Heart Preservation in HTK Solution: Role of Coronary Vasculature in Recovery of Cardiac Function

Yuhei Saitoh, MD, PhD, Michio Hashimoto, PhD, Kwansong Ku, MD, PhD, Seikon Kin, MD, PhD, Seishi Nosaka, MD, PhD, Sumio Masumura, PhD, and Kengo Nakayama, MD, PhD

First Department of Surgery, Shimane Medical University, Shimane, Japan

Background. **Poor myocardial tolerance to prolonged cold ischemia remains a major concern in heart transplantation. In this study, we estimated superiority of Histidine-Tryptophan-Ketoglutarate (HTK) over University of Wisconsin (UW) as a cardiac preservation solution.**

Methods. **Isolated rat hearts were mounted on a Langendorff apparatus to estimate the baseline cardiac function. The hearts were arrested and stored at 4°C in UW and HTK solution for 8 hours, and then reperfused. The aortic flow, coronary flow, cardiac output, rate pressure product, and left ventricular dp/dt in the HTK group recovered significantly more than the UW group. The values of myocardial total adenine nucleotides and the adenosine triphosphate to adenosine diphosphate ratio were higher in the HTK than in the UW group. We also examined coronary vascular responsiveness using left coronary arteries dissected from the rat hearts before flushing, before storage, after storage, and after reperfusion.**

Results. **The maximal relaxation response to acetylcholine was significantly higher in the HTK than in the UW group after reperfusion, although there were no significant differences at each stage before reperfusion. In addition, the endothelium-independent relaxation response to sodium nitroprusside in the HTK group was also well preserved after reperfusion.**

Conclusions. **These results indicate that HTK is superior to UW solution for cardiac preservation. HTK protects coronary vasculature during preservation, which together with reperfusion might lead to improved functional cardiac recovery following preservation.**

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Poor myocardial tolerance to prolonged cold ischemia remains a major concern in heart transplantation. Although selection of an ideal storage solution and innovations in storage techniques may extend the current safe limits of graft preservation, and increase the number of potential donor hearts clinically, acceptable graft protection still does not exceed 4 to 6 hours [1]. The University of Wisconsin solution (UW) has extended the preservation of organs such as kidney, liver, and pancreas, but the clinical graft viability is still currently limited for 4 to 6 hours in heart preservation [2]. Histidine-tryptophan-ketoglutarate solution (HTK) was initially developed as a cardioplegic solution by Bretschneider and coworkers, and it is also effective in kidney and liver transplantation [3]. HTK was also reported as a good preservative for human atrial myocardium [4]. Thus, in heart transplantation, several preservation solutions have been proposed, but these remain controversial.

Loss of endothelial responsiveness during storage could compromise blood supply to the donor heart [5], resulting in mechanical failure. Thus, coronary arterial vasoactivity, which is modulated by the vascular endothelium may regulate cardiac function. Despite the importance of coronary regulation, few investigators have assessed in vitro coronary arterial function after prolonged ischemia followed by reperfusion.

In this study, functional cardiac recovery was examined in isolated rat hearts preserved in UW or HTK, after prolonged cold ischemia. In addition, by using these solutions, the mechanical responses of coronary endothelium and smooth muscle in the in vitro isolated coronary artery were also examined before flushing, before storage just after cardiac arrest, after storage, and after reperfusion, to understand the mechanisms and effects of coronary vasculature on functional cardiac recovery. We tried to determine which solution is more suitable for cardiac preservation, UW or HTK, with respect to cardiac functional recovery and coronary arterial function following prolonged cold ischemia.

Material and Methods

Animal experimentation proceeded in accordance with institutional guidelines and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health. Male Wistar rats (250 to 350 g) were

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Address reprint requests to Dr Saitoh, First Department of Surgery, Shimane Medical University, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan.

