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Effects of Celsior and University of Wisconsin preservation solutions on hemodynamics and endothelial function after cardiac transplantation in humans: a single-center, prospective, randomized trial

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Abstract Optimal preservation of the myocardium remains a major concern in clinical and experimental heart transplantation. The present study compared the efficacy of University of Wisconsin (UW) and Celsior preservation solution with respect to myocardial performance, epicardial and microvascular endothelial vasomotor function and myocardial expression of endothelin and nitric oxide synthases in humans. Forty-one cardiac transplant recipients received either UW ($n = 20$) or Celsior ($n = 21$) preserved hearts. Catecholamine and vasodilator requirements were assessed within the first 5 postoperative days. Left ventricular performance and endothelial function was assessed 1 month after transplantation. Endothelin and nitric oxide synthase gene expression were detected in myocardial biopsy samples. Celsior preserved hearts required significantly more catecholamines and vasodilators within the first 5 postoperative days. Myocardial performance and endothelial function were comparable 1 month after transplantation. Total ischemic time correlated with impaired endothelial function in the Celsior but not in the UW group. Endothelin and inducible nitric oxide synthase gene expression were significantly higher in the Celsior group. The results of the study show that both solutions provide myocardial protection with regard to left ventricular performance and endothelial function 1 month after cardiac transplantation. The necessity for higher vasodilator and catecholamine therapy in Celsior preserved hearts suggests post-ischemic myocardial stunning within the first 5 postoperative days. The positive correlation between impaired endothelial function and total ischemic time in the Celsior group requires longitudinal investigation in particular with regard to the development of allograft vasculopathy.

Key words Cardiac transplantation · Myocardial preservation · Endothelin · Nitric oxide synthases · Humans

Introduction

Optimal preservation of the myocardium remains a major concern in clinical and experimental heart transplantation. Acceptable myocardial protection can be obtained for 4–7 h with perfusion of and storage in cold cardioplegia. Recently, University of Wisconin solution (UW) has been proven to be effective in myocardial protection for clinical heart transplantation [1]. However, recent data suggest that UW may not effectively preserve endothelial function an important determinant of regional myocardial blood flow regulation [2]. Moreover, there is evidence that early development of endothelial vasomotor dysfunction predicts the subsequent development of transplant coronary artery disease (TVP) at 1 year post transplant [3]. Hypothermic pres-

ervation solutions, such as UW may contribute to the development of endothelial dysfunction; potential mechanisms may be the high potassium content, insufficient antioxidant capacity and/or temperature effects as well as perfusion pressure, subsequently leading to alterations of vasoactive mediators. Vasomotor dysfunction of the endothelium has been defined as an imbalance between relaxing and contracting factors and growth-inhibiting and growth-promoting factors [4]. Several studies have shown that dysfunction of the endothelium may be due to impaired release or increased inactivation of endothelium-derived nitric oxide (NO), thereby enhancing the vasoconstrictor response to various stimuli. In this regard, Okada et al. reported that endothelial cell damage caused by UW leads to enhanced release of endothelin, a potent vasoconstrictor peptide, with subsequent increase in coronary vascular resistance and diminished myocardial blood flow. They reported improved post-ischemic functional recovery after supplementation of an endothelin receptor antagonist in isolated rabbit hearts [5].

Celsior preservation solution has been introduced as a new formula for clinical heart transplantation. It was designed to provide a single solution used during the subsequent steps of the transplantation procedure from organ harvest to ultimate reperfusion in the recipient. Recently it has been shown that Celsior is superior when compared to St Thomas' Hospital solution with respect to left ventricular systolic and diastolic function in the rat heart [6]. Moreover, Celsior has been shown to have powerful antioxidant effects, which resulted in better diastolic and systolic function when compared to a cardioplegic solution in isolated heart experiments [7]. However, the effects of Celsior preservation solution on endothelium dependent and independent vasomotion in humans early after cardiac transplantation have not been evaluated. In addition, a direct functional comparison of donor hearts preserved with either UW or Celsior solution early after cardiac transplantation has not been assessed to date.

The present study investigated the effects of UW and Celsior preservation solutions on myocardial hemodynamics and epicardial and microvascular endothelial function early after cardiac transplantation in humans. In addition, we determined transcardiac plasma levels of endothelin and nitric oxide, two important endothelium-derived vasoactive substances.

Materials and methods

The study protocol was reviewed and approved by the Human Subjects Research Committee of the Ludwig-Maximilians University. All participants received orthotopic heart transplantation (HTx) in our clinic and gave written informed consent. Recipients enrolled in the study were randomly chosen to receive hearts perfused either with Celsior or UW solution.

