Development and Evaluation of a Novel Solution, Somah, for the Procurement and Preservation of Beating and Nonbeating Donor Hearts for Transplantation

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Background—Injury to myocytes, endocardium, and the coronary endothelium during harvesting and storage can compromise outcomes after heart transplantation. Safeguarding of structure and function of cardiomyocytes and endothelium in donor hearts may lead to improved patient survival after transplantation. Information gained from porcine hearts stored in standard transplant solution was used to design a superior preservation solution that would optimally protect and maintain organs from beating heart and/or nonbeating heart donors during long-term storage.

Methods and Results—Multiphoton microscopy was used to image deep within cardiac biopsies and coronary artery tissue harvested from porcine hearts obtained from beating heart and nonbeating heart donors for analysis of myocyte and endothelial cell structure and function. Cell structural integrity and viability, calcium mobilization, and nitric oxide generation were determined with fluorescence viability markers, immunofluorescence, and Western blots. During hypothermic storage in standard preservation solution, Celsior, myocyte, and endothelial viability was markedly attenuated in hearts obtained from beating heart donors. In contrast, hearts from beating and nonbeating heart donors stored in the newly formulated Somah solution demonstrated an increase in high-energy phosphate levels, protection of cardiac myocyte viability, mitochondrial membrane polarization, and structural proteins. Similarly, coronary artery endothelial organization and function, calcium mobilization, and nitric oxide generation were well maintained during temporal storage in Somah.

Conclusions—The Celsior preservation solution in clinical use today has led to a profound decline in cardiomyocyte and endothelial cell viability, whereas the newly designed Somah solution has safeguarded myocyte and endothelial integrity and function during organ storage. Use of Somah as a storage medium may lead to optimized graft function and long-term patient survival after transplantation. (Circulation. 2009;120:1704-1713.)

Key Words: endothelium • myocytes • nitric oxide synthase • transplantation

In the face of advances in transplantation, postoperative outcomes have improved only moderately in the past decade. This is due perhaps to increased acceptance of older recipients with morbidities and, as a result of persistent organ shortage, the inclusion of older, marginal, and nonbeating heart organ sources in the donor pool. Use of organs from marginal and older donors has resulted in decreased graft function and survival compared with organs obtained and used for transplantation from younger donors in previous years. Preserving organ viability during storage is an important prerequisite for successful posttransplantation long-term outcome. Most transplantation centers use cold static storage in either extracellular- or intracellular-type storage solutions to preserve organs, with variable outcomes.

Clinical Perspective on p 1713

The conventional preservation strategy for explanted hearts involves storage of the organ in hyperkalemic, electrolyte solutions that contain impermeants to maintain osmoregulation and control edema, reactive oxygen species scavengers to prevent free radical damage, substrates to maintain energy, and buffers to prevent acidosis. University of Wisconsin Solution, Celsior, Histidine-Tryptophan-Ketoglutarate Solution, and other storage solutions were designed to potentially counterbalance these adverse effects. However, damage to cardiomyocytes and the endothelium by hypothermia, hyperkalemia, edema, energy depletion, and reperfusion injury remains a problem. To offset the negative effects associated with hyperkalemia-induced depolarized arrest and
storage, the concept of polarized or hyperpolarized arrest and storage has been introduced and is being tested through the design of new solutions that contain adenosine, ATP-sensitive potassium channel agonists, and/or sodium channel blockers to potentially prevent ionic imbalance and consequent energy depletion and edema during storage.7

We have previously demonstrated that our surgical conduit storage solution, GALA, is able to preserve the endothelial and smooth muscle function in ex vivo–stored vascular conduits for an extended period.8 Therefore, using a similar strategy and GALA as the foundation, we have formulated a novel organ storage solution, Somah (ambrosia of rejuvenation in Sanskrit), to counterbalance many of the impairing factors encountered by the heart and other organs during storage. We hypothesized that Somah solution will provide optimum cell protection and thus preserve the structure and function of cardiac myocytes and the coronary endothelium not only during short-term hypothermic storage but also for a longer duration. Our objective was to evaluate the comparative efficacy of Somah solution with the commonly used organ preservation solution, Celsior, a solution shown to be equivalent and/or superior2–5 to other marketed solutions used in transplantation.

