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Prolonged Preservation with University of Wisconsin Solution[1]

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Previous studies from this institution using human cell cultures have suggested that University of Wisconsin Solution may be preferred for prolonged cardiac storage. University of Wisconsin Solution (UWS) contains adenosine (5 mmole/liter) which could maintain adenine nucleotides better than other storage fluids. Human cardiomyocytes were isolated from left ventricular biopsies. Cells (seven to nine dishes/group) were rinsed of culture media and placed in one of four solutions: Stanford cardioplegia, phosphate-buffered saline, modified EuroCollins', or UWS. Metabolites were assessed using high-performance liquid chromatography (units = nmole/μg DNA) after 24 hr of storage at 0°C and compared to baseline controls (BASE). Adenosine triphosphate ($P < 0.0001$, ANOVA), adenosine diphosphate ($P < 0.0001$), and adenosine monophosphate ($P < 0.01$) decreased with each solution compared to BASE but were maintained best with UWS ($P < 0.05$). Adenosine increased in the UWS cells only (BASE, 0.029 ± 0.118; UWS, 1.836 ± 1.110; $P < 0.0001$, ANOVA). Adenosine in the UWS cells was largely degraded to inosine (UWS, 1.013 ± 0.779; BASE, 0.034 ± 0.032; $P < 0.0001$) and hypoxanthine (UWS, 0.124 ± 0.091; BASE, 0.005 ± 0.005; $P < 0.001$). University of Wisconsin Solution does preserve adenine nucleotides better than other storage fluids and may improve the clinical results of cardiac transplantation. © 1991 Academic Press, Inc.

INTRODUCTION

Previous studies from this institution using human cell cultures have suggested that University of Wisconsin Solution (UWS) is preferred for prolonged cardiac preservation [1, 2]. Other investigations using isolated hearts [3–5] or heterotopic transplantation [6] are consistent with these findings. University of Wisconsin Solution contains adenosine (5 mmole/liter) which could maintain adenine nucleotides better than other storage fluids. This study was designed to assess purine metabolites following prolonged storage using UWS compared to other preservation fluids.

METHODS

Cell Cultures

The techniques of human cardiomyocyte culture have been previously reported [1, 2]. Cardiomyocytes were obtained from skinny needle left ventricular biopsies acquired during elective aortocoronary bypass surgery. Patients signed a consent form approved by the institutional human ethics committee. Tissue was minced following which the cells were dissipated with collagenase (0.1%) and trypsin (2%). The supernatant was collected. Cells were separated by centrifugation for 5 min at 600*g*. The precipitated cells were suspended in culture medium containing fetal bovine serum and DMEM (GIBCO Laboratories Life Technologists, Inc., Grand Island, NY) at 37°C with an environment of 5% CO_2 and PO_2 of 150 mm Hg.

Cells were grown until confluent and then passage with trypsin was performed. Fibroblasts and myocytes were manually separated and the identity of the myocytes was determined using antiactin [7] and anti-ventricular light chain 1 antibodies [8].

Protocol

Four solutions were evaluated: Stanford cardioplegia (STA), phosphate-buffered saline (PBS), modified EuroCollins' (COL), and UWS. The composition of the storage fluids is presented in Table 1. For the baseline control studies (BASE), the culture media was discarded, and the cells were rinsed with chilled phosphate-buffered saline and then frozen with liquid nitrogen. For the experimental groups, the culture media was discarded and the cells were rinsed with phosphate-buffered saline. Ten milliliters of chilled storage solution was then added to each dish. Cells were stored at 0°C for 24 hr. At the end of the preservation period, the storage fluids were discarded, and the cells were rinsed with phosphate-buffered saline and then frozen with liquid

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TABLE 1

Storage Solutions

	STA	PBS	COL	UWS
K^+ (mmole/liter)	27	4.2	114	125
Na^+ (mmole/liter)	20	145	10	30
Mg^{2+} (mmole/liter)	—	5	5	5
Ca^{2+} (μmole/liter)	—	—	25	—
Cl^- (mmole/liter)	140	—	15	—
Phosphate (mmole/liter)	—	9.6	57.5	25
$HCO3^-$ (mmole/liter)	20	—	10	—
$SO4^{2-}$ (mmole/liter)	—	—	5	5
Glucose (mmole/liter)	225	—	139	—
Lactobionate (mmole/liter)	—	—	—	100
Mannitol (mmole/liter)	63	—	—	—
Raffinose (mmole/liter)	—	—	—	30
Allopurinol (mmole/liter)	—	—	—	1
Adenosine (mmole/liter)	—	—	—	5
Glutathione (mmole/liter)	—	—	—	3
Pentastarch (%)	—	—	—	5

Note. STA, Stanford cardioplegia; PBS, phosphate-buffered saline; COL, modified EuroCollins'; UWS, University of Wisconsin Solution.

nitrogen. The cells were lyophilized and stored at −80°C until analysis. There were seven to nine dishes/group.