Table 1 Compositions of the two preservation solutions: **A** Celsior, **B** University of Wisconsin (UW). Both solutions were administered as ready-to-use solutions and no additional additives were added. The final concentrations are given as mmol per liter of solution

A Celsior solution	
Mannitol	60 mmol
Lactobionic acid	80 mmol
Glutamic acid	20 mmol
Sodium hydroxide	100 mmol
Calcium chloride	0.25 mmol
Potassium chloride	15 mmol
Magnesium chloride	13 mmol
Histidine (base)	30 mmol
Glutathion (reduced)	3 mmol
Osmolarity	320 mOsmol/kg
pH	7.3
B University of Wisconsin solution	
Pentafraction (modified hydroxyethyl starch)	5%
Lactobionic acid	100 mmol
Potassium phosphate	25 mmol
Magnesium sulfate heptahydrate	5 mmol
Raffinose pentahydrate	30 mmol
Adenosin	5 mmol
Allopurinol	1 mmol
Total glutathion (glutathion/glutathionbisulfide)	3 mmol
Potassium hydroxide	100 mmol
Sodium hydroxide (pH adjustment)	30 mmol
Osmolarity	320 mOsmol/kg
pH	7.4

Donor heart preservation and cardiectomy

Donor hearts were either perfused with UW solution (1000.0 ml; $n = 20$; Belzer UW, DuPont Pharma, Bad Homburg, Germany) or with Celsior preservation solution (1000.0 ml; $n = 21$, Pasteur Merieux, Lyon, France). Compositions of solutions used in this study are given in Table 1.

Systemic heparinization of the donor was followed by separation of the ascending aorta from the pulmonary artery and dissection of the superior vena cava (SVC) extrapercardial. A catheter was inserted into the ascending aorta for delivery of cardioplegia. The SVC was ligated. Perfusion of the heart with cold preservation solution (1000 ml; 4°C) was started with constant pressure of 50 mmHg and the inferior vena cava and the right upper pulmonary vein were divided to avoid distention of the cardiac chambers. Additional topical cooling was perfomed with ice-cold saline solution poured into the pericardial cradle. After completion of perfusion the heart was excised and stored during transportation in cardioplegia in a sealed plastic bag on ice. During implantation the graft was cooled with ice-cold saline solution.

Patient demographics and exclusion criteria

Recipients ($n = 41$) were chosen for transplantation by donor dependent clinical criteria such as weight, height and blood group exclusively, which resulted in a comparable study population in both groups (Table 2). Post-operative exclusion criteria were impaired renal function (serum creatinine levels > 1.6 mg/dl), acute signs of infection 10 days or less prior to blood sample collection, rejection

Table 2 Donor and recipient demographics. No significant differences between donor dependent criteria were observed between the two groups. With respect to recipient data, significantly lower plasma cholesterol levels were observed in the UW group 1 month after transplantation (* $P < 0.05$). However, with respect to pre- and post-transplant data evaluated no significant differences were determined (*CMV* cytomegalovirus, *Dx* diagnosis, *CAD* coronary artery disease, *TPG* transpulmonary gradient, *PVR* pulmonary vascular resistance, *LDL* low-density lipoproteins, *HDL* high-density lipoproteins)

	Celsior $n = 21$	UW $n = 20$
Donors		
Age (years)	33.5 ± 9.8	32.3 ± 8.3
Weight (kg)	71.7 ± 3.2	77.2 ± 2.7
Height (cm)	171.1 ± 10.1	175 ± 8.3
Sex (% male)	62	63
CMV positive (%)	52	40
Graft ischemic time (min)	197.4 ± 13	209.7 ± 13.4
Recipients		
Age (years)	51.4 ± 2.7	49.3 ± 2.5
Weight (kg)	71.3 ± 3.6	76.8 ± 2.4
Height (cm)	174 ± 2.3	176.8 ± 7.6
Sex (% male)	79	100
CMV positive (%)	28.5	30
CMV mismatch, *n* [%]	9 [42.8]	6 [30]
Pre-transplant Dx (% CAD)	42	35
TPG (pre-transplant)	8.7 ± 3.1	10.2 ± 3.6
PVR (pre-transplant; Wood units)	2.08 ± 0.2	2.53 ± 0.22
Tacrolimus (ng/ml)	16.3 ± 1.6	18.5 ± 1.3
Creatinine (mg/dl)	1.2 ± 0.1	1.3 ± 0.08
Cholesterol (mg/dl)	202.2 ± 10	163.2 ± 12.7*
LDL (mg/dl)	109.3 ± 11.4	120 ± 8.8
HDL (mg/dl)	54.9 ± 4.2	53.01 ± 9.1
Native CAD risk factors		
Diabetes, *n* [%]	3 [14.2]	3 [15]
Hypertension, *n* [%]	4 [19]	4 [20]
Hyperlipidemia, *n* [%]	11 [52]	11 [55]
Tobacco, *n* [%]	3 [14.2]	5 [25]
Hyperuremia, *n* [%]	4 [19]	2 [10]
Alcohol, *n* [%]	1 [5]	0 [0]
Number of CAD risk factors per patient	1.2	1.35
Immunosuppressive regime		
Tacrolimus + mycophenolate + prednisolone	15	14
Tacrolimus + azathioprine + prednisolone	2	3
Cyclosporine + azathioprine + prednisolone	4	3
Number of rejection episodes (≤ 1b; ISHLT)	1.3 ± 0.2	1.2 ± 0.4

grade ≥ 1 b (according to International Society for Heart and Lung Transplantation) 10 days or less prior to collection of biopsy samples.