Methods

Materials

Celsior was purchased from Genzyme Corp (Cambridge, Mass). Creatine orotate was obtained from Man Sports Products Inc (San Diego, Calif), and micronized creatine monohydrate and citrulline malate were purchased from True Protein Inc (Oceanside, Calif). Diaminofluorescein AM ester (DAF-2/DA) was purchased from Calbiochem (La Jolla, Calif). Calcein AM ester, ethidium homodimer (live-dead assay kit), JC-1 mitochondrial polarization dye, and calcium orange were obtained from Molecular Probes Inc (Eugene, Ore).

Animal Protocol

Heart Storage

The study was conducted in 9 female pigs, each weighing 40 to 50 kg, in accordance with a protocol approved by our Animal Studies Subcommittee. The animals were divided into 3 groups of 3 animals. Two different solutions were compared for their ex vivo preservation of the heart: Celsior, the solution currently used in organ transplantation, and the newly formulated Somah solution. In groups 1 and 2, hearts were harvested and immediately stored in Celsior (Celsior beating heart [BH]) and Somah solution (Somah BH), respectively, for 4 hours at 4°C. In group 3, hearts were stored in Somah solution for 4 hours at 4°C (Somah nonbeating heart [NBH]). Cardiomyocyte biopsies were obtained from the anterior and posterior regions of the left ventricle at 0, 60, 120, 180, and 240 minutes. Left anterior descending coronary artery (LAD) samples were collected from the hearts at the end of storage time (240 minutes).

Surgical Procedure

General anesthesia was induced with intramuscular injections of telazol 4 to 6 mg/kg and xylazine 2 mg/kg. After intubation, animals were maintained with isoflurane (1% to 2%) inhalation anesthetic with 100% oxygen and mechanically ventilated. The carotid and femoral artery were cannulated for intravenous access and blood pressure monitoring, respectively. External ECG leads were placed, and the chest was opened via a median sternotomy. The pericardium was opened and elevated with 2-0 silk sutures to the skin. In experimental groups 1 and 2, systemic heparinization with 300 mg/kg was achieved through the arterial line. Ten minutes after heparinization, a 9F aortic vent cannula was placed in the aortic root for infusion of cardioplegia. The aorta was cross-clamped, and 1 L cardioplegia (4°C) was infused rapidly with a pressure bag at a system pressure of 150 mm Hg (coronary pressure, 60 to 70 mm Hg) in all 3 groups. The cardioplegia solution was rapidly vented through the inferior vena cava and left superior pulmonary vein. The hearts of group 1 were arrested and flushed by rapid infusion of Celsior until the hearts became uniformly pale and the effluent was relatively clear. In contrast, hearts of pigs in group 2 were initially perfused with 500 mL of 15 mmol KCl Somah cardioplegia solution, followed by a washout with 500 mL of 7 mmol KCl Somah storage solution. In group 3, the animals were prepared to the point of application of the aortic cross-clamp as described above. The animals were quickly exsanguinated through the superior vena cava until cardiac arrest. One hour postmortem, the aorta was cross-clamped and cannulated as above, and the hearts were rapidly perfused with 7 mmol ice-cold KCl Somah storage solution (4°C). The hearts from all 3 groups were quickly harvested, stored in Celsior or Somah solution at 4°C, and transported to the laboratory for evaluation.

Formulation of Somah Solution

Somah was formulated with the GALA solution8 as the base. GALA is a physiological salt solution that has been shown to effectively preserve the blood vessel endothelium9 but lacks key ingredients to sustain complex organs (heart, liver, kidney, etc) over long-term ex vivo storage. GALA was modified by changing the concentration of some of its ingredients and by including additional key components to formulate Somah solution (Table 1). The solution was freshly made and chilled to 4°C, and the pH was adjusted to 7.5 with Tris-hydroxymethyl aminomethane. The composition of Celsior solution used in this study is given in Table 2.

