
K+	25.0	125.0	25.0	70.0	-
Ca++	0.5	-	0.05	0.05	1.5
Mg++	5.0	5.0	15.0	15.0	5
Cl−	1.0	-	30.1	30.1	122.0
SO_4-	-	5.0	-	-	1.0
pH Buffers					
H_2PO_4-	25.0	25.0	2.5	2.5	1.2
HPO_42-	-	-	-	-	-
HCO_3-	-	-	5.0	5.0	25.0
HEPES	10.0	-	35.0	35.0	25.0
Impermeants					
Lactobionate-	-	100.0	30.0	30.0	-
Raffinose	-	30.0	-	-	-
Sucrose	-	-	15.0	15.0	-
Mannitol	30.0	-	25.0	25.0	-
Glucose	10.0	-	5.0	5.0	5.0
Gluconate	85.0	-	70.0	70.0	-
Ribose	0.5	-	-	-	-
Colloids					
HES	5%	5%	-	-	-
Dextran 40	-	-	6%	6%	6%
Pharmacologics					
Adenosine	-	5.0	2.0	2.0	1.0
Glutathione	3.0	3.0	3.0	3.0	3.0
Allopurinol	1.0	7.4	-	-	-
Dexamethasone (mg/L)	-	8.0	-	-	-
Adenine	5.0	-	-	-	-
Insulin (U/L)	40.0	100.0	-	-	-
Osmolality (mOsm/kg)	-	320	350	350	315
pH (25°C)	-	7.4	7.6	7.6	7.5
[K+] [Cl−]	25	0	752	2100	732

BMPS, Belzer machine perfusion solution (KPSI from Organ Recovery Systems); UW, University of Wisconsin solution (Viaspan from Barr Laboratories); Unisol-I (ULK), Unisol-intracellular base-low potassium (Organ Recovery Systems); Unisol-I (UHK), Unisol-intracellular base-high potassium (Organ Recovery Systems); Unisol-E, Unisol-extracellular base (Organ Recovery Systems).

validation of this method have been published.[6] The performance of the experimental animals was compared to 15 control animals that were used to establish this training protocol.

Brain Fixation and Histology

Six weeks postexperiment, the animals were anesthetized and their brains were fixed in situ by infusion of ice-cold saline followed by 4% buffered paraformaldehyde through the carotid arteries. The brains were then kept in the same fixative overnight at 4°C, dissected, and examined for gross lesions. Brain blocks were embedded in paraffin and 10 μm frontal sections of cortical, striatal, and hippocampal areas were cut and stained with hematoxylin-eosin and examined for the presence of ischemic changes.

RESULTS

Control animals (n = 10) subjected to normothermic arrest (maximum ischemic insult) universally resulted in brain death

and there were no survivors. In sharp contrast, 9 of 10 of the hypothermically arrested animals (90%) survived, all were neurologically intact at 6 weeks postoperatively, and the animals displayed normal learning capacity (details published elsewhere[11]). One animal in the experimental group was neurologically intact but died on the 24th postoperative day. Autopsy revealed empyema and septic pericarditis. Intraoperatively, profound hypothermia markedly diminished total body metabolic activity, as evidenced by significantly lower build-up of lactic acid during the periods of hypothermia and rewarming compared with the normothermic controls (data reported elsewhere[11]).

The focus of this study is posthypothermic organ function and Table 2 shows the biochemical profiles for the surviving animals. Any biochemical abnormalities detected during the immediate postoperative period were transient and improved rapidly. These included a significant increase in the levels of liver aminotransferases, serum creatine kinase, and lactate dehydrogenase, and a nonsignificant increase in serum creat-

