Cardiac Storage With University of Wisconsin Solution and a Nucleoside-Transport Blocker

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Findings from previous investigations conducted at this institution and others have suggested that University of Wisconsin solution (UWS) is preferable for the prolonged hypothermic storage of hearts before transplantation. The benefit seen with UWS may in part be related to the inclusion of adenosine (5 mmol/L) in the UWS. To investigate whether further manipulations of adenosine metabolism might enhance myocardial protection, studies were initially conducted using cultured myocytes, followed by confirmatory experiments using isolated rat hearts. Cultured human ventricular myocytes (7 to 8 dishes/group) were stored for 12 hours at 0°C in unmodified UWS or UWS supplemented with increasing concentrations (1 to 100 μmol/L) of the nucleoside-transport blocker *p*-nitrobenzylthioinosine. The adenosine triphosphate concentrations were found to be enhanced with nucleoside-transport inhibition, with the best results achieved with the 1- and 3-μmol/L groups (control, 3.37 ± 0.41 nmol/μg DNA; UWS, 2.89 ± 1.31 nmol/μg DNA; 1 μmol/L, 5.91 ± 3.23 nmol/μg DNA; 3 μmol/L, 7.86 ± 3.45 nmol/μg DNA; $p < 0.05$ versus control or UWS group). Isolated rodent hearts from Sprague-Dawley rats were prepared on a Langendorff apparatus with an intraventricular balloon and subsequently stored for 8 hours at 0°C in unmodified UWS (13 hearts/group) or UWS supplemented with 1 or 3 μmol/L of *p*-nitrobenzylthioinosine (9 to 10 hearts/group). In separate experiments (5 to 6 hearts/group), the tissue levels of purine metabolites were monitored. The addition of the nucleoside-transport blocker was associated with an increased postischemic developed pressure (UWS, 66.2% ± 11.1%; 1 μmol/L, 75.8% ± 6.4%; 3 μmol/L, 78.3% ± 10.7%; $p < 0.05$ UWS versus 1- or 3-μmol/L group) while diastolic compliance was reduced after storage in all groups ($p = 0.01$). Coronary flow was increased in association with the nucleoside-transport blocker (UWS, 71.3% ± 16.7%; 1 μmol/L, 78.5% ± 11.0%; 3 μmol/L, 86.6% ± 13.3%; $p < 0.05$ UWS versus 3 μmol/L). The *p*-nitrobenzylthioinosine did seem to block nucleoside transport, as the hypoxanthine content was unmeasurable after perfusion in the treated groups compared with the findings in the group receiving unmodified UWS ($p < 0.01$).

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Findings from previous investigations conducted at this institution [1, 2] and at others [3–5] recommend the use of University of Wisconsin solution (UWS) for the prolonged hypothermic storage of hearts before transplantation. University of Wisconsin solution contains the cardioprotective agent adenosine (5 mmol/L), which may contribute to the improved results [6]. The benefits of exogenous or endogenous adenosine observed in various ischemia–reperfusion models are enhanced by adenosine deaminase inhibition [7], acadesine [8], or blockers of transmembrane nucleoside transport that result in increased levels of tissue nucleosides [9]. To determine whether the addition of a nucleoside-transport blocker can augment cardiac recovery after prolonged hypothermic storage with UWS, we carried out experiments using *p*-nitrobenzylthioinosine (NBMPR), initially using cultured human cardiomyocytes as a screening test. These results subsequently were applied in the setting of an isolated rodent heart preparation.

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Methods

Human Cardiomyocyte Experiments

Cultured human ventricular cardiomyocytes were prepared from infundibular myocardium of the right ventricular outflow tract, as previously described [1, 2]. Myocardium was obtained from patients with tetralogy of Fallot, aged 2 to 4 years, whose parents had signed a consent form approved by the institutional human ethics committee. All experiments were performed using cell cultures established from a single patient. Control results were obtained by discarding the culture medium, rinsing the cells with phosphate-buffered saline solution, and then freezing the cells with liquid nitrogen. To obtain the storage results, after removal of the culture media and rinsing the cells with phosphate-buffered saline solution, 12 mL of a chilled test solution was placed in a culture dish. The dishes were stored for 12 hours at 0°C, after which the preservation fluid was discarded, the cells were rinsed with phosphate-buffered saline, and the cells were then frozen with liquid nitrogen.

The NBMPR (*s*-[*p*-nitrobenzyl]-6-thioinosine) was obtained from Sigma Chemical Company (St. Louis, MO).