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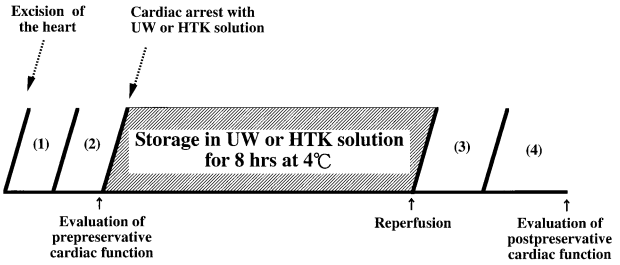

Fig 1. In the experimental protocol of functional cardiac recovery hearts were excised and mounted on a Langendorff apparatus. They were perfused for 3 minutes Langendorff mode (1) and for 2 minute working mode (2). They were arrested and stored for 8 hours in UW or HTK solution at 4°C. Following cold storage, they were reperfused for 15 minutes Langendorff mode (3) and 25 minutes working mode (4).

systemically heparinized (500 U, ip) and anesthetized with sodium pentobarbital (65 mg/kg, ip). The heart was excised and immediately immersed in Krebs-Henseleit bicarbonate buffer (KHB; consisting of: NaCl [118 mmol/L], KCl [4.7 mmol/L], $MgSO_4$ [1.2 mmol/L], KH_2PO_4 [1.2 mmol/L], $CaCl_2$ [2.5 mmol/L], $NaHCO_3$ [25.0 mmol/L], and glucose [11.0 mmol/L]) at 37°C. The hearts were then mounted on a Langendorff apparatus (IPH-W, Labo Support, Osaka, Japan) through the aorta, and perfused at a constant pressure of 60 mm Hg for several minutes (Langendorff mode [L-mode]) with filtered (0.22 μm) KHB equilibrated with 95% O_2 and 5% CO_2, and maintained at 37°C. Excised hearts were rapidly cannulated to minimize ischemia.

Experimental Protocol 1

The experimental protocol is shown in Figure 1. Following cannulation of the left atrium through the pulmonary vein, the hearts were switched to working mode with a preload of 13 mm Hg and an afterload of 60 mm Hg. Prepreservative baseline cardiac function was determined after 20 minutes in the working (W) mode. Aortic flow (AF) (mL/min), coronary flow (CF) (mL/min), cardiac output (CO) (mL/min), rate pressure product (RPP) and left ventricular (LV) dp/dt values were measured. The hearts were divided into UW (n = 6) and HTK groups (n = 6). The hearts in both groups were then arrested by flushing each preservative (60 mL/kg at 4°C) through an aortic cannula at a pressure of 60 mm Hg and stored in UW or HTK (30 mL at 4°C). Table 1 shows the composition of the preservatives. The hearts were mounted on the Langendorff apparatus and reperfused for 20 minutes on L-mode and 40 minutes on W-mode, and then the recovery of cardiac function was evaluated.

The adenylate content of myocardial samples frozen in liquid nitrogen after storage in UW (n = 6) or HTK (n = 6) was analyzed. Frozen myocardium was centrifuged (11,000 g for 5 minutes at 4°C). The supernatant was decanted and neutralized with potassium hydroxide (2 mol/L). Aliquots (20 mL) were then analyzed by high performance liquid chromatography (HPLC) (LC-9A liquid chromatograph, Shimadzu) with a column of STR ODS-M (Shimadzu) [6]. The HPLC values were used to calculate the following indices of myocardial energy status: total adenine nucleotides (TAN) = adenosine triphosphate (ATP) + adenosine diphosphate (ADP) + adenosine monophosphate (AMP) and adenylate energy charge and ATP/ADP ratio.

Table 1. Components of the Preservation Solutions[a]

UW		HTK	
KH_2PO_4	25	NaCl	15
$MgSO_4$	5	KCl	9
KCl	15	$MgCl_2$	4
Adenosine	5	α-ketoglutarate	1
Glutathione	3	Tryptophan	2
Raffinose	30	Histidine	180
Allopurinol	1	Histidine-HCl	18
K-lactobionate	110	Mannitol	30
Pentastarch	5%		

[a] Concentrations are given as millimoles per liter (mmol/L).

Experimental Protocol 2

MECHANICAL FUNCTION OF ISOLATED CORONARY ARTERIES. To account for the influence of flushing and reperfusion injury, coronary endothelial function was examined at four stages: (1) before flushing, (2) after cardiac arrest by flushing with each preservative (flush injury), (3) after storage without reperfusion (flush/storage injury), and (4) after reperfusion (flush/storage/reperfusion injury) (Fig 2). The left main trunks of male Wistar rats were divided into the following: group 1 (n = 11), nonflushed (control); group 2 (n = 10), flushed with UW, group 3 (n = 8), flushed with HTK; group 4 (n = 10), after simple cold storage in UW; group 5 (n = 11), after simple cold storage in HTK; group 6 (n = 5), storage in UW followed by