Nucleotide Extraction from Myocytes

The nucleotide extraction technique was based on the method of Shryock *et al.* [9]. An aqueous methanol solution (80%, 5 ml, 70–75°C) was added to each dish of cells. The frozen cells were then transferred to a glass homogenizer and the homogenate was transferred to centrifuge tubes. The glassware was kept on ice at all times. The tubes were centrifuged for 10 min at 2500 rpm and 4°C. The supernatant was evaporated to dryness under nitrogen at room temperature. The pellet was used for DNA quantification. Water (0.5 ml) and 0.5 ml Freon:heptane (4:1) solution were added to the dried nucleotide residues. After vortexing, the upper layer of the resulting two phases was transferred to a microcentrifuge tube and frozen in liquid nitrogen. This extract was lyophilized and reconstituted in 100 m*M* $NH_4H_2PO_4$ buffer prior to analysis by high-performance liquid chromatography (HPLC).

The protocol for DNA quantification was based on the method of Maniatis *et al.* [10]. The samples were assayed on a Beckman DU-40 spectrophotometer (Beckman Instruments, Inc., Irvine, CA) at 260 nm.

HPLC Protocol

The levels of adenine nucleotides and their degradation products were measured using HPLC according to a modification of the method of Hull-Ryde and associates [11] and employing the Waters Maxima 820 Chromatography Workstation computer program as previously described [12]. Samples were maintained at 4°C and injected with an automatic injector (Model 700 Satellite WISP, Waters Associates, Mississauga, Ontario, Canada). A step-gradient solvent delivery was accomplished via a Waters Solvent Select Valve and two reciprocating pumps (Waters Associates, Models 501 and 510). The chromatographic column was a Radial-Pak Resolve C18 Column with a 5-μm particle size (Waters Associates) which was operated in a radial compression module (Waters Associates, Model RCM 100) at 175 kg/cm^2. A programmable multiwave length detector (Waters Associates, Model 490) was used to monitor the peaks obtained at an absorbance of 254 nm. The previously mentioned equipment was controlled by a System Interface Module (Waters Associated, Model I-200).

The system measured the levels of uric acid, adenosine triphosphate (ATP), inosine monophosphate (IMP), adenosine diphosphate (ADP), hypoxanthine, xanthine, adenosine monophosphate (AMP), inosine, adenosine, and 2′-*O*-methyladenosine at 254 nm. Peak areas were integrated by the Waters Maxima 820 computer program. The concentrations of the adenine nucleotides and their degradation products were expressed as nanomoles per microgram of DNA.

Statistical Analysis

Data analysis was facilitated using Statistical Analysis System software (SAS Institute, Cary, NC) and a microcomputer. Data are summarized as the mean ± standard deviation in the text and figures. The treatment effect was tested initially with analysis of variance [12] and the differences between means were specified with Duncan's multiple range test. Statistical significance is assumed for $P < 0.05$.

RESULTS

The results of the adenine nucleotides ATP, ADP, and AMP are summarized in Fig. 1. ATP, ADP, and AMP were all severely depressed after 24 hr storage with each of the solutions tested, although they were better maintained with UWS compared to other preservation fluids. ATP values following 24 hr storage were less than 10% of BASE values while ADP and AMP results were approximately 25% of BASE results with UWS.

The adenosine, inosine, and hypoxanthine results are depicted in Fig. 2. Adenosine increased significantly in the UWS group from 0.029 ± 0.018 to 1.836 ± 1.110. Adenosine in the UWS cells was largely degraded to inosine and to a lesser degree to hypoxanthine. Inosine concentrations were elevated with COL and hypoxanthine was increased with PBS and STA storage. Values for xanthine or uric acid were always small ($P < 0.01$ nmole/μg DNA) and generally unmeasurable.

The proportional contribution of each of the purine metabolites is presented (Fig. 3). It demonstrates that total purine metabolites were increased with UWS com-

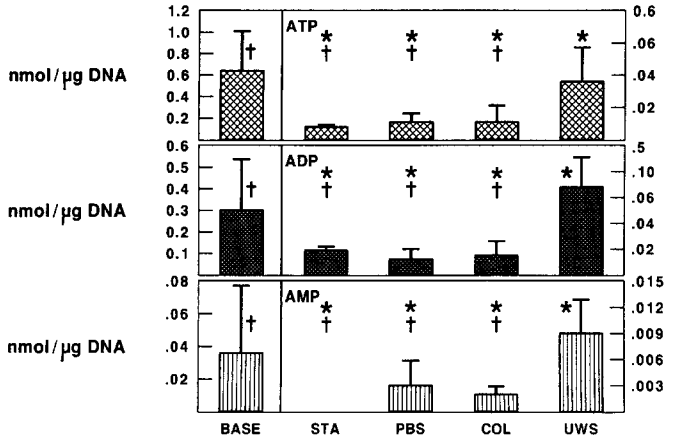

FIG. 1. The results of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) are displayed for baseline controls (BASE), Stanford cardioplegia (STA), phosphate-buffered saline (PBS), modified EuroCollins' (COL), and University of Wisconsin Solution (UWS). The vertical scales on the left-hand side refer to BASE values only, while the right-hand scales refer to the various storage solution results. *$P < 0.05$ vs BASE. †$P < 0.05$ vs UWS.