Cell Viability Assay

Structural and functional viability of cardiomyocytes and endothelial cells was assessed with a fluorescence-based live-dead assay and multiphoton microscopy as described.9–11

Table 1. Composition of Somah Organ Preservation Solution

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water, L</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>1.30</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>7.00</td>
</tr>
<tr>
<td>Potassium phosphate (monobasic)</td>
<td>0.44</td>
</tr>
<tr>
<td>Magnesium chloride (hexahydrate)</td>
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</tr>
<tr>
<td>Magnesium sulfate (heptahydrate)</td>
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<tr>
<td>Sodium chloride</td>
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<tr>
<td>Sodium bicarbonate</td>
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<tr>
<td>Sodium phosphate (dibasic; heptahydrate)</td>
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</tr>
<tr>
<td>d-Glucose</td>
<td>11.00</td>
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<tr>
<td>Glutathione (reduced)</td>
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<tr>
<td>Ascorbic acid</td>
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<tr>
<td>L-Arginine</td>
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<tr>
<td>L-Citruline malate</td>
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<tr>
<td>Creatine orotate</td>
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<td>Creatine monohydrate</td>
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<tr>
<td>L-Carnosine</td>
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</tr>
<tr>
<td>L-Carnitine</td>
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<tr>
<td>Dichloroacetate</td>
<td>0.50</td>
</tr>
<tr>
<td>Insulin 10 mg/mL, mL/L</td>
<td>( \text{pH is adjusted to 7.5 with sodium bicarbonate or Tris-hydroxymethyl aminomethane at desired temperature.} )</td>
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</tbody>
</table>
Table 2. Composition of Celsior

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
</tr>
<tr>
<td>Mannitol</td>
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<tr>
<td>Lactobionic acid</td>
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<td>Glutamic acid</td>
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<tr>
<td>Histidine</td>
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<td>Calcium chloride</td>
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<tr>
<td>Magnesium chloride</td>
<td>13.00</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>100.00</td>
</tr>
<tr>
<td>Glutathione SH</td>
<td>3.00</td>
</tr>
</tbody>
</table>

PH 7.3 at 20°C.

Measurement of Esterase Activity
Conversion of Calcein AM ester (nonfluorescent) to fluorescent Calcein by esterases in living cells was used as a marker of esterase activity in the heart tissues. The enzyme activity was measured and quantified as described.10,11

JC-1 Assay: Evaluation of Mitochondrial Membrane Potential
Cardiac myocytes and LAD segments were labeled with JC-1 membrane potential dye and imaged with multiphoton microscopy. Fluorescence was excited at 790 nm and emission was measured at 520 and 585 nm with narrow bandpass filters. JC-1 forms j-aggregates (red) in polarized mitochondria but remains dissociated into monomers (green) in the cytoplasm in depolarized mitochondria.12 The ratio of red to green fluorescence (polarized to depolarized) was estimated with MetaMorph software. To reduce intersample and intrasample variability, the subsequent fluorescence values were normalized to the values obtained at the start of the experiments for each sample.

Immunofluorescence
Cardiomyocyte biopsy sections were labeled with primary anti-myosin heavy chain, anti–myosin light chain, anti-actinin, anti-actin, and anti–troponin C. LAD sections were labeled with caveolin 3, endothelial nitric oxide synthase (eNOS), and von Willebrand factor (vWF) antibodies as described.11

Western Blotting
Protein Extraction
Left ventricular tissue or LAD sample (20 mg) was suspended in extraction buffer containing a protease inhibitor cocktail. Tissue was homogenized for 30 seconds and centrifuged at 16,100g for 10 minutes, and the supernatant was collected. Equal amounts of total protein (50 μg) from different samples were mixed with Laemmli sample buffer containing 5% β-mercaptoethanol and heated at 100°C for 3 minutes.