Patients were kept on standard immunosuppressive therapy, which included either tacrolimus (FK 506) or cyclosporine in combination with either mycophenolate mofetil or azathioprine and prednisolone. The immunosuppressive regimes did not differ between the two groups (Table 1). Other medication consisted of calcium antagonists, diuretics and lipid lowering drugs. Except for the immunosuppressive regime, all medications were discontinued 12–24 h prior to data and sample collection.

Evaluation of the inotropic state of the heart on postoperative days 1–5

Measurement of hemodynamics including endsystolic or end-diastolic pressures depend on loading and inotropic conditions including the regime of catecholamines and vasodilators administered and the amount of fluids being given. In order to evaluate postoperative cardiac function we determined the amount of catecholamines and vasodilators required to maintain mean arterial pressure (MAP) at between 80 and 100 mmHg with systolic pressures between 110 and 150 mm/Hg and diastolic pressures between 65 and 90 mmHg. In addition, left ventricular filling pressures were maintained at between 10 and 15 mmHg. Systemic vascular resistance was maintained at between 800 and 1500 dynes-s-cm^{-5} and pulmonary vascular resistance at below 300 dynes-s-cm^{-5}. Each patient was monitored continuously using a femoral arterial line and a Swan-Ganz catheter; the dosages of catecholamines and vasodilators were adjusted as appropriate. Mean dosages of catecholamines (epinephrine, norepinephrine, dobutamine and dopamine) and vasodilators including nitroglycerine, enoximone and epoprostenol (PGI_2) required for maintenance of pre-defined hemodynamics were calculated within the first 120 h after transplantation; it resulted in comparable values for MAP, systolic and diastolic pressures as well as systemic and pulmonary vascular resistance within this time frame.

A second evaluation of the inotropic state of the heart was assessed 35 ± 6 days (UW) and 38 ± 5 days (Celsior) after HTx. A left heart catheterization was performed in each patient. Left ventricular maximum rate of rise of pressure (+ dP/dt), endsystolic pressure (LVESP), end-diastolic pressure (LVEDP), mean arterial pressure (MAP; mmHg) and ejection fraction (EF; %) were evaluated. All data are shown in Table 3.

Table 3 Hemodynamics 24 h and 1 month after transplantation. No significant differences with respect to left ventricular hemodynamics were observed between the two groups 24 h and 1 month after cardiac transplantation (*MAP* mean arterial pressure, *CVP* central venous pressure, *PAP* pulmonary artery pressure, *SVR* systemic vascular resistance, *PVR* pulmonary vascular resistance, *CO* cardiac output, *CI* cardiac index, *LVESP* left ventricular endsystolic pressure, *LVEDP* left ventricular end-diastolic pressure, *+dP/dt max* maximal rate of rise of left ventricular pressure, *EDV* end-diastolic volume, *ESV* endsystolic volume)

	Celsior $n = 21$	UW $n = 20$
24 h post-transplant		
MAP (mm Hg)	94.7 ± 10.9	89 ± 8.5
CVP (mm Hg)	10.4 ± 1.3	11.2 ± 0.9
PAP (mm Hg)	20.4 ± 3.1	24.9 ± 2.1
SVR (dynes-s-cm^{-5})	1385 ± 267	1321 ± 157
PVR (dynes-s-cm^{-5})	221 ± 29	267 ± 30
CO (L/min)	6.2 ± 0.5	6.4 ± 0.3
CI (L/min/m^2)	3.2 ± 0.3	3.2 ± 0.1
1 month post-transplant		
LVESP (mm Hg)	136.9 ± 5.3	135.7 ± 4.2
LVEDP (mm Hg)	12.3 ± 3.7	14.5 ± 3.2
+dP/dt max (mm Hg/s)	2694 ± 225	2236 ± 158
Ejection fraction (%)	74.6 ± 1.7	78.8 ± 1.6
Stroke volume (ml)	74.4 ± 6.2	88.8 ± 5.3
EDV (ml)	96.9 ± 7	113.5 ± 6.5
ESV (ml)	23.2 ± 1.8	28.7 ± 4.9

Endothelium dependent and independent epicardial vasomotion

Quantitative coronary angiography with a computerized automatic-analysis system (Hicor, Siemens) was used to assess the vasomotor response (epicardial diameter changes) of the coronary arteries in an early but stable phase after Htx – 35 ± 6 days post Htx (UW) and 38 ± 5 days post Htx (Celsior). Proximal and distal coronary artery segments identified between easily visualized branch points were selected for analysis in the left anterior descending and circumflex coronary arteries. The mean proximal and distal diameter was calculated for each coronary segment from a series of at least two measurements. Endothelium dependent vasomotor response was measured with intracoronary administration of acetylcholine (1.0 and 30.0 μg/min for 5 min each). Endothelium independent vasomotion was determined with intracoronary infusion of adenosine (80.0 and 160.0 μg/min for 5 min each) and intracoronary bolus application of 0.2 mg nifedipine. Data were expressed as a percentage of the difference between stimulated and non-stimulated diameter of the proximal and distal segments.