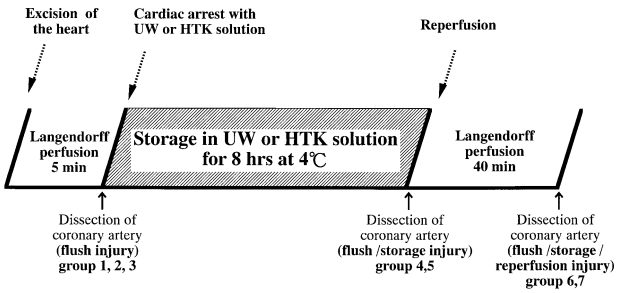

Fig 2. In the experimental protocol of mechanical function of coronary endothelium the hearts were excised and mounted on a Langendorff apparatus. They were perfused for 5 minutes Langendorff perfusion. They were arrested and stored for 8 hours in UW or HTK solution at 4°C. Following storage, they were reperfused for 40 minutes Langendorff perfusion. The left main trunks were dissected out and the endothelial function was examined at four stages; (1) before flushing (group 1) and before storage following cardiac arrest with each solution (flush injury: group 2 and 3); (2) just after storage without reperfusion (flush/storage injury: group 4 and 5); and (3) after storage with reperfusion (flush/storage/reperfusion injury: group 6 and 7).

reperfusion; and group 7 (n = 5), storage in HTK followed by reperfusion.

PREISCHEMIC FUNCTION OF CORONARY ARTERY (FLUSH INJURY). Following experimental preparation, the hearts in group 1 (nonflushed group) were immediately immersed in KHB bubbled with a 95% O_2 and 5% CO_2 gas mixture at 37°C in a petri dish. The left main trunks were carefully dissected out, and then cleaned of fat and adherent connective tissue under the microscope.

As much of the surrounding tissue as possible was removed and stretching, and rubbing of the intimal surface or the opposite wall of the vessel was scrupulously avoided. The arteries were cut into rings approximately 0.5 mm long. The ring preparations were mounted horizontally on two L-shaped tungsten wires (50 μm in diameter) in a microvascular apparatus, which was a minor modification of the model described by Högestätt and colleagues [7]. The microorgan bath was filled with 5 mL of KHB at 37°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. Contractile and relaxation responses of the muscles were recorded isometrically with a force-displacement transducer (transducer: Sanei, Japan; recorder: LR-4220, Yokogawa, and 8K21-1-L, Sanei, Japan) as described by Hashimoto and associates [8]. The muscle was equilibrated under aerobic conditions for over 30 minutes before each experiment. During equilibration, basal tension was adjusted to 100 mg. Initially, we measured potassium chloride (KCl, 80 mM)-induced maximal contraction twice, followed by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced maximal contraction. Following a 60-minute rinse with KHB, the muscle was stimulated with $PGF_{2\alpha}$ at the concentration required for half-maximal contraction. The muscle was then relaxed using cumulative amounts of acetylcholine (ACh) or sodium nitroprusside (SNP). The arteries in groups 2 and 3 were dissected out after cardiac arrest by flushing with UW and HTK (60 mL/kg body weight at 4°C through the aortic cannula at a pressure of 60 mm Hg), respectively. Mechanical function was assessed in the manner described for group 1.

POSTPRESERVATION CORONARY FUNCTION WITHOUT REPERFUSION (FLUSH/STORAGE INJURY). The hearts in group 4 and 5 were arrested by flushing, then stored at 4°C for 8 hours with UW and HTK, respectively. The arteries were dissected out just at the end-point of the storage and the mechanical function was measured.

POSTPRESERVATIVE CORONARY FUNCTION WITH REPERFUSION (FLUSH/STORAGE/REPERFUSION INJURY). The hearts in groups 4 and 5 were again mounted on the Langendorff apparatus and reperfused with oxygenated KHB at 37°C for 40 minutes on L-mode (groups 6 and 7, respectively). After reperfusion, the arteries were dissected out and coronary function was measured.

ISOMETRIC FORCE. Smooth muscle contraction was measured as the increase in tension above the initial baseline. Relaxation responses to ACh and SNP are expressed as the percent relaxation from the contraction generated by $PGF_{2\alpha}$.