Electrophoresis
Proteins were resolved on 7.5%, 10%, or 12% SDS-PAGE and electrophoresed onto the nitrocellulose membrane. Proteins were identified through the use of antibodies (anti–myosin heavy and light chain, anti-actinin, anti-actin, and anti–troponin C for cardiac tissue; anti–caveolin 1, anti-eNOS, and anti-vWF for LAD samples) as described.11

ATP and Creatine Phosphate Assay
ATP and creatine phosphate (CP) were measured in tissue extracts from Somah BH and NBH as described.13 In short, 20 mg heart tissue, cut into 300 small pieces, was suspended in 500 mL of 0.4 mol/L ice-cold perchloric acid and homogenized for 30 seconds. The homogenate was centrifuged at 1970g for 10 minutes at 0°C. An aliquot of the supernatant was neutralized with an equal volume of 0.4 mol/L ice-cold KHCO3 solution and centrifuged as mentioned above. The supernatant was stored at −80°C for ATP and CP measurements. The pellet was dissolved in an equal volume of 0.1 mol/L NaOH, centrifuged as above, and used for protein assay. ATP and CP were measured with a bioluminescent assay kit and spectrophotometer (PerkinElmer, Waltham, Mass) according to the manufacturer’s protocol.

Calcium Mobilization and Generation of Nitric Oxide
Bradykinin-induced calcium mobilization, eNOS activation, and nitric oxide production in the endothelium of LAD were measured with calcium orange dye and DAF-2/DA, respectively, with multiphoton microscopy, and the fluorescence data were normalized as described.8–11

Multiphoton Microscopy
Imaging and semiquantitative fluorescence measurements were performed with a BioRad MRC 1024ES multiphoton imaging system (BioRad, Hercules, Calif) coupled with a MaiTai laser (Spectra-Physics, Mountain View, Calif) tuned to 790 to 820 nm in the transmission and epifluorescence mode as described.8–11 The lumen endothelium and myocytes were identified by XYZ scanning and imaged at depths of 50 μm in artery cross sections and 50 to 100 μm in left ventricular biopsies from the site of excision.8–11

Statistical Analyses
The measurements and data extraction were performed in a blinded fashion. The analyses were focused on comparing the Celsior BH (n = 3) with the Somah BH (n = 3) and Somah NBH (n = 3) groups to evaluate the ability of preservation solutions to maintain viability of the hearts. The esterase activity differences between groups were evaluated by a linear mixed model to account for the potential correlation between repeated measurements over time. Mitochondrial polarization was compared as a ratio change from baseline between the 3 groups. Differences between groups were analyzed with 1-way ANOVA, and the Tukey test was used to identify specific differences between groups. A 2-sample t test was used to evaluate the differences in normalized fluorescence intensity of calcium mobilization and nitric oxide production at 10 minutes between the Celsior BH and Somah BH and between the Celsior BH and Somah NBH groups, respectively. A paired t test was used to assess the difference in ATP and CP values between time 0 and 240 minutes in the Somah BH and Somah NBH groups, respectively. Statistical significance was accepted at the 95% confidence level (P < 0.05). All values used were mean ± SD unless otherwise indicated. All analyses were performed with SAS statistical software version 9.1(SAS Institute, Cary, NC).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Cardiomyocyte Viability in Explanted Hearts
The effect of preservation solutions on myocardial viability in donor hearts was investigated. As shown in Figure 1A, myocytes obtained from all 3 groups showed robust green fluorescence at the start of storage. However, within 60 minutes, Celsior BH myocytes began to exhibit a red fluorescence pattern and a simultaneous decrease in green fluorescence, indicating deterioration of myocyte structure. In contrast, both Somah BH and Somah NBH demonstrated minimal red fluorescence and a robust increase in green
fluorescence during 4 hours of storage, indicating viability and recovery of the cardiomyocytes (Figure 1A).

Functional viability of cardiomyocytes was estimated by measuring the quantum yield of Calcein green fluorescence as a measure of esterase activity in living cells (Figure 1B). The green fluorescence in the tissue decreased temporally in Celsior BH but remained robust and increased with time in both Somah BH and Somah NBH. Time-adjusted esterase activity in Celsior BH, Somah BH, and Somah NBH was 99.03, 162.80, and 177.50 (arbitrary units), respectively. Both Somah BH and Somah NBH groups had significantly higher esterase activity compared with the Celsior BH group ($P=0.0012$ and $P=0.0004$, respectively).