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The storage solutions consisted of UWS or UWS supplemented with 1, 3, 10, 30, or 100 μmol/L of NBMPR (groups UWS, N1, N3, N10, N30, and N100, respectively). The NBMPR was solubilized in a vehicle composed of dimethyl sulfoxide (Anachemia, Toronto, Ontario, Canada). A stock solution with an NBMPR concentration of 20 mg/mL was prepared, and aliquots of this stock were employed to formulate the various concentrations of NBMPR in UWS, with appropriate corrections made for the final volume. There were seven to eight dishes per group. Additionally, cells were stored in UWS supplemented with the vehicle but without NBMPR.

The methods for purine extraction and analysis have been described elsewhere in extensive detail [10]. The DNA in the pellets remaining from the adenine nucleotide analysis was assayed using the method of Burton [11]. Cold (4°C) 5% perchloric acid was added to each sample, followed by mixing and incubation on ice for at least 10 minutes. After centrifugation at 12,000 *g* for 10 minutes, the supernatants were removed and the pellets were resuspended in cold 5% perchloric acid and kept on ice for 10 minutes. All samples and a DNA standard (1.0 mg/mL of calf thymus DNA [Sigma]) in 5 mmol/L of sodium hydroxide and an equal volume of cold 10% perchloric acid were placed in a 70°C water bath for 15 minutes. The samples and standard were then spun at 12,000 *g* for 10 minutes and the supernatants transferred to new tubes. A diphenylamine reagent consisting of diphenylamine (88.6 mmol/L), glacial acetic acid (98%), concentrated sulfuric acid (1.5%), and a 16-mg/mL acetaldehyde solution (0.5%) was added to each of the samples and DNA standard aliquots and to a tube containing 5% perchloric acid (blank) in a ratio of two parts of the diphenylamine reagent to one part of the sample. All of the samples and standards were then incubated at room temperature in darkness for 16 hours. The optical densities of the standards and samples were then measured on a spectrophotometer (DU-40; Beckman Instruments, Irvine, CA) at a spectral wave length of 600 nm. The concentrations of adenine nucleotides and their degradation products were expressed as nanomoles per microgram of DNA.

Isolated Rodent Heart Experiments

Hearts were obtained from Sprague-Dawley rats (weight, 250 to 500 g), and all animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication number 85-23, revised 1985). Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. Heparin (200 units) was administered intravenously. A median sternotomy was performed, and the hearts were rapidly excised and immersed in chilled normal saline solution. The experimental preparation has been described previously [12]. After excision the hearts were subsequently perfused in a Langendorff apparatus with filtered Krebs-Henseleit buffer at a pressure of 100 cm H_2O. The reservoirs and conduits were placed in a water jacket kept at 37°C. The perfusate was aerated with 95% oxygen and 5% carbon dioxide and the pH adjusted to 7.4.