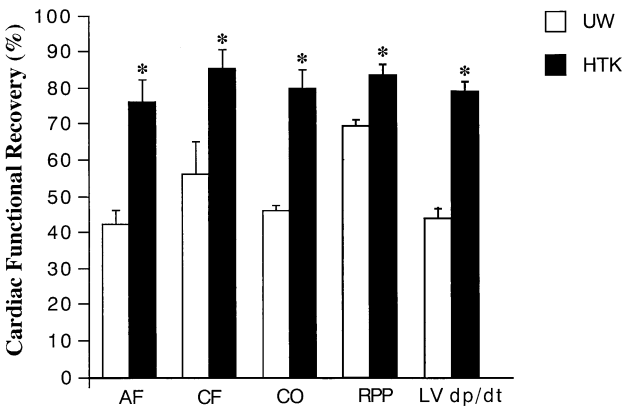

*Fig 3. The functional cardiac recovery of AF, CF, CO, RPP, and LV dp/dt after 8 hours of preservation was significantly better in the group stored in HTK than in UW (*p < 0.05). (AF = aortic flow; CF = coronary flow; CO = cardiac output; RPP = rate pressure product; LV = left ventricle.)*

Statistical Analysis

All results are expressed as means ± standard error of the mean. Statistical analysis was performed by the one-way analysis of variance (ANOVA) and Scheffe's method for multiple comparisons. Students' unpaired *t*-test was used for simple comparison. A p value of less than 0.05 was considered statistically significant. Data processing was performed with the computer software package Stat View™ SE + Graphics (Abacus Concepts, Inc, Berkeley, CA).

Results

Baseline Cardiac Function

Baseline cardiac function (n = 12) was measured prior to preservation: AF, 46.2 ± 3.4 mL/min; CF, 15.4 ± 0.7 mL/min; CO, 65.4 ± 1.7 mL/min; RPP, 24,550 ± 450; and LV dp/dt, 2450 ± 150 mm Hg/sec.

Functional Cardiac Recovery

Figure 3 shows the percent recovery of hemodynamic function (percentage values to the prepreservative baseline function) following 8 hours of preservation in each group. The percent recoveries of cardiac function in the UW and HTK hearts were as follows: 42.5 ± 3.8 and 76.3 ± 6.0% of AF, 56.5 ± 8.4 and 85.5 ± 5.0% of CF, and 46.2 ± 1.1 and 79.9 ± 5.2% of CO, 70.2 ± 2.1 and 86.5 ± 3.8% of RPP, and 44.5 ± 5.9 and 81.3 ± 3.9% of LV dp/dt, respectively. The recovery of AF, CF, CO, RPP, and LV dp/dt was significantly better in the group stored in HTK than in UW ($p < 0.05$).

Myocardial Adenylate Contents After Storage

Figure 4 shows the values of total adenine nucleotides and the ATP/ADP ratio in the myocardium after storage in UW and HTK. The TAN values and the ATP/ADP

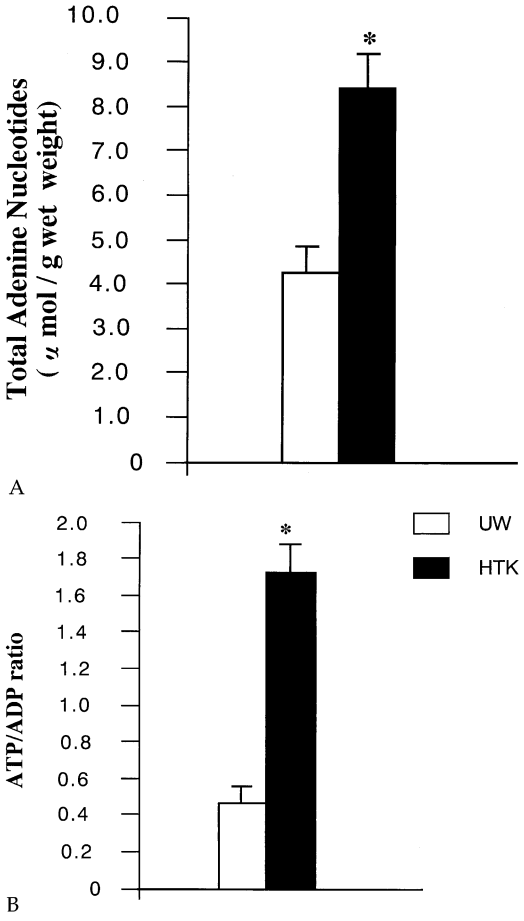

*Fig 4. The total adenine nucleotides (TAN) values (A) and the ATP/ADP ratios (B) in the myocardium of HTK group were significantly higher than those of the UW group (*p < 0.05) following storage. (ATP = adenosine triphosphate; ADP = adenosine diphosphate.)*

ratios in the myocardium of HTK group were significantly higher than those of the UW group.