**Polarization State of Mitochondria in Cardiomyocytes**

Functionality of the cardiomyocytes was further evaluated by assessing the polarization state of the mitochondria. The mitochondria in tissue samples from all 3 groups remained initially polarized, as shown by the fluorescence pattern (red fluorescence more than green fluorescence; Figure 2A). However, in Celsior BH, the mitochondria depolarized within 60 minutes of storage. Mitochondrial function deteriorated progressively with time, with a substantial attenuation of total fluorescence (red and green) at the end of storage. In contrast, in both Somah BH and Somah NBH, mitochondria remained polarized during the time of storage (Figure 2B). These results were confirmed by estimation of the mitochondrial membrane polarization ratio (polarized to depolarized) in these samples. As shown in Figure 2B, mitochondria in Celsior BH depolarized progressively during the course of the experiment and remained depolarized. In contrast, mitochondria in hearts stored in Somah solution remained polarized with significant increases in polarization with time of storage (Figure 2B).

**Structural and Contractile Components of Cardiomyocytes**

The immunofluorescence patterns of myosin, actin, actinin, and troponin were not different between the 3 groups (Figure 3). However, the immunofluorescence intensity of these la-
beled proteins appeared to decrease in Celsior BH, indicating a potential loss or degradation during storage. In contrast, Somah BH and Somah NBH showed robust fluorescence labeling of these proteins, indicating preservation of the structural components.

We observed a sizable decrease in myosin, actinin, actin, and troponin C and a substantial loss in myosin light chain proteins in extracts from Celsior BH (Figure 4A). In contrast, structural and contractile proteins were well preserved, clearly resolved, and well labeled in cardiomyocyte extracts from Somah BH (Figure 4A) and Somah NBH over the entire course of storage (Figure 4B), confirming our observations with immunofluorescence (Figure 3).

**Viability of Coronary Arteries**
The LAD obtained from the Celsior BH showed thinning of the endothelial cells and substantial disruption of the smooth muscle medial layer in the artery (Figure 5A). In contrast, the endothelium and smooth muscle cells were well preserved, intact, and morphologically typical in the LAD obtained from Somah BH and Somah NBH (Figure 5A).

Viability of the coronary arteries was assessed in a manner similar to that used for cardiomyocytes mentioned above. Substantial membrane damage and extensive cell death were observed in LAD from the Celsior BH, demonstrated by red fluorescence and decreased green fluorescence. Additionally, myocytes in the medial region also showed sizable damage, characteristic of altered permeability of smooth muscle membranes (Figure 5B). In contrast, both endothelial and smooth muscle cells exhibited robust green fluorescence, indicating preservation of structural integrity, in Somah BH and Somah NBH (Figure 5B). Similarly, esterase activity and mitochondrial polarization remained robust in LAD from Somah BH and Somah NBH; in contrast, both these activities were compromised in Celsior BH (not shown).

Caveolin, eNOS, vWF, and cadherin, components of endothelial cells, were identified with immunofluorescence in the LADs (Figure 5C). In Celsior BH, LAD samples showed noticeable disruption of caveolin and vWF and a decreased labeling of eNOS and cadherin in the endothelium, with potential attenuation of cell function, including nitric oxide production, vasomotor regulation, and cell adhesion. In con-

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**Figure 2.** Mitochondrial polarization in stored hearts. A, Mitochondria were polarized in all heart groups at the start of storage. Mitochondria progressively depolarized in Celsior BH (red fluorescence less than green fluorescence) but remained polarized in Somah BH and Somah NBH during storage. Magnification ×320. B, Mitochondria in Celsior BH progressively depolarized. Mitochondria in Somah BH and Somah NBH showed significant increases in polarization. Each bar represents the mean±SEM of measurement/time point; *P<0.05, Celsior BH vs Somah BH; **P<0.05, Celsior vs Somah NBH.
Contrast, these proteins were robustly labeled and well preserved in the LAD segments obtained from Somah BH and Somah NBH (Figure 5C). Similarly, protein extracts of arteries from Celsior BH showed complete absence of high-molecular-weight vWF and a decrease in extractable eNOS and caveolin proteins (Figure 6). However, these proteins are well preserved in LAD from Somah BH and Somah NBH, thus substantiating our immunofluorescence results (Figure 5C).