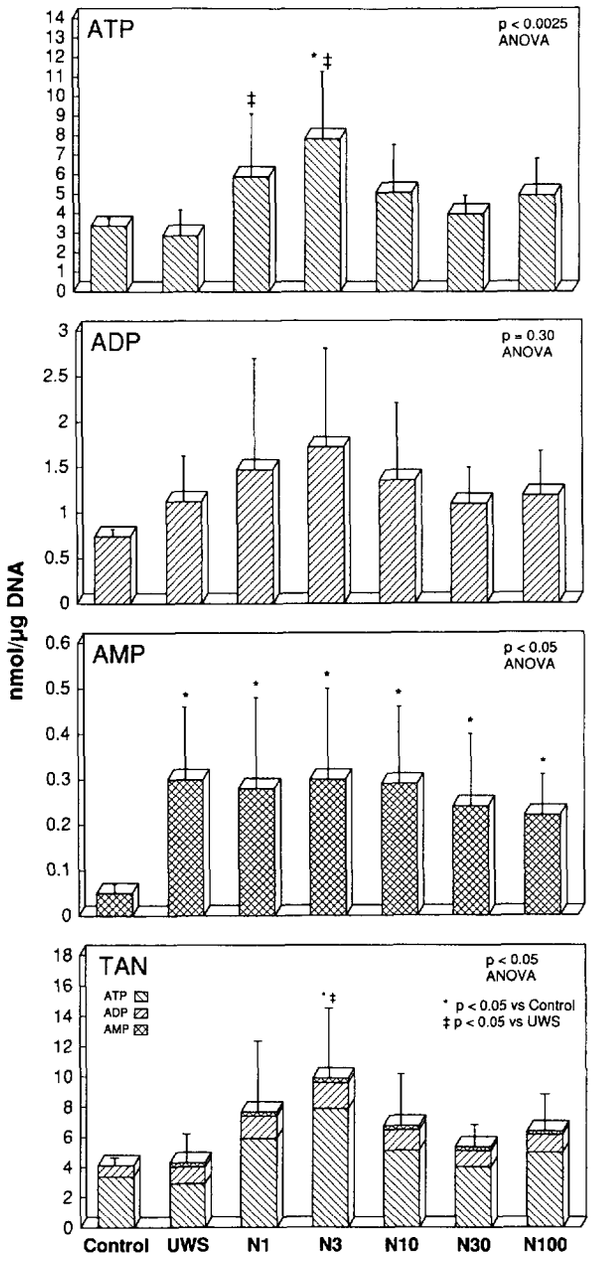

Fig 1. Adenine nucleotide results (mean ± standard deviation; 7 dishes/group) are presented for control samples and after 12-hour storage at 0°C for unmodified University of Wisconsin solution (UWS) or that supplemented with 1, 3, 10, 30, or 100 μmol/L of the nucleoside-transport blocker p*-nitrobenzylthioinosine (groups N1, N3, N10, N30, N100). The addition of the blocker augmented the recovery of adenosine triphosphate (ATP) and total adenine nucleotides (TAN), with an optimal improvement observed using 1 to 3 μmol/L. (ADP = adenosine diphosphate; AMP = adenosine monophosphate;* N1, N3, N10, N30, N100 = *1, 3, 10, 30, and 100 μmol/L of* p*-nitrobenzylthioinosine.)*

A saline-filled balloon was inserted into the left ventricle through a left atriotomy and fixed to the mitral valve ring with a pursestring suture. The balloon volume was varied in 0.02-mL increments from 0 to 0.4 mL, but

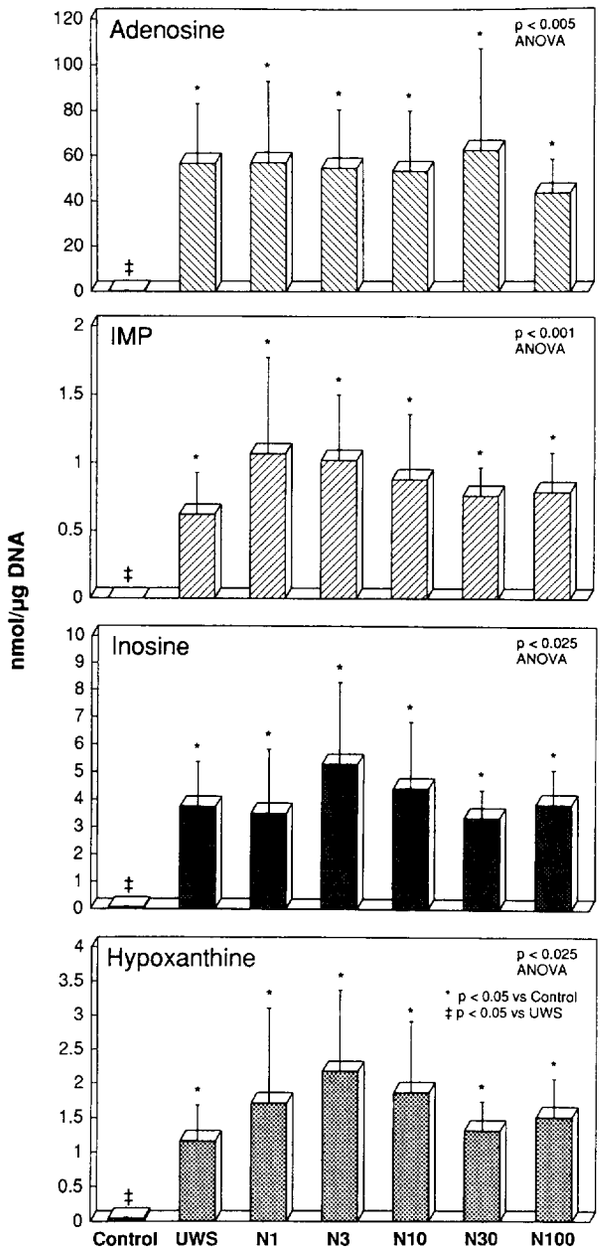

Fig 2. Adenosine, inosine monophosphate (IMP), inosine, and hypoxanthine values were increased after storage with unmodified and modified preparations of University of Wisconsin solution. (See Fig 1 for abbreviations.)

not to exceed an end-diastolic pressure of 30 mm Hg. Data were obtained after a 30-minute stabilization period before storage and after 45 minutes of reperfusion following storage.