Mechanical Function of Isolated Coronary Arteries

There were no significant differences between the HTK and UW groups in the KCL-induced (Fig 5A) and prostaglandin $F_{2\alpha}$-induced maximal contractions (Fig 5B) at any of the stages. There were no significant differences in the relaxation responses to Ach and SNP before flushing, before storage, or after storage between the HTK and UW groups. However, the relaxation response to Ach after reperfusion was significantly improved in the HTK group compared with the UW group (Fig 6A). In addition, the endothelium-independent relaxation response to SNP in the HTK group was also well preserved after reperfusion (Fig 6B). The median effective dose (ED_{50}) values for Ach were similar at all stages in the HTK and UW groups (Fig 6C).

Weight of the Rings of the Coronary Arteries

The coronary artery rings weighed the same in all of the groups (0.5 to 0.7 mg, mean: 0.57 mg).

Comment

Hearts have been successfully preserved in UW, but the long-term effectiveness of this solution remains controversial [2, 9, 10]. HTK was initially developed by Bretschneider and coworkers for cardioplegia [11], and it is a good preservative for human atrial myocardium [4]. However, few investigators have examined the effects of HTK on cardiac function in the preserved heart.

In the present study, the cardiac function of hearts preserved in HTK was significantly improved following cold storage, compared with those stored in UW. The efficacy of HTK is attributed to the high buffering capacity during prolonged ischemia provided by histidine/histidine-hydrochloride, which suppresses ischemia-induced tissue acidosis [12, 13]. Decreased acidosis might also prevent ATP degradation and improve energy metabolism during hypothermic storage. In fact, in the present study, the myocardial ATP level was significantly

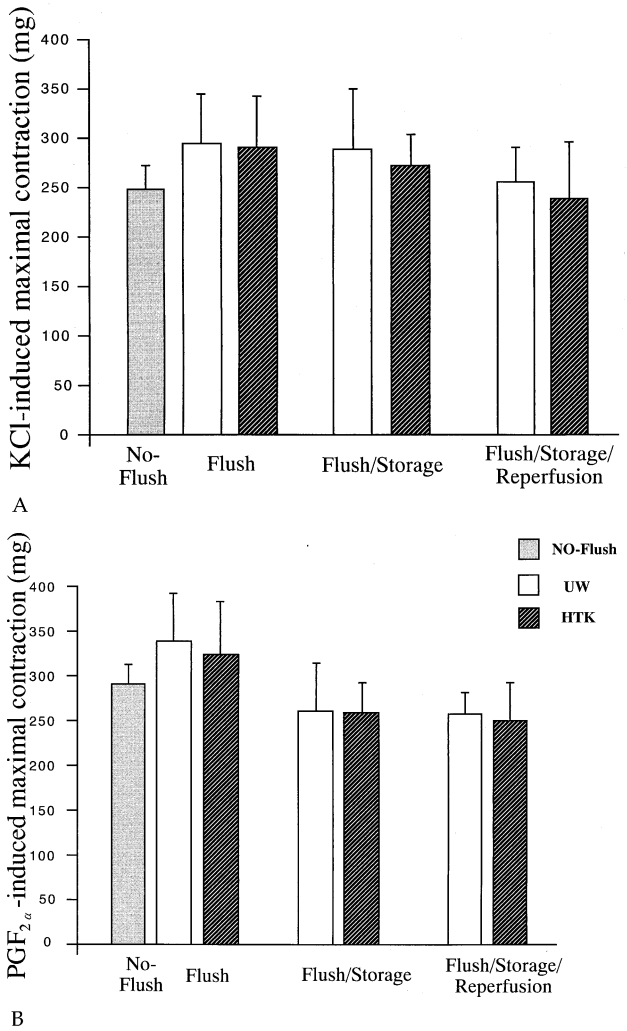

Fig 5. There were no significant differences between the HTK and UW groups of isolated coronary arteries in the KCL-induced (A) and prostaglandin $F_{2\alpha}$-induced (B) maximal contractions at any of the stages. (KCl = potassium chloride; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$.)