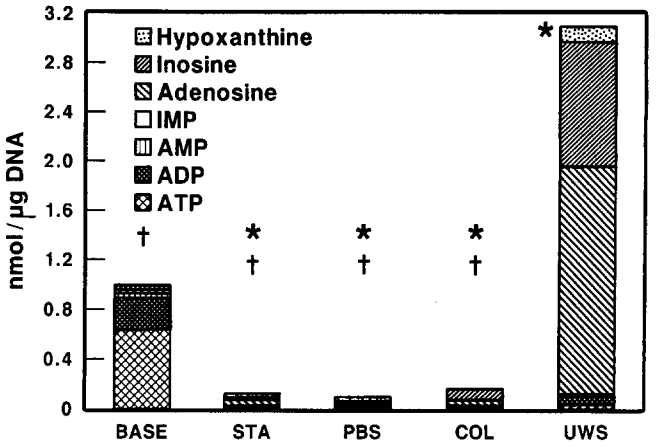

FIG. 3. The proportional contribution of each of the purine compounds are depicted. Abbreviations are as for Fig. 1. IMP, inosine monophosphate. *$P < 0.05$ vs BASE. †$P < 0.05$ vs UWS.

pared to BASE, largely due to the increase in adenosine and inosine. Total purine compounds were depleted with other methods of storage.

DISCUSSION

Information supplied from the Registry of the International Society for Heart Transplantation indicates that the 30-day mortality is approximately 10% [14–16] and that cardiac causes are responsible for 40% of operative deaths [16]. Prolonged ischemic time is an important univariate [14] and multivariate [15] predictor of operative mortality for cardiac transplantation. Operative mortality is increased for ischemic times in excess of 4 hr.

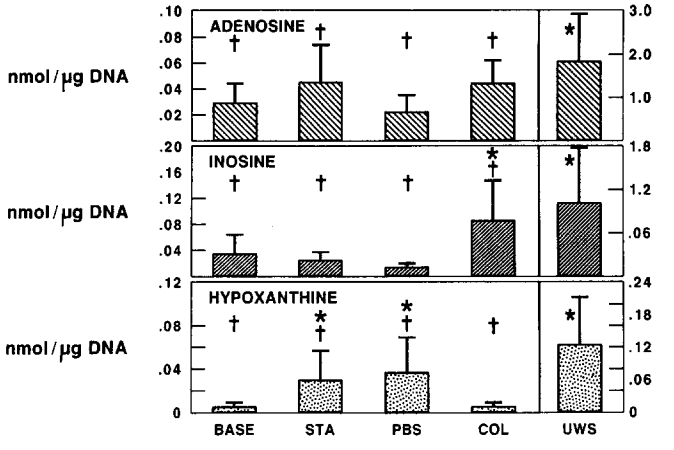

FIG. 2. The results of adenosine, inosine, and hypoxanthine are demonstrated for each of the storage conditions. Abbreviations are as for Fig. 1. The right-hand scales refer to UWS results, while the scales on the left side refer to all other values. Xanthine and uric acid samples were generally unmeasurable. *$P < 0.05$ vs BASE. †$P < 0.05$ vs UWS.

The favorable results of cardiac transplantation have resulted in expanded indications for cardiac recipients. This, in association with an increase in the number of heart transplantation centers [16], has resulted in greater disparity between the number of available organs and potential recipients. One solution to this problem is to broaden the criteria of donor acceptability. Improving the safety or prolonged hypothermic storage should extend the benefits of cardiac transplantation to more organ recipients by reducing the mortality associated with prolonged cross-clamp times and perhaps by increasing the acceptability of donor organs considered to be at high risk by nature of advanced age, excessive inotropic utilization, or small size [17–19].

Isolated cell cultures provide an excellent model for metabolic investigations of myocardial tissue [20]. Human cells were employed for these studies, obviating concerns relating to species differences [21]. The cell culture experiments cannot, however, replace studies involving the intact organ. The adult myocytes are quiescent in culture. The cells are submerged in a relatively large volume of storage solution and therefore exogenous substrates may be depleted more slowly and end products of metabolism accumulate less rapidly than in the intact organ. We suggest that results obtained in cell culture studies are relevant as a screening test for cardiac preservation.