The developed pressure was recorded before and after storage at the preischemic balloon volume associated with an end-diastolic pressure of 5 mm Hg. Compliance curves were assessed by linear regression analysis of the end-diastolic pressure data to calculate a slope and X-intercept. Linear regression provided a reasonable model for the diastolic function curves (R^2 of 0.85 to 0.99

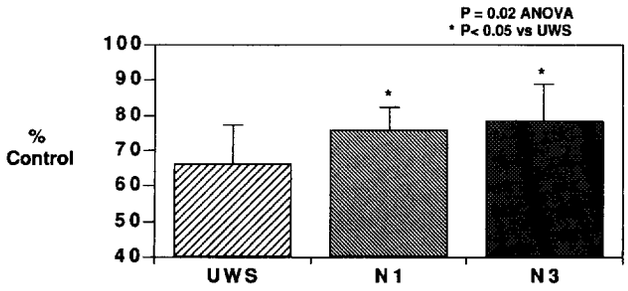

Fig 3. Postreperfusion developed pressure (mean ± standard deviation; 9 to 13 hearts/group) as a percentage of prestorage values. Developed pressure was increased in the nucleoside blocker groups (N1 [group receiving 1 µmol/L of p-benzylthioinosine] and N3 [group receiving 3 µmol/L of p-benzylthioinosine]) compared with group receiving unmodified University of Wisconsin solution (UWS).

for individual curves). Coronary flow was obtained in duplicate by a timed collection carried out in the emptying beating state. Hearts were rejected for subsequent storage if they exhibited a developed pressure of less than 90 mm Hg (4 animals) or a coronary flow of greater than 30 mL/min (4 animals), or a cardiac arrest occurred during the baseline stabilization period (2 animals).

The adenine nucleotide contents were measured in control hearts after baseline perfusion in the Langendorff apparatus before storage, immediately after storage, or following 45 minutes of reperfusion after storage (6 hearts/group). Hearts were submerged immediately in liquid nitrogen. The purine metabolite levels were measured by high-performance liquid chromatography using a previously described method [13], and results were expressed as micromoles per gram dried weight. The creatine kinase release and lactate dehydrogenase release were assessed for the 45-minute reperfusion period after the storage interval. The entire coronary effluent for the reperfusion phase was collected. Enzyme release was determined spectrophotometrically using a Hitachi Automatic Analyzer 737 and Olympus AU800 at a spectral

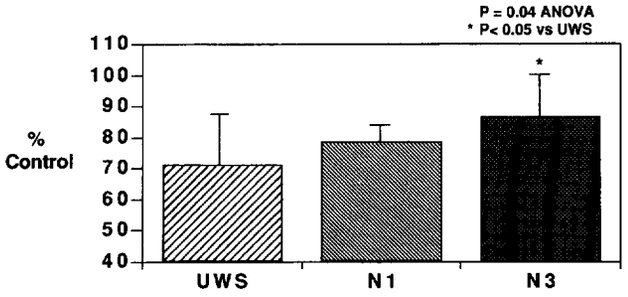

Fig 4. Postreperfusion coronary flow results (mean ± standard deviation; 9 to 13 hearts/group) as a percentage of prestorage values. Coronary flow was increased in the group receiving 3 µmol/L of p-nitrobenzylthioinosine in the preservation solution (N3) compared with that receiving the unmodified University of Wisconsin solution (UWS). (N1 = group receiving 1 µmol/L of p-nitrobenzylthioinosine in preservation solution.)

Ann Thorac Surg
1995;59:1127–33

Table 1. Diastolic Function[a]

Variable	UWS	N1	N3	*p* Value (pre versus post)
Slope (mm Hg/mL)				
Pre	155 ± 71	180 ± 184	105 ± 27	0.01
Post	212 ± 194	227 ± 193	220 ± 182	
X-intercept (mL)				
Pre	0.15 ± 0.09	0.05 ± 0.14	0.11 ± 0.10	0.50
Post	0.12 ± 0.15	0.08 ± 0.11	0.18 ± 0.10	

[a] The results (mean ± standard deviation; 9 to 13 hearts/group) of the slopes and X-intercepts derived from linear regression analysis of the diastolic function curves obtained before storage (pre) and after reperfusion (post). There was a significant increase in slope detected for all groups, but no significant changes for the X-intercept. There were no significant differences among the groups for the slope or X-intercept.

N1 = 1 μmol/L of *p*-nitrobenzylthioinosine added to UWS; N3 = 3 μmol/L of *p*-nitrobenzylthioinosine added to UWS; UWS = University of Wisconsin solution.

wavelength of 340 nm. The results of these studies were expressed as international units per gram dried weight.

Control functional data were obtained after 30 minutes of perfusion in the Langendorff apparatus before ischemia. Hearts were preserved with unmodified UWS or UWS supplemented with the optimal NBMPR concentrations identified by the preliminary cell culture studies. After aortic root flushing (15 mL/kg), hearts were stored for 8 hours at 0°C in 15 to 20 mL of storage solution.