Table 2. Biochemical Profiles of Surviving Animals

Parameter	Baseline	Upon Resuscitation	Week 1	Week 3	Week 6
Alkaline Phos	133.6 ± 10.8	181.7 ± 18.3	103.3 ± 15.7	114.7 ± 11.2	114.0 ± 12.8
Amylase	738.1 ± 57.6	584.6 ± 59.3	602.4 ± 61.6	784.2 ± 50.6	832.4 ± 81.7
CK	1376 ± 558.4	5306 ± 1062*	760.3 ± 406.5	446.1 ± 93.4	550.4 ± 76.5
GGT	65.7 ± 8.3	55.5 ± 10.1	64.0 ± 9.7	98.0 ± 30.0	61.5 ± 11.1
LDH	1385 ± 112.1	5143 ± 924.9*	4024 ± 712.0*	2251 ± 494.9	1359 ± 151.0
Total bilirubin	0.144 ± 0.04	1.129 ± 0.9	0.122 ± 0.01	0.600 ± 0.23	0.610 ± 0.48
Triglycerides	23.4 ± 5.2	18.0 ± 4.9	25.0 ± 4.12	32.6 ± 10.0	22.7 ± 4.6
Uric acid	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
ALT	62.7 ± 3.3	90 ± 10.1	75.8 ± 13.9	60.7 ± 6.7	70.6 ± 10.4
AST	38.9 ± 3.8	273.0 ± 48.6*	57.4 ± 15.3	76.5 ± 25.3	49.5 ± 10.4
Cholesterol	70.7 ± 4.1	52.4 ± 2.3*	73.4 ± 6.9	81.2 ± 1.9	77.1 ± 4.6
Creatinine	1.4 ± 0.1	1.67 ± 0.13	1.22 ± 0.06	1.37 ± 0.07	1.64 ± 0.09
Urea nitrogen	7.43 ± 1.30	10.6 ± 1.12	8.57 ± 1.39	8.87 ± 1.11	9.00 ± 0.88

N = 10, mean ± SEM.

Alkaline Phos, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase; GGT, gamma glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

*$P < .01$ compared to baseline.

inine (Table 2). All of these returned to baseline within the first week. Histological examination of brains from the HBS survivors after 6 weeks revealed no ischemic damage in any of the animals, in marked contrast to the brains from normothermic control animals, which all showed diffuse ischemic damage.

DISCUSSION

In considering the efficacy of a solution for universal tissue preservation, there is no better test than to expose all the tissues of the body to cold ischemia. Moreover, protection of those tissues most exquisitely sensitive to an ischemia/hypoxia insult, namely the heart and brain, provides the greatest challenge. Current interests in the development of hypothermic arrest techniques to facilitate resuscitation of hemorrhagic shock victims in trauma medicine has parenthetically provided an opportunity to examine the efficacy of new HBS solutions for universal tissue preservation. The studies outlined here were undertaken to evaluate this approach based on the Unisol concept,[5] in which new maintenance and purge solutions have been tested for separate roles in the procedure. In accordance with earlier baseline models,[4,12,13] these studies validate the unequivocal benefit of profound hypothermia combined with specially designed blood replacement fluids for the protection of heart, brain, and the other major organs during several hours of cardiac arrest and ensuing ischemia. Moreover, the preservation of higher cognitive functions in these animals subjected to hypothermic arrest further corroborates previous reports of the high level of neuroprotection provided by these HBS solutions.[14]

The general in vivo tissue preservation qualities of this HBS substitution technique are clearly demonstrated by the consistent resuscitation of exanguinating animals after traumatic hypovolemic shock. More specifically, biochemical profiles (Table 2) of the surviving animals showed that, apart from a transient elevation in liver enzymes that normalized within the first postoperative week, there were no metabolic signs of organ dysfunction. This is consistent with previously published observations in a canine model of clinical suspended animation in which specific markers of heart, brain, and muscle injury (creatine kinase isozymes) all showed transient increases in the immediate postoperative period and returned to normal baseline levels within the first postoperative week.[3,4] Also, the present study showed that, even in the control animals that did not survive due to brain injury, their other major organs were well preserved during the ischemic procedure. Interestingly, 50% of the normothermic control animals regained excellent cardiac function after 60 minutes of arrest on minimal CPB flow rates. Moreover, metrics for organ function were not significantly different upon resuscitation than the hypothermic counterparts.[11]

In conclusion, the demonstrated efficacy of these synthetic, acellular HBS solutions for the protection of all tissues and organs in the body during clinical suspended animation justifies their consideration for multiple-organ harvesting from cadaveric and living donors. Furthermore, these observations support the findings of parallel studies for longer-term hypothermic storage of a variety of cell types derived from vascular tissues and kidney in which the Unisol-UHK maintenance solution has provided excellent cell survival compared with a variety of commonly employed solutions.[5,7]

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