Calcium Mobilization and Generation of Nitric Oxide in Coronary Arteries

Bradykinin stimulation of LAD resulted in a minimal change in calcium and nitric oxide fluorescence in Celsior BH (Figure 7A and 7B), indicating attenuated eNOS activity and potentially depressed vasomotor function. In contrast, a significant, time-dependent 2.5- to 3-fold increase in endothelial calcium and nitric oxide fluorescence was observed in LAD from both Somah BH and Somah NBH (Figure 7A and 7B). These results show that both the tonic- and agonist-responsive components of eNOS were fully functional in the artery endothelium of these hearts, very similar to our observation in freshly harvested porcine LAD.10 Normalized fluorescence intensity for calcium mobilization and nitric oxide generation at 10 minutes was significantly higher in the Somah BH than in the Celsior BH group (2.51 ± 0.64 versus 0.85 ± 0.14, P < 0.026; and 2.37 ± 0.25 versus 1.04 ± 0.19, P < 0.013, respectively). Similarly, the Somah NBH group showed greater calcium mobilization and nitric oxide production compared with the Celsior BH group (2.63 ± 1.70 versus 0.85 ± 0.14, P = 0.046; and 1.70 ± 0.2 versus 1.04 ± 0.1, P = 0.042, respectively).

Measurement of High-Energy Phosphates in Somah Hearts

ATP and CP concentrations of Somah BH and Somah NBH are given in Table 3. Interestingly, the low temperature of storage notwithstanding, the hearts stored in Somah solution were able to synthesize ATP and CP and thus increase their concentrations (Table 3). ATP levels increased by 35% and CP levels increased by 60% in the Somah BH. Similarly, ATP levels increased by 75% and CP levels by 243% in the Somah NBH during 4 hours of storage.

Discussion

The objective of this study was to find a solution for extended temporal, extracorporeal preservation, and revitalization of organs on implantation from beating and nonbeating heart.
donors. We hypothesized that the loss of structural and functional integrity in hearts stored in standard storage solutions can be avoided by developing a synergistic physiological salt solution (Somah) that combines various energy substrates, metabolic modulators, free radical scavengers and antioxidants, ammonia chelators and nitric oxide synthase substrates, intracellular and extracellular H+ chelators, and a physiological concentration of calcium. We postulated that optimizing these components would prevent edema, ionic imbalance, and energy depletion and provide a favorable environment and cellular support during ex vivo storage and during reperfusion. The protection of structure and function of cardiomyocytes and the endothelium that we observed in BH and NBH donor hearts stored in Somah confirms this hypothesis (Figures 1 through 7 and Table 3).

Formulation of Somah solution met the energy requirements of cardiomyocytes and coronary endothelium, accentuating the cardioprotective mechanisms during hypothermic and hypoxic storage. The components of Somah also prime the organs with substrates and metabolites during storage to facilitate resumption of biochemical, physiological, and mechanical work on reperfusion after transplantation. Preloading the organs with selective synergistic constituents helps counterbalance the detrimental effects of initial hyperoxic state and allows an uneventful transition to normoxic functional state after transplantation.

The preservation of anaerobic metabolism in organs during hypoxic storage is of critical importance. High-energy phosphates generated through the glycolytic pathway are crucial in maintaining the functional state of cell membranes, during periods of hypoxia and/or ischemia, and during normoxia in the working heart.14 Maintenance of membrane polarity, activity of electrogenic Na/K ATPase, calcium uptake by storage organelles, ionic homeostasis, and membrane asymmetry is crucially dependent on ATP generated via the glycolytic pathway.15,16 A relatively small amount of glycolytic ATP is essential to maintain homeostasis and to prevent irreversible damage to the organ during hypoxic storage. Our results show that Somah solution clearly provides these metabolic requirements (Table 3).

Insulin translocates glucose transporters to the cell surface, facilitating glucose uptake, and synthesis of glycolytic ATP.15–19 Insulin also stimulates intermediary pathways of metabolism via phosphorylation, activating glucose influx, glycogen synthesis, and growth.15 These activities stimulated by insulin in Somah solution were further accentuated by the addition of dichloroacetate, which exerts pluripotent effects by stimulating glucose metabolism and reducing intracellular lactate concentration by accelerating its conversion to pyruvate, facilitating the production of alkali by the conversion of carbon dioxide to bicarbonate.20–22 Thus, dichloroacetate simultaneously enhances both ATP generation and the buffering capacity of the tissue during storage.