Statistical Analysis

Data analysis was facilitated using Statistical Analysis System software (SAS Institute, Cary, NC) and a microcomputer. Variables are expressed as the mean ± standard deviation of the original values, or as a percentage of the control value. Data analysis was performed with a one-way analysis of variance and between-group differences specified with Duncan's multiple-range test. Diastolic function was analyzed additionally using a multivariate analysis of variance, testing simultaneously both the slope and X-intercept. Statistical significance is assumed for a p value of less than 0.05.

Results

Human Cardiomyocyte Experiments

The recovery of DNA after storage was reduced for all test solutions ($p < 0.0001$, analysis of variance) compared with the control results, but was similar among the different groups (UWS, 25.5% ± 9.6%; N1, 27.0% ± 12.1%; N3, 26.3% ± 12.1%; N10, 27.3% ± 10.4%; N30, 27.5% ± 7.3%; N100, 28.5% ± 7.6%) and the NBMPR vehicle group (28.0% ± 12.8%).

Figure 1 depicts the poststorage values of adenine nucleotides. The adenosine triphosphate (ATP) levels were increased in association with NBMPR supplementation, and the improvement was statistically significant for the N1 and N3 groups. The adenosine diphosphate level was unchanged with storage, but the adenosine monophosphate values were increased for all test solutions. The levels of total adenine nucleotides were increased in association with nucleoside-transport inhibition, with an optimal improvement identified for the cells stored in UWS with 3 μmol/L of NBMPR. There was no significant effect noted for the NBMPR vehicle (ATP, 3.87 ± 1.63 nmol/μg DNA; total adenine nucleotides, 5.96 ± 2.74 nmol/μg DNA).

The levels of inosine monophosphate, adenosine, inosine, and hypoxanthine were increased after storage, but were similar for the NBMPR and UWS groups (Fig 2).

Because of the improvement noted for the preservation fluids containing 1 or 3 μmol/L of NBMPR, subsequent rodent experiments compared the effects of unsupplemented UWS with the effects of UWS supplemented with NBMPR at these two concentrations.

Isolated Rodent Heart Experiments

The developed pressure and coronary flow were similar for all groups before storage (developed pressure: 128.9 ± 16.0 to 133.3 ± 15.4 mm Hg, $p = 0.90$; coronary flow: 20.3 ± 6.4 to 21.2 ± 3.8 mL/min; $p = 0.97$). The developed pressure was reduced after storage for all preservation media groups, but increased in the N1 and N3 groups compared with the unmodified UWS group (Fig 3). Coronary flow was decreased after storage for all groups but increased in the N3 group relative to the UWS group (Fig 4). The diastolic function results are presented in Table 1. The poststorage slopes were increased for each of the study conditions but did not differ among the groups. There was no significant change in the X-intercept, although it tended to be decreased for the UWS group and to be increased for the NBMPR groups.

Biopsy results are presented in Table 2. The ATP, diphosphate, and total adenine nucleotide levels were reduced compared with the control poststorage and postreperfusion values, but did not differ among the groups. The increase in the adenosine monophosphate level after storage was greater in the N3 group. The recovery of creatine phosphate after reperfusion observed in the UWS and N3 hearts exceeded the control values, but the values in the N1 hearts were similar to the control values. The NBMPR did seem to block nucleoside transport, as the hypoxanthine content was unmeasurable in the N1 and N3 groups after reperfusion.

There were no significant differences in the creatine kinase or lactate dehydrogenase release among the groups during the reperfusion phase (Table 3), although both values were increased in the N3 hearts. This trend was also seen when the results were expressed as concentrations (creatine kinase: UWS, 49.4 ± 35.9 IU/L; N1, 40.2 ± 30.5 IU/L; N3, 70.7 ± 72.7 IU/L. Lactate dehydrogenase: UWS, 12.3 ± 10.3 IU/L; N1, 9.2 ± 7.6 IU/L; N3, 15.9 ± 18.0 IU/L).

Comment

In our initial studies we attempted to determine whether the addition of a nucleoside-transport blocker would

Table 2. Rodent Heart Biopsy Results[a]