For clinical transplantation in the United States, 45% of programs employ Stanford cardioplegia, while 42% use extracellular solutions as determined by a questionnaire evaluated by Gott and colleagues [5]. The early results reported are similar (operative mortality 9.2% with STA, 10% with extracellular solutions), and were dependent upon the organ ischemic time (1–2 hr, <8%; 4–5 hr, >15%).

Several groups have suggested that UWS may be preferred for cardiac preservation compared to conventional storage techniques. Swanson and colleagues [3] evaluated early results of canine hearts stored for either 5 or 12 hr with UWS, modified Collins', or Stanford cardioplegia. Similar preservation was achieved with each of the methods after 5 hr storage. Following 12 hr, tissue sodium and water were significantly lower and postischemic recovery of ventricular function was improved with UWS. Yeh and colleagues [4] compared UWS and St. Thomas' solution in rodent hearts stored for 6 hr. Tissue electrolyte compositions and histology were better maintained with UWS. Recovery of diastolic compliance and developed pressure was more complete with UWS. Gott and colleagues [5] determined that 6 hr canine preservation was superior with Stanford cardioplegia or UWS compared to extracellular solutions with respect to recovery of ventricular function but not significantly different between UWS and Stanford cardioplegia. Okouchi and co-workers [6] studied heterotopic transplantation in inbred rats after 16 hr cold storage. Graft survival at 1 week was five of six hearts with UWS, two of six with Bretschneiders' solution, and one of six with either saline or a glucose–insulin–potassium solution. Interstitial fibrosis was present but the myocardium was well preserved with UWS.

University of Wisconsin Solution contains several ingredients, the importance of each of these components for prolonged preservation being unclear. Experiments conducted by Jamieson and associates [22] using isolated perfused rabbit livers suggested that lactobionate, raffinose, and glutathione were essential, that the concentrations of potassium and sodium could be reversed, and that several components could be eliminated. Swanson and associates [3], Gott and co-workers [5], and previous studies from this institution [1, 2] employed the unmodified version. Yeh and colleagues added calcium [4] to their formulation while Okouchi *et al.* [6] reversed the sodium and potassium concentrations and eliminated hydroxyethyl starch and adenosine. Experiments have not identified the necessary, essential or optimal component concentrations for cardiac transplantation.

We evaluated adenine nucleotides following 24 hr storage using STA, PBS (an extracellular solution), modified EuroCollins' (an intracellular solution), and UWS. University of Wisconsin Solution contains 5 mmole/liter adenosine. Adenosine entered the cardiac myocyte in the UWS experiments and was converted into inosine and subsequently hypoxanthine. We did not identify any significant amounts of xanthine or uric acid in our samples. Other studies conducted in adults undergoing coronary bypass surgery did not identify any appreciable quantities of xanthine or uric acid despite elevations of tissue hypoxanthine [23]. These results support the concept that xanthine oxidase activity is minimal in human myocardium [24]. Alternative explanations regarding the absence of xanthine and uric acid relate to the presence of allopurinol (1 mmole/liter) in UWS or the lack of reperfusion in these studies.

Total purine compounds increased with UWS and were depleted with the other preservation techniques. Total purine compounds increased in the UWS cells essentially due to adenosine and inosine concentrations. Adenine nucleotides were depleted with all storage solutions but were significantly increased in the UWS studies compared with the STA, PBS, or COL results. This may be related to the augmented cellular adenosine observed following UWS storage, despite the preferential production of inosine. Alternatively, increased adenine nucleotides may only reflect improved viability secondary to enhanced storage conditions unrelated to the presence or absence of adenosine in the solution. Additional experiments with and without adenosine are required to clarify the role of adenosine for hypothermic cardiac storage.

Adenosine supplementation appears protective in other models of cardiac ischemia–reperfusion [25–29]. The mechanism of action is thought to be restoration of nucleoside concentrations depleted during ischemia/reperfusion to augment postischemic adenine nucleotide levels and postischemic ventricular function recovery [30]. Other adenosine effects—such as nodal depression [31], vascular dilatation [29], or inhibition of neutrophils or platelets [32, 33]—may be important *in vivo*. The benefits of adenosine appear enhanced by the addition of adenosine deaminase inhibitors [24–26, 28] or by nucleoside transport blockers [35, 36].

University of Wisconsin Solution preserved adenine nucleotide levels better than other preservation solutions using isolated human myocardial cells as a screening test for cardiac preservation. The present data and previous experiments using human tissues [1, 2] and other published results [3–6] involving animal models are consistent with respect to the recommendation that UWS is preferred for cardiac transplantation. University of Wisconsin Solution may extend the present limits of organ ischemia and expand the current criteria for donor acceptability.

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