Endothelium dependent and independent microvascular vasomotion

Microvascular vasomotor response was assessed by flow velocity measurements with an intracoronary doppler flow wire [0.018 in. (0.04 cm); Flo Wire, Cardiometrics Inc., USA)]. The flow wire was introduced in a 6-F Judkins catheter and positioned in the proximal part of the left anterior descending or circumflex coronary artery. The technical details of the system and its validation for accurate measurements have been described in detail elsewhere [8]. After successful positioning of the flow wire yielding a stable and optimal velocity tracing, baseline flow velocity readings were obtained. Hyperemic flow velocity data were determined with intracoronary adenosine infusion (Ad; 80.0 and 160.0 μg/min over 5 min each). Endothelium dependent changes in flow velocity were measured with intracoronary acetylcholine (Ach; 1.0 and 30.0 μg/min over 5 min each). Finally, a long-acting bolus of nifedipine was given i.c. to obtain endothelium independent vasomotor information in addition to adenosine. Coronary flow velocity reserve was assessed by calculation of the ratio of peak (Ad, Ach or nifedipine) to baseline blood flow velocity. Heart rate, mean arterial pressure, coronary flow velocity and electrocardiogram were monitored continuously throughout the procedure.

RNA extraction and cDNA preparation

Samples were homogenized with OMNI 200 homogenizer (Süd-Laborbedarf, Gauting, Germany) in 600 μl of lysis buffer (Quiagen, Hilden, Germany). Insoluble material was separated from the lysate by centrifugation at 10,000 g for 3 min. Total RNA was extracted from the supernatant using spin columns with a selective binding silica-based membrane (RNeasy Kit, Quiagen, Hilden, Germany). The total RNA was quantified by measuring the optical density at A_{260} and confirmed by gel electrophoresis. Complementary DNA (cDNA) was prepared from 2 μg of total RNA in 30 μl reverse transcription buffer (Gibco BRL, Paisley, Scotland) supplemented with 0.6 mM each of dATP, dGTP, dCTP and dTTP (all New England Biolabs, Shwalbach, Germany), 32 U RNase inhibitor (Boehringer, Mannheim, Germany), 400 U of Moloney murine leukemia virus reverse transcriptase (MMLV-RT, Gibco BRL, Paisley, Scotland), 10 mM dithiotreitol, and 1.5 μM $p(dt)_{15}$ primer (Boehringer, Mannheim, Germany) at 37 °C for 60 min. Subsequently, the reaction mixture was heat-inactivated for 10 min at 95 °C.

Polymerase chain reaction (PCR) procedure

An aliquot (3 μl) of cDNA was amplified by PCR with a DNA thermal cycler (Perkin Elmer 480, Cetus Corp., Norwalk, Conn.). The amplification reaction was carried out in a total volume of 50 μl of PCR buffer containing 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl, 50 mM KCl, 200 μM each of dATP, dGTP, dCTP and dTTP, 400 nM each of 3' and 5' primers, and 1 U Taq DNA polymerase (Boehringer Mannheim, Germany). The oligonucleotide primers had been synthesized on a DNA synthesizer (Applied Biosystems, Norwalk, Conn.) and purified using Sephadex columns (NAP 5, Pharmacia, Freiburg, Germany). The primers were designed such that the expected products were only obtained from cDNA, but not from genomic DNA. The GAPDH (glycerolaldehyde-3-phosphate dehydrogenase) transcript was used as an internal control of the processed RNA. The nucleotide sequences of the primers used in the amplification reaction are shown in Table 4. The PCR reaction mixture was covered with 50 μl of light mineral oil (Sigma Chemie, Deisenhofen, Germany). After 1 min pre-denaturation at 94 °C, the PCR conditions were as follows: denaturation at 94 °C for 45 s, annealing at 62 °C for 45 s, and extension at 72 °C for 1 min. To ensure detection of low-abundance mRNA, 35 amplification cycles were performed. No data were obtained after 40–45 amplification cycles because by then the reaction had reached the concentration pleateau making it impossible to account for semi-quantitative results. A sample of (10 μl) of each amplified product was subjected to electrophoresis in a 1% agarose gel (Promega, Madison, Wisc.), stained with ethidium bromide and visualized by UV illumination.

Table 4 Sequences of the primers used for detection of gene expression. Due to the primer design, specific detection of the product and not of genomic DNA was achieved (*GAPDH* glyceralaldehyde-3-phosphate dehydrogenase, *iNOS* inducible nitric oxide synthase, *cNOS* constitutive nitric oxide synthase, *ET* endothelin)

Primer	Sequence 5′ to 3′	Product size (bp)
GAPDH	TGAAGGTCGGAGTCAACG-GATTTGGT and CATGTGGGCCATGAGGTCCACCAC	983
iNOS	GGCCTGGAAACGCACAAGCTG and TTGGGGTTGAAGGCACAGCTG	506
cNOS	GAAGAGGAAGGAGTCCAGTAACAC and GGTGGCCCTCGTGGACTTGCTG	451
ET-1	GTCAACACTCCCGAGCACGTT and CTGGTTTGTCTTAGGTGTTCCTC	350

Semiquantitative analysis of detected signals was performed by a computer supported densitometric analysis image system. The data obtained were normalized for the DNA standard of each gel to account for variation between gels. In addition, the scores were normalized for the corresponding signal for the housekeeping gene GAPDH, which served as a internal standard. Data were scored between 0 (no detection) and 5 (maximum detection).