Substance (μmol/g)	Control (prestorage)	UWS		N1		N3		*p* Value (timing)	*p* Value (solution)	*p* Value Timing[b] Solution
		Storage	Reperfusion	Storage	Reperfusion	Storage	Reperfusion			
ATP	17.1 ± 0.5	3.2 ± 1.0[b]	12.0 ± 2.8[b]	2.6 ± 0.9[b]	13.4 ± 0.9[b]	1.7 ± 0.9[b]	13.3 ± 1.1[b]	<0.0001	NS	NS
ADP	4.6 ± 0.5	3.6 ± 0.5[b]	3.6 ± 0.4[b]	4.3 ± 0.6	3.8 ± 0.3[b]	4.2 ± 0.9	3.3 ± 0.4[b]	0.0094	NS	NS
AMP	0.28 ± 0.08	1.81 ± 0.79[b]	0.29 ± 0.03	2.21 ± 0.56[b]	0.23 ± 0.07	3.73 ± 1.67[b,c]	0.23 ± 0.3	<0.0001	0.0202	0.0169
TAN	22.0 ± 0.6	8.6 ± 0.6[b]	15.8 ± 3.1[b]	9.1 ± 1.0[b]	17.5 ± 0.9[b]	9.7 ± 2.2[b]	16.8 ± 1.4[b]	<0.0001	NS	NS
IMP	0	1.84 ± 0.38[b]	0.61 ± 0.84	1.70 ± 0.49[b]	0	2.43 ± 1.32[b]	0	<0.0001	NS	NS
CP	24.3 ± 6.0	1.4 ± 0.2[b]	32.2 ± 6.1	1.3 ± 0.2[b]	24.1 ± 6.7[c]	1.6 ± 0.5[b]	30.3 ± 3.4[b]	<0.0001	0.0346	0.0504
Adenosine	0.14 ± 0.02[c]	1.24 ± 0.16[b]	0.11 ± 0.01	1.13 ± 0.12[b]	0.13 ± 0.01	1.20 ± 0.19[b]	0.12 ± 0.01	<0.0001	NS	NS
Inosine	0.56 ± 0.25	1.43 ± 0.07[b]	0.74 ± 0.07	1.54 ± 0.09[b]	0.74 ± 0.12	1.56 ± 0.24[b]	0.77 ± 0.23	<0.0001	NS	NS
Hypo-xanthine	0.08 ± 0.08	0.20 ± 0.04[b]	0.09 ± 0.07	0.21 ± 0.03[b]	0[b,c]	0.21 ± 0.06[b]	0[b,c]	<0.0001	0.0254	0.0086

[a] Values are the mean ± standard deviation (5 to 6 hearts/group). [b] $p < 0.05$ versus control. [c] $p < 0.05$ versus UWS.

ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate; CP = creatine phosphate; IMP = inosine monophosphate; N1 = 1 μmol/L of *p*-nitrobenzylthioinosine in UWS; N3 = 3 μmol/L of *p*-nitrobenzylthioinosine in UWS; NS = not significant; TAN = ATP + ADP + AMP; UWS = University of Wisconsin solution.

augment the recovery of ATP in human cardiomyocytes after storage in UWS. These results were subsequently tested in isolated rat heart experiments. This investigative strategy provided evidence supporting the addition of NBMPR to UWS.

The primary rationale for the use of cultured human cardiomyocytes stems from the potential concerns about discrepancies introduced by species differences. Second, the use of cultured human cardiomyocytes allows one to perform experiments on specific cell types (eg, myocytes, fibrocytes, and endothelial cells). We have used right ventricular outflow tract myocardium from patients with tetralogy of Fallot as a convenient source of a large amount of ventricular myocardium to initiate the cultures. We emphasize the importance of confirming the cell culture findings in an intact cardiac preparation; however, using similar experimental protocols, we have previously shown that the supplementation of UWS with glucose also improved the poststorage results in the settings of both human cultured cardiomyocytes [14] and isolated rodent hearts [12]. We acknowledge that buffer-perfused systems are not ideal and that blood-perfused models are more physiologic.

There exists considerable interest in the application of UWS to cardiac allograft storage [1–6]. Clinical trials have been conducted in patients in the setting of conventional organ ischemic times, that is, less than 4 hours [15–17]. A reduction in the requirement for electrical defibrillation was noted in all three studies. The trials comparing UWS and Stanford cardioplegia [15, 16] revealed additional evidence for the superiority of the myocardial protection conferred by UWS, as shown by the cardiac levels of ATP or creatine phosphate and by the postoperative enzyme release. The one study that compared UWS and St. Thomas' Hospital solution [17] did not reveal any other evidence of improved preservation with UWS. Studies conducted on organs from high-risk donors by virtue of the prolonged organ ischemic time anticipated, the high level of inotropic agents utilized, small donor size, or advanced age may better discriminate the protective properties of alternative storage solutions.

The beneficial properties of UWS with respect to cardiac storage may relate to the inclusion of adenosine in its formulation [6]. Adenosine originally was documented to facilitate ATP synthesis during hypothermic kidney perfusion [18]. However, the composition of UWS is unique in many regards, and, furthermore, multiple different UWS-like formulations have been employed during experimental evaluations. Recent studies conducted by Lasley and Mentzer [6] directly evaluated the contribution of adenosine to UWS. The resultant interstitial adenosine concentrations were 20 to 40 times greater in the adenosine-treated hearts, as shown by cardiac microdialysis, and functional recovery was enhanced.