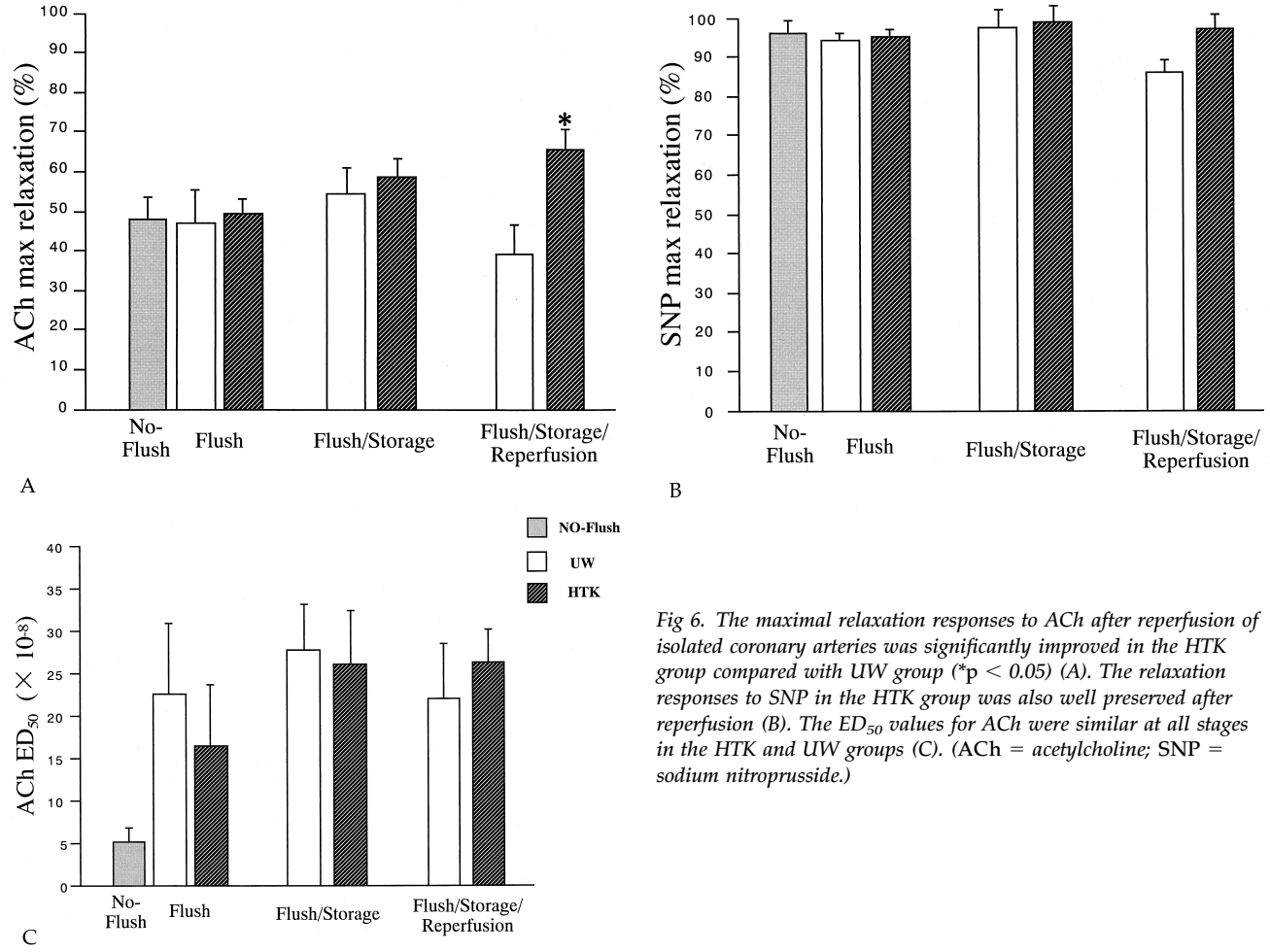

Fig 6. The maximal relaxation responses to ACh after reperfusion of isolated coronary arteries was significantly improved in the HTK group compared with UW group ($p < 0.05$) (A). The relaxation responses to SNP in the HTK group was also well preserved after reperfusion (B). The ED_{50} values for ACh were similar at all stages in the HTK and UW groups (C).* (ACh = *acetylcholine;* SNP = *sodium nitroprusside.)*

higher in hearts preserved in HTK than in UW. The assumed effects of HTK could explain the improved recovery of myocardial contractility and the preserved myocardial ATP level following preservation.