Determination of endothelin plasma levels

To account for actual myocardial production of endothelin, coronary sinus and aortic plasma levels were measured. Blood samples (10.0 ml each) were taken from the coronary sinus prior to endomyocardial biopsy and during left heart catheterization procedures from the ascending aorta, stored on ice and centrifuged immediately. Plasma (approx 5.0 ml) was frozen at –80 °C. Endothelin plasma concentration was determined by radio immuno assay (RIA) as described previously [9] In brief, samples were lyophilized and re-dissolved in 350 µl of RIA buffer (containing 13.75 g NaH_2PO_4, 43.8 g NaCl, 0.5% bovine serum albumin, 5 g gelatin and 0.5% Triton X-100 in 5 l deionized water at pH 7.3) to which 100 µl ET-1 antiserum (RAS 6901 N; rabbit; Peninsula, Heidelberg, Germany) was added. Cross-reactivity of the antiserum was 100% to the human antigen as specified by the manufacturer. [3-^{125}I-Tyr-]-ET-1 (50 µl) was added to the assay reaction and samples were incubated overnight. Separation of bound and unbound tracer was achieved by addition of 500 µl charcoal suspension (containing 0.5 g bovine serum albumin and 1.25 g activated charcoal in 100 ml RIA buffer without Triton X-100). Bound radioactivity was measured in a gamma counter and calculated in fmol/ml.

Determination of total plasma nitrite

Plasma of aortic root and coronary sinus blood samples was stored frozen at –80 °C. Total nitrate/nitrite levels were measured by the Gries reaction. In brief, plasma was deproteinized by ultrafiltration (Centrifree micropartition system, Amicon, Beverly, Mass.). The nitrate content of the sample was reduced to nitrite with a nitrate reductase. Sample aliquots were incubated in the presence of 0.1 U/ml nitrate reductase in 2.5 mM phosphate buffer containing 5.0 µM FAD and 50 µM NADPH (all from Boehringer, Mannheim) in final sample volume of 106.7 µl and incubated for 30 min at 37 °C. NADPH was oxidized by adding 6.7 µl of a reaction mix containing lactatdehydrogenase (dilution 1:10; 60 µl) and pyruvate (140 µl) followed by 5 min incubation at 37 °C. Finally the Gries reagent was added followed by incubation at 37 °C for 10 min. The reaction was stopped by adding 333.0 µl of cold TCA (1.2 M). Samples were measured by spectrophotometric analysis at 540 nm. A standard curve was performed in each experiment. The nitrite content of the samples was calculated from the standard curve, which was linear within this range.

Statistical analysis

Group data are presented as arithmetic mean values ± SD. One way analysis of variance (ANOVA) was performed for comparison of functional parameters. Paired *t*-test was used to assess differences within each individual. Correlation coefficient was determined by simple regression analysis. *P*-values of 0.05 or less were considered statistically significant.

Results

Baseline demographics and clinical characteristics

No significant differences regarding donor dependent clinical criteria such as age, weight, height, medication requirement, or cytomegalovirus (CMV) status were observed between the two groups. Detailed patient data are summarized in Table 2. As shown, no significant differences among recipient data were noted with respect to pre- and post-operative parameters except plasma cholesterol. In addition, total ischemic time (min) and the number of rejection episodes (> 1b ISHLT) did not differ significantly between the two groups. Nor were any differences noted between groups with respect to histological findings including cellular infiltration, morphology of cardiomyocytes and cellular necrosis as examined by hematoxilin & eosin staining of biopsy samples.

Catecholamines and vasodilators required on post-operative days 1–5

Patients who received hearts preserved with Celsior solution required significantly more catecholamines and vasodilators to achieve a hemodynamic status within the pre-defined range (Fig. 1). This included dobutamine, enoximone and nitroglycerine. This occurred despite comparable pre-transplant pulmonary vascular resistance (PVR) and transpulmonary gradients (TPG) in patients with Celsior preserved hearts when compared to UW patients (PVR: Celsior: 2.08 ± 0.2 vs UW: 2.53 ± 0.22, P = n.s.; TPG: Celsior: 8.7 ± 3.1 vs UW: 10.2 ± 3.6, P = n.s.).