**Figure 5.** Evaluation of structural integrity of coronary arteries. A, Thinning of endothelium and disruption of smooth muscle layers was observed in Celsior BH. Endothelium and smooth muscle layers were well maintained in Somah BH and Somah NBH. B, Live-dead assay. Extensive cell membrane damage was observed in endothelial and smooth muscle cells in the Celsior BH. Cell viability was well preserved in Somah BH and Somah NBH. C, Celsior BH showed disruption in the labeling pattern of caveolin and vWF and decreased staining of eNOS and cadherin in the endothelium. These proteins showed robust, smooth, uniform labeling in Somah BH and Somah NBH. Magnification ×320.

**Figure 6.** Structural and functional components of the endothelium in stored hearts. There was no vWF protein immunoreactivity and a 20% and 35% decrease in eNOS and caveolin proteins in artery extracts from the Celsior BH. vWF, eNOS, caveolin were well preserved in Somah BH and Somah NBH. Representative blot from n=3.
The normal oxygen tension of arterial blood is \(
\approx 100 \text{ mm Hg (2} \times 10^{-4} \text{ mol/L)23,24}\). Therefore, although the supply of oxygen is diminished in excised hearts, mitochondria need only minute amounts of oxygen (0.05 mm Hg; \(10^{-7}\) mol/L) for an operational oxidative phosphorylation.14,23,25 However, once a critical anoxic state is reached, decreased mitochondrial oxygen tension leads to membrane depolarization, release of cytochrome C, and induction of apoptosis.15 Interestingly, the concentration of dissolved oxygen in Somah solution was 140 mm Hg (2.8\(\times 10^{-4}\) mol/L) at 4°C. We speculate that this oxygen concentration was sufficient to maintain the mitochondrial respiration and oxidative phosphorylation in the tissue during storage. Our data corroborate this observation in that the mitochondria in the cardiomyocytes from both the Somah BH and Somah NBH remained polarized compared with those stored in Celsior (Figure 2). Furthermore, both the ATP and CP concentrations increased significantly in hearts preserved in Somah (Table 3).

**Table 3. ATP and CP Concentrations in Somah-Stored Porcine Hearts**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ATP, mmol/mg Protein</th>
<th>CP, mmol/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>240 min</td>
</tr>
<tr>
<td>Somah BH (n=3)</td>
<td>0.20±0.10</td>
<td>0.27±0.10</td>
</tr>
<tr>
<td>Somah NBH (n=3)</td>
<td>0.40±0.05</td>
<td>0.70±0.20</td>
</tr>
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</table>

*Paired t test.
a pyrimidine intermediate in the generation of ATP via the pentose-phosphate shunt, and enhances glucose uptake and ATP synthesis.\textsuperscript{7,28}

Carnosine, levocarnitine, and citrulline malate formed important constituents of Somah solution. Carnosine, composed of \(\beta\)-alanine and \(L\)-histidine, provides cardioprotection by intracellular buffering and calcium regulation and serves as an antioxidant, antiglycating, aldehyde-quenching, metal-chelating agent.\textsuperscript{29,30} Levocarnitine (\(L\)-carnitine) facilitates fatty acid translocation across the mitochondria, enhances glucose/carbohydrate metabolism during hypoxia/ischemia,\textsuperscript{31} stimulates anaerobic ATP generation required for maintenance of membrane function during organ storage,\textsuperscript{32} and acts as an antioxidant during reperfusion. Citrulline mediates in urea and nitric oxide cycle. Combined with malate, it has been used in antiasthenic treatment\textsuperscript{33} and been shown to reduce fatigue and improve muscle performance by increasing ATP production. It can also reduce lactic acid and ammonia and increase recovery of ATP and CP.\textsuperscript{34,35} Additional organ-specific, synergistic effects of the components of Somah include the breakdown and/or synthesis products of carnosine, citrulline malate and creatine orotate, into \(\beta\)-alanine, histidine, malate, and fumarate, which enter the Kreb cycle to generate additional ATP. Subsequently, histidine is also converted to glutamine, which then chelates ammonia (from the transaminase reactions) to form carbamoyl phosphate, augmenting the nitric oxide cycle.