Adenosine supplementation has been found to be effective in models of regional ischemia [19], global ischemia [20], cardioplegia [7], ventricular assist [21], and transplantation [6]. Adenosine has been implicated as the

Table 3. Enzyme Release[a]

Enzyme	UWS	N1	N3	*p* Value (ANOVA)
Creatine kinase (IU/g)	115.5 ± 43.9	123.1 ± 54.8	246.3 ± 214.0	0.09
Lactate dehydrogenease (IU/g)	27.3 ± 15.8	28.0 ± 17.1	52.6 ± 53.2	0.23

[a] The results (mean ± standard deviation; 6 hearts/group) of creatine kinase and lactate dehydrogenase release collected over the entire 45-minute reperfusion period and expressed as IU/g dried weight.

ANOVA = analysis of variance; N1 = 1 μmol/L of *p*-nitrobenzylthioinosine added to UWS; N3 = 3 μmol/L of *p*-nitrobenzylthioinosine added to UWS; UWS = University of Wisconsin solution.

mediator of ischemic preconditioning through the process of A_1-selective receptor activation [22]. The ultimate effector of this phenomena is uncertain, although evidence supports the role of pertussis-sensitive G_i proteins linked to K^+_{ATP} channels [23]. The potential mechanisms responsible for the improved tolerance to ischemia-reperfusion, extensively covered in previous reviews [24], relate to enhanced tissue adenine nucleotide levels, the modification of glucose metabolism, vascular dilatation, a cardioplegia effect secondary to the depression of nodal and conductive tissue function, an inhibitory activity directed against neutrophils or platelets, or an antiadrenergic effect.

Masuda and associates [9] investigated the role of the experimental nucleoside-transport blocker R75231 for 24-hour cardiac storage. The best results were obtained when R75231 was added to both the cardioplegic-storage solution and the reperfusion medium. Improved recovery was associated with greater levels of ATP and total adenine nucleotides after storage and reperfusion in the nucleoside transport–blocker groups. The adenosine and inosine levels were greater after storage in the control group, but these were not maintained during reperfusion. The hypoxanthine concentrations were increased after storage and increased further during reperfusion in the untreated hearts, but nucleoside-transport inhibition was associated with maintained levels of adenosine and inosine during reperfusion and with minimal hypoxanthine values. Hypoxanthine can act as a substrate for the formation of oxygen free radicals associated with the xanthine–xanthine oxidase reaction.

In our cardiomyocyte experiments, we identified that the addition of 1 and 3 μmol/L of NBMPR to the UWS was associated with increased levels of ATP and total adenine nucleotides after storage. Based on these data, rodent heart experiments were performed with 1 and 3 μmol/L of NBMPR. The adenosine concentrations were markedly elevated in the cellular preparations related to the high adenosine concentrations in the storage solutions, as we have previously reported [3, 22], without any differences identified among the groups. The inosine and hypoxanthine values were similar to those achieved with unmodified UWS.

The NBMPR appeared to be biologically active in the rodent hearts, as the postreperfusion hypoxanthine levels were unmeasurable. The reasons for the discrepancy in the adenine nucleotide levels between the rodent and human cardiomyocyte experiments may relate to model or species differences. A beneficial effect of nucleoside-transport blockade was noted in terms of the developed pressure and coronary flow, as compared with the effects of unmodified UWS. These findings support the addition of NBMPR to UWS and are compatible with the concept that nucleoside-transport blockade is the mechanism responsible for the improved results. The optimal concentration of NBMPR shown by the cell culture experiments was 3 μmol/L. The coronary flow results from the rodent heart experiment recommend the addition of 3 μmol/L of NBMPR. Other end points (tissue levels of creatine phosphate and adenosine, and the cardiac release of creatine kinase and lactate dehydrogenase) suggest that a concentration of 1 μmol/L may be preferable.

In summary, we have provided evidence that the putative nucleoside transport–blocking agent NBMPR enhances the recovery of cardiac tissue stored with UWS. Additional relevant questions that remain unanswered are whether exogenous adenosine is necessary for this benefit and whether supplementing other storage solutions with NBMPR is advantageous.

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References

1. Fremes SE, Li R-K, Weisel RD, Mickle DAG, Tumiati LC. Prolonged hypothermic cardiac storage with University of Wisconsin solution. J Thorac Cardiovasc Surg 1991;102: 666–72.
2. Fremes SE, Furukawa RD, Li R-K, Weisel RD, Mickle DAG, Tumiati LC. Prolonged preservation with University of Wisconsin solution. J Surg Res 1991;50:330–4.
3. Swanson DK, Pasaoglu I, Berkoff HA, Southard JA, Hegge JO. Improved heart preservation with UW preservation solution. J Heart Transplant 1988;7:456–67.
4. Okouchi Y, Shimizu K, Yamaguchi A, Kamada N. Effectiveness of modified University of Wisconsin solution for heart preservation as assessed in heterotopic rat heart transplant model. J Thorac Cardiovasc Surg 1990;99:1104–8.