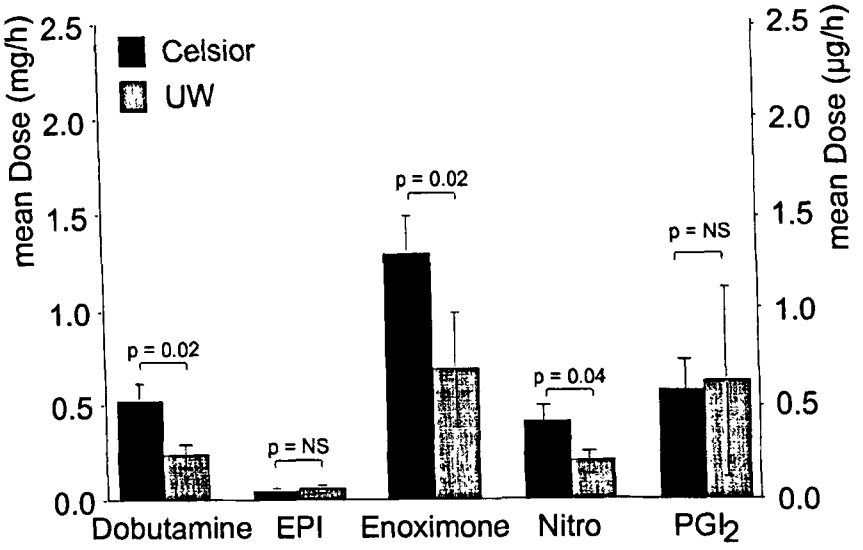

Fig. 1 Mean dosages for various catecholamines and vasodilators. In order to maintain pre-defined clinical hemodynamics within the first 120 h after transplantation, significantly more milligrams per hour of dobutamine, enoximone and prostaglandins (PGI_2) were administered in those patients who received Celsior preserved hearts. No significant differences were noted for nitroglycerine or epinephrine (EPI). Patients responded well to the drugs administered. In particular, the myocardial response to catecholamines was sufficient, suggesting myocardial stunning which may have developed during the ischemic period

Left ventricular hemodynamics 24 h and 1 month after transplantation

Hemodynamics measured 24 h post Htx did not differ significantly between the two groups. In addition, left ventricular hemodynamics evaluated 1 month after transplantation showed no significant differences between the groups (Table 3).

Epicardial and microvascular vasomotor response

No significant differences between the groups were noted with respect to proximal epicardial luminal diameter changes (%) in response to adenosine (160 µg/min; Celsior: 8.3 ± 2.6 vs UW: 7.1 ± 1.9, P = n.s.) and acetylcholine (30.0 µg/min; Celsior: −5.3 ± 3.5 vs UW: −5.4 ± 2.9, P = n.s.). Moreover, comparable data were obtained with respect to distal epicardial luminal diameter changes (%) between both groups in response to adenosine (160 µg/min; Celsior: 20.6 ± 2.3 vs UW: 16.3 ± 2.1, P = n.s.) and acetylcholine (30.0 µg/min; Celsior: 7.9 ± 3.9 vs UW: 11.3 ± 3.2, P = n.s.) The overall slight epicardial vasoconstriction observed in both groups in response to Ach indicates a mild epicardial endothelial vasomotor dysfunction.

No differences were noted between the groups with respect to coronary microvascular response (flow velocity reserve) to adenosine (160 µg/min; Celsior: 2.48 ± 0.1 vs UW: 2.6 ± 0.2, P = n.s.) and acetylcholine (30 µg/min; Celsior: 2.51 ± 0.16 vs UW: 2.7 ± 0.2, P = n.s.).

Despite the fact that overall CFVR was comparable between the two groups, it appeared that in Celsior but not in UW perfused hearts, total ischemic time was associated with CVFR to Ach (Fig. 2).

Myocardial endothelin, constitutive and inducible NOS gene expression

Gene expression analysis did not reveal significant differences with respect to constitutive nitric oxide synthase (cNOS) (Celsior: 2.1 ± 0.6 vs UW: 2.1 ± 0.6, P = n.s). However, densitometric analysis showed a significant difference in myocardial inducible NOS (iNOS) gene expression (Celsior: 1.9 ± 0.2 vs UW: 1.3 ± 0.2, P = 0.04). In addition, a significantly higher endothelin (ET) gene expression was noted in hearts preserved with Celsior solution (Celsior: 2.9 ± 0.2 vs UW: 2.1 ± 0.3, P = 0.01).

Transcardiac release of endothelin and nitrite

Overall, ET plasma levels in both groups were significantly higher when compared to healthy subjects as shown previously by others [10]. However, coronary sinus ET levels did not differ between groups (Celsior: 10.0 ± 0.4 vs UW: 9.7 ± 0.0.7, P = n.s.). In addition, in both groups ET plasma levels were significantly lower in coronary sinus when compared to aortic values, indicating transcardiac ET net extraction (Celsior, aorta:

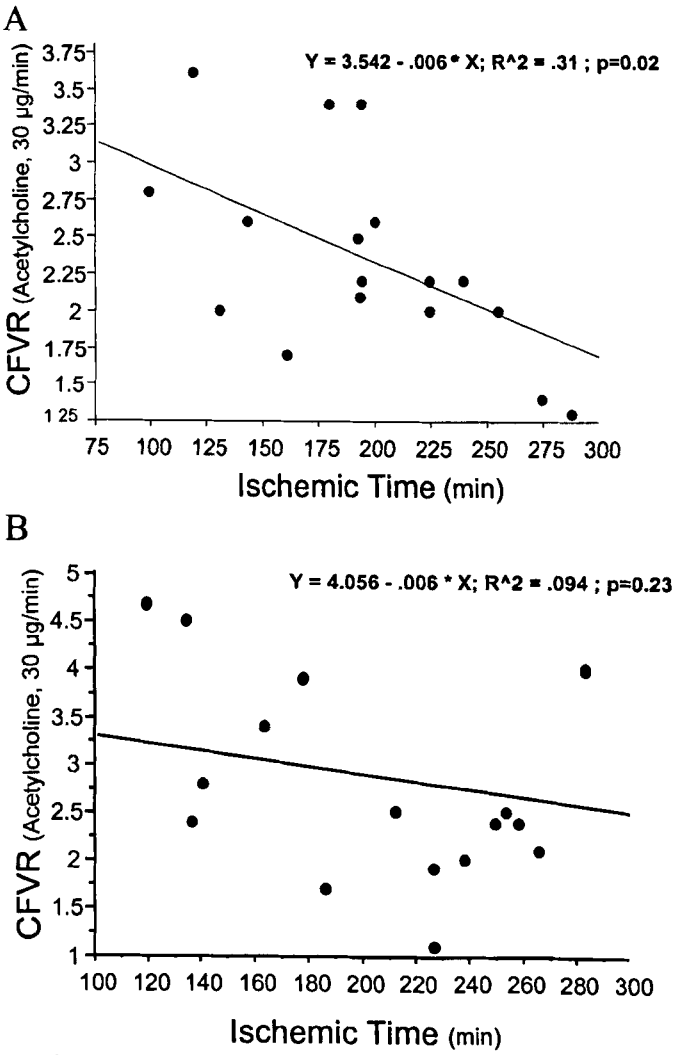

Fig. 2 A, B A significant correlation was found between total ischemic time (min) and endothelium dependent coronary flow velocity reserve (CFVR) in response to acetylcholine (Ach), with impaired response to Ach in patients with longer total ischemic time. Seven patients in the Celsior group showed an impaired flow velocity response to Ach (increase in flow velocity < factor 2.0) (**A**). In contrast, total ischemic time and endothelium dependent coronary flow reserve did not correlate significantly in the UW group (**B**)

11.8 ± 0.38 vs CS: 10.0 ± 0.4, $P = 0.0003$; UW, aorta: 12.6 ± 0.9 vs CS: 9.7 ± 0.7, $P = 0.003$).

No significant difference was found in coronary sinus nitrite levels between the two groups (Celsior: 38.7 ± 7.0 vs UW: 43.5 ± 6.6, P = n.s.). However, there was a significant increase of transcardiac nitric oxide production (nitrite concentration in coronary sinus as compared to aortic levels) in Celsior preserved hearts (Celsior, aorta: 27.0 ± 6.1 vs CS: 38.7 ± 7.0, $P = 0.04$; UW, aorta: 39.6 ± 7.9 vs CS: 43.5 ± 6.6, P = n.s.).

Discussion

The present study is the first single-center, prospective and randomized clinical report showing that both Celsior and UW preservation solutions preserve myocardial performance and endothelial function in epicardial and microvascular compartments 1 month after cardiac transplantation.

The major findings are

1. Myocardial function (LVESP, + dP/dt, LVEDP, EF) assessed 1 month after HTx showed regular function of the grafts, which did not differ significantly between the two groups (Table 3);

2. Epicardial and microvascular endothelial function, potential precursors of the development of transplant vasculopathy seen in later stages after HTx, did not differ between the groups;

3. The development of early right ventricular dysfunction in patients with Celsior preserved hearts, which required significantly more catecholamines and vasodilators when compared to UW perfused hearts (Fig. 1). Allografts responded well to the drugs administered, indicating that myocardial stunning may have developed during the ischemic period from explantation to initial reperfusion in the recipient.

4. Cold ischemic time correlated negatively with endothelium dependent CFVR in Celsior but not in UW perfused hearts (Fig. 2);

5. ET and nitric oxide systems are expressed and activated in both groups 1 month after HTx, as shown on the transcriptional level (gene expression) and by determination of the end products of the two systems. All sample and data collection were performed in the absence of acute rejection and infection, indicating a chronic high (smolder) immunologic activation even under sufficient immunosuppressive therapy.

As shown in Table 2, the two groups received different immunosuppressive regimes. As to what extent this may have an impact on myocardial and endothelial function early after transplantation remains uncertain. However, the different treatment regimes were found in both groups (Table 2). Moreover, no significant intra- or intergroup differences were noted with respect to myocardial and endothelial function.