Adenosine in Somah solution is a precursor of ATP synthesis and exerts cardioprotective effects via vasodilation, catecholamine antagonism, and preconditioning by activating protein kinase C and mitochondrial potassium channels.\textsuperscript{7,15,36} Adenosine also maintains the heart in a polarized and/or hyperpolarized state, thereby contributing to the reduction in ionic imbalance and associated degenerative changes in the stored heart.\textsuperscript{7,36}

Edema in the transplanted organ is a major clinical problem that impairs the proper functioning of the organs. Storage solutions used in current practice have not addressed the role of activation of hemichannels and/or aquaporins in the heart in the development of edema during organ storage.\textsuperscript{37–39} Conditions during storage may lead to opening of hemichannels and aquaporins, thereby disrupting ionic homeostasis and excessively increasing water uptake and cell volume.\textsuperscript{37} To compensate for increased volume and to avoid rupture, the cells rearrange the cytoskeleton, causing cell stiffness.\textsuperscript{37} Edema and stiffness can lead to organ failure after transplantation. Specific components of Somah solution, including a physiological concentration of calcium (Table 1), address these issues and thus prevent any of these detrimental changes from taking place in the stored hearts.

Long-term outcomes of transplanted hearts are limited by the development of cardiac allograft vasculopathy resulting from immunologic and nonimmunologic risk factors precipitated by injury to and dysfunction of the endothelium.\textsuperscript{40} Therefore, the structural and functional viability of endothelium in coronary vasculature during storage is critical to the long-term survival of a transplanted heart. Our results show that in contrast to Celsior, the LAD endothelium is not injured and remains functionally viable in both groups of Somah hearts (Figures 4 through 6). Similarly, the physiological processes of calcium mobilization, activation of eNOS, and nitric oxide generation are also well preserved (Figure 7). We have previously shown that these protective effects on endothelium and smooth muscle cells are a result of synergism of properties of glutathione, ascorbic acid, and arginine in vessels preserved in GALA,\textsuperscript{3} which also holds true for Somah solution (Table 1).

Currently, numerous patients are awaiting cardiac (organ) transplantation in the United States because of a lack of donor organs or because of the age criterion. Of those patients who receive heart transplants, survival at 5 years after transplantation is 68%.\textsuperscript{1–3} The majority of deaths are due to the accelerated vasculopathy that occurs after cardiac transplantation. However, significantly increasing the storage time, improving the condition of donor hearts/organs during storage, and allowing the use of NBH donors will have a profound impact on the current landscape of transplant surgery. We believe that replacing current preservation solutions with Somah would immediately affect the practice of transplant surgery. Studies are ongoing to evaluate long-term, in vitro preservation of organs in Somah to translate our experimental observations into a clinical reality.

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Disclosures

None.

References

Organ donation is limited by the number of organs available for transplantation, with organs being derived largely from brain-dead, beating heart donors. In addition, the length of storage continues to be a limiting factor with currently available solutions and devices, and the viability of stored organs and their cellular substructures, even within currently accepted storage timeframes, may be compromised. In the case of heart transplantation, hearts must be transplanted within 4 to 6 hours of harvesting, thereby severely restricting the number of organs harvested for transplantation. A new capability to store organs longer may be necessary to increase the number of organs available for transplantation. This could be achieved by developing new solutions and devices that can better preserve the viability of stored organs.

In summary, the ability to preserve hearts for transplantation is a critical issue that needs to be addressed to improve the outcome of heart transplantation. The development of new solutions and devices that can better preserve the viability of stored organs is essential to increase the number of organs available for transplantation and improve the outcome of heart transplantation.

Reference:
Development and Evaluation of a Novel Solution, Somah, for the Procurement and Preservation of Beating and Nonbeating Donor Hearts for Transplantation
Hemant S. Thatte, Laki Rousou, Bader E. Hussaini, Xiu-Gui Lu, Patrick R. Treanor and Shukri F. Khuri

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