5. Human PA, Holl J, Vosloo S, et al. Extended cardiopulmonary preservation: University of Wisconsin solution versus Bretschneider's cardioplegic solution. Ann Thorac Surg 1993;55:1123–30.
6. Lasley RD, Mentzer RM. The role of adenosine in extended myocardial preservation with the University of Wisconsin solution. J Thorac Cardiovasc Surg 1994;107:1356–63.
7. Bolling SF, Bies LE, Bove EL, Gallagher KP. Augmenting intracellular adenosine improves myocardial recovery. J Thorac Cardiovasc Surg 1990;99:469–74.
8. Galinanes M, Mullane KM, Bullough DB, Hearse DJ. Acadesine and myocardial protection. Studies of time of administration and dose-response relations in the rat. Circulation 1992;86:598–608.
9. Masuda M, Chang-Chun C, Mollhoft, Van Belle H, Flameng W. Effects of nucleoside transport inhibition on long-term ex vivo preservation of canine hearts. J Thorac Cardiovasc Surg 1992;104:1610–7.
10. Fremes SE, Li R-K, Weisel RD, Mickle DAG, Furukawa RD, Tumiati LC. The limits of cardiac preservation with University of Wisconsin solution. Ann Thorac Surg 1991;52:1021–2.
11. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation level of deoxyribonucleic acid. Biochem J 1956;62:315–23.
12. Fremes SE, Guo LR, Furukawa RD, Mickle DAG, Weisel RD. Cardiac storage with UW solution and glucose. Ann Thorac Surg 1994;58:1368–73.
13. Weisel RD, Mickle DAG, Finkle CD, Tumiati LC, Madonik MM, Ivanov J. Delayed myocardial metabolic recovery after blood cardioplegia. Ann Thorac Surg 1989;48:503–7.
14. Fremes SE, Furukawa RD, Li R-K, Tumiati LC, Weisel RD, Mickle DAG. In vitro assessment of the effects of glucose added to the University of Wisconsin solution on myocyte preservation. Circulation 1992;86(Suppl 2):289–94.
15. Stein DG, Drinkwater DC, Laks H, et al. Cardiac preservation in patients undergoing transplantation. A clinical trial comparing University of Wisconsin solution and Stanford solution. J Thorac Cardiovasc Surg 1991;102:657–65.
16. Jeevanandam V, Barr ML, Auteri JS, et al. University of Wisconsin solution versus crystalloid cardioplegia for human donor heart preservation. A randomized blinded prospective clinical trial. J Thorac Cardiovasc Surg 1991;103: 194–9.
17. Demertzis S, Wippermann J, Schaper J, et al. University of Wisconsin versus St. Thomas' hospital solution for human donor heart preservation. Ann Thorac Surg 1993;55:1131–7.
18. McAnulty FJ, Southard JH, Belzer FO. Improved maintenance of adenosine triphosphate in five-day perfused kidneys with adenine and ribose. Trans Proc 1987;19:1376–9.
19. Norton ED, Jackson EK, Virmani R, Forman MB. Effect of intravenous adenosine on myocardial reperfusion injury in a model with low myocardial blood flow. Am Heart J 1990;122: 1283–91.
20. Janier MF, Vanoverschelde JL, Bergmann SR. Adenosine protects ischemic and reperfused myocardium by receptor-mediated mechanisms. Am J Physiol 1993;264:H163–70.
21. Demmy TL, Magovern JA, Kao RL, Magovern GJ. Resuscitation of injured myocardium with adenosine and biventricular assist. Ann Thorac Surg 1991;52:1044–51.
22. Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 1991;84:350–6.
23. Auchampach JA, Gross GJ. Adenosine A_1 receptors, K_{ATP} channels and ischemic preconditioning in dogs. Am J Physiol 1993;264:H1327–36.
24. Downey JM, Forman MB. Spotlight on: the cardioprotective properties of adenosine. Cardiovasc Res 1993;27:1–140.

Notice Regarding "Our Surgical Heritage" Section

Readers are solicited to contribute recollections, vignettes, interviews, and incidents involving surgical pioneers. The items need not be lengthy, but they should have involved the author directly.

Humorous incidents will not be excluded, and the style of writing can be such as to provide a few minutes of entertainment.

Full articles of historic nature will, of course, continue to be welcome.

Anthony R. C. Dobell, MD
Associate Editor
Our Surgical Heritage Section