Celsior preservation solution is a new compound that is currently under clinical investigation for organ transplantation in humans. The formulation was designed to prevent cell swelling and oxygen-free radical-induced injury, both being mediated by static hypothermic storage, and to enhance tissue preservation, which can be achieved by prevention of contracture. It is a single solution which can be administered during all the various steps of the transplantation procedure from organ harvest to reperfusion in the recipient. In several experimental settings Celsior has been shown to preserve myocardial function effectively, to protect against free rad-

ical mediated cell damage and to prevent ischemia-reperfusion induced pulmonary microvascular permeability [6, 7, 11]. In the present study significantly more patients in the Celsior group developed early myocardial dysfunction and required significantly more catecholamines and vasodilators when compared to patients in the UW group (Fig. 1). This was observed despite comparable pulmonary and systemic vascular resistance, and comparable mean arterial and central venous pressure (Table 2). The exact mechanism(s) can not be identified in the present study. However, the fact that myocardial function in the Celsior group improved over time (comparable hemodynamics after 1 month) and the adequate hemodynamic response of the grafts to the drugs administered within the first 5 postoperative days, indicate a reversible dysfunction (stunning), which may have occurred during the ischemic period [12, 13]. Most experimental and clinical studies evaluate left ventricular function as markers for myocardial preservation. However, the right ventricle may be even more sensitive to lack of protection. In this regard, Van Trigt et al. have reported that the donor right ventricle is exposed to various factors that are detrimental to its mechanical performance before facing an increased afterload in the recipient [14]. In their studies they showed a 35% decrease in right ventricular versus a 19% decrease in left ventricular performance after cardiac transplantation in dogs [15]. Similar results were obtained by Shuhaiber et al. who reported that the right ventricle suffers more from ischemia and benefits more from the cardioplegic protection [16].

Semiquantitative analysis revealed significantly higher ET gene expression in Celsior preserved hearts. In addition, ET plasma levels were significantly elevated in transplant recipients of both groups when compared to healthy controls (unpublished observations). Upregulation of the ET system after cardiac transplantation has been shown in various experimental and clinical settings [10, 17]. It leads to vasoconstriction and mitogenesis, important factors in the development of myocardial dysfunction and transplant vasculopathy [18, 19]. Moreover, in nearly all our patients in both groups we observed a net extraction of ET over the coronary circulation. Whether or not this is indicative of activation of ET receptors within the myocardium remains elusive. Comparable data of enhanced ET plasma levels in transplant recipients were shown in a recent publication by Sudhir et al. [17]. Enhanced ET gene expression and plasma levels may contribute to the myocardial stunning observed in our patients. In fact, there is experimental evidence that activation of the ET system contributes to myocardial stunning [20, 21]. Therefore, with respect to the clinical situation, future studies are required using selective receptor antagonists to further evaluate the role of the ET system in these patients.

We also observed increased iNOS expression and enhanced myocardial NO production in the Celsior group. Nitric oxide produced by the inducible isoform may lead to oxyradical damage due to the formation of peroxynitrite subsequently contributing to allograft dysfunction. Worral et al. have recently shown that selective inhibition of iNOS by aminoguanidine improved myocardial function in a heterotopic transplant model in rats. They also showed a decrease in the number of infiltrating cells and improved vascular barrier function [22, 23]. Yang et al. reported similar findings in a rat model of acute allograft rejection [24]. These data indicate that selective modulation of iNOS, while preserving endothelial NO production, may be of benefit in the treatment of early allograft dysfunction.

University of Wisconsin (UW) solution has proven to be an effective myocardial protector in experimental and clinical transplantation [25, 26]. However, recent data suggest that UW may not preserve endothelium dependent relaxation in isolated rat hearts [2]. To the best of our knowledge, no clinical data with respect to the effects of both UW and Celsior preservation solutions on endothelial function in humans are available to date. In the present study, overall epicardial and microvascular endothelial function was comparable between both groups. However, there was an inverse correlation between total ischemic time and endothelium dependent vasomotor response to Ach in the Celsior group but not in UW preserved hearts (Fig. 2). This association may be of importance in particular when total ischemic time is prolonged. However, this finding does not proove causation. In the present clinical setting relatively short preservation times were examined; it is of major importance to further analyze the efficacy of Celsior solution with regard to endothelial function, in particular after longer preservation times.

Vasomotor dysfunction of the epicardial endothelium may precede and predict allograft vasculopathy seen in later stages after transplantation [3, 27]. However, microvascular dysfunction occurs independently of epicardial dysfunction, which indicates a heterogeneous development of the disease process within the cardiac allograft. Various factors may contribute to early endothelial dysfunction. It is known that increased ET levels may contribute to severe vasoconstriction and early endothelial dysfunction due to activation of ET-A receptors [28]. Moreover, Okada et al. showed enhanced maintenance of coronary blood flow and reduction of tissue edema as well as improvement of post-ischemic functional recovery using UW solution containing ET-A receptor antagonists in isolated rabbit hearts. Lerman et al. reported enhanced ET immunoreactivity in the coronary and systemic circulation in humans with early atherosclerosis, and suggested that it is an important participant in and marker for the disease process in humans [29].

In conclusion, the present data provide evidence that both preservation solutions provide appropriate myocardial protection evaluated early after heart transplantation. Whether or not an upregulation of the inducible NO pathway or endothelin system alters myocardial or endothelial long-term function remains uncertain. It is therefore of major importance to further evaluate both solutions, in particular with respect to activation of both vasoactive mediators and their role in reperfusion injury and the development of cardiac allograft vasculopathy.

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