The vascular endothelium plays a major role in vasoregulation and coronary arterial vasoactivity regulates cardiac function [5]. Decreased coronary blood flow may lead to ischemia and additional tissue damage during reperfusion [14, 15]. Despite the importance of coronary regulation, few investigators have assessed in vitro coronary arterial function after prolonged ischemia followed by reperfusion. In the present study, we examined the performance of coronary vasculature from which myocardial metabolic and neurogenic influences were excluded, and the influences of coronary vasculature with the recovery of cardiac function after simple cold storage. According to our results, CO recovered well after preservation in HTK. Two explanations for this result are (1) the coronary vessels remained intact thus improving coronary flow, which improved CO recovery, and (2) the improved recovery of AF due to the well-preserved myocardial contractility. To examine the notion that preventing coronary vessel injury during preservation improves the recovery of cardiac function, we investigated coronary endothelial function in vitro. In addition, to account for the influence of flushing, flush/storage and flush/storage/reperfusion, we also investigated coronary arterial function under these conditions.

Flush injury before heart preservation for transplantation is controversial. Mankad and colleagues [16] and Saldanha and associates [17] have reported that a hyperkalemic cardioplegic solution impairs endothelial cell production of endothelium-derived relaxing factor. On the other hand, the high viscosity of UW increases shear stress and vascular resistance during perfusion, which might enhance endothelial injury [18]. In the present model, coronary arterial function before preservation did not differ among the nonflushed, UW (high potassium concentration; 130 mmol/L) flushed, and HTK (low potassium concentration; 10 mmol/L) flushed groups. Furthermore, the present study showed that after flushing with HTK or UW, the relaxation response to ACh did not differ between the groups and were identical to that of the nonflushed group. Therefore, flushing with HTK or UW does not appear to impair endothelial function and prevent flush injury under carefully controlled situations.

Other factors affecting the preserved heart are storage injury and preservation/reperfusion injury. Ku and asso-

ciates have reported that ischemia/reperfusion alters coronary vascular tone [19]. Quillen and coworkers have reported that although ischemia alone produces mild alterations in coronary microvascular reactivity in a dog model, ischemia followed by reperfusion produces a marked and selective impairment of endothelium-dependent responses in the coronary microcirculation [20]. In the present study, the maximal relaxation of coronary artery to ACh in the HTK group was higher than in the UW group after reperfusion, although no differences were noted in the before flush stage, both before and after storage (without reperfusion). In addition, the endothelium-independent relaxation response to SNP in the HTK group was retained following preservation and reperfusion. Thus, preservation/reperfusion could also alter the coronary vascular response in a like manner of ischemia/reperfusion injury. These findings suggest that reperfusion is the most causative phase to damage coronary circulation of preserved hearts than flush and flush/storage in heart transplantation. Several pathways for the production of oxygen-derived free-radicals, which may contribute to the death of cells, can be activated during reperfusion after a period of ischemia. Mizukawa and associates have reported that histidine protects endothelium-dependent relaxation against rose bengal-derived activated oxygen, the superoxide anion-derived singlet [21]. The high buffering capacity of histidine, and the membrane-protecting effects of tryptophan and a-ketoglutarate in HTK might also protect the endothelial membrane against free-radicals following reperfusion.

In the isolated heart model, neutrophils and platelets are not present in the perfusion, and therefore all findings cannot be extrapolated to other conditions such as the blood-perfused transplanted heart model. However, HTK attenuates preservation injuries, not only in the myocardium, but also in coronary epicardial arteries in the present study. The cardioprotective effects of HTK could be that HTK might keep well-preserved coronary circulation after storage following reperfusion, which might consequently protect myocardium against preservation/reperfusion injury.

In conclusion, HTK may be more suitable for cardiac preservation than UW. Reperfusion following flushing and storage may be the most causative factor of preservation injuries to the coronary artery. Further experimental and clinical trials of solutions and methods for myocardial and coronary vascular protection and preservation may increase donor heart availability.

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