Celsior Versus Custodiol: Early Postischemic Recovery After Cardioplegia and Ischemia at 5°C

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Background. **The aim of this experimental study was to compare the protective efficacy of the cardioplegic solutions Celsior and Custodiol. Canine hearts were examined with regard to energy metabolism and early postischemic recovery after 8 or 12 hours of ischemia at 5°C.**

Methods. **Canine hearts were preserved with Celsior or Custodiol (each n = 19). Five hearts of each group were used to determine myocardial content of energy-rich phosphates immediately after preservation and after 8 and 12 hours of ischemia at 5°C; the remainder were reperfused after 8 and 12 hours of ischemia. Control variables during reperfusion were myocardial content of energy-rich phosphates, myocardial K^+ uptake, left ventricular dP/dt_{max} and dP/dt_{min}, and incidence of arrhythmias in percentage of heart rate.**

Results. **Custodiol-preserved hearts contained more ATP than Celsior-preserved hearts after 8 and 12 hours of ischemia (8 hours p = ns, 12 hours, $p < 0.05$). During reperfusion after 8 hours of ischemia, dP/dt_{max} and dP/dt_{min} showed the same values for both solutions, after 12 hours values were significantly higher in Custodiol-preserved hearts ($p < 0.005$). The incidence of reperfusion arrhythmias was higher in hearts of the Celsior group (8 hours $p < 0.01$, 12 hours p = ns). Myocardial K^+ uptake during reperfusion after 8 and 12 hours of ischemia was about twice as high in Celsior-preserved compared to Custodiol-preserved hearts ($p < 0.005$).**

Conclusions. **In the Langendorff model of the canine heart, cardioplegia with Celsior showed no advantage over cardioplegia with Custodiol. Differences were observed, however, which may be clinically important, especially in the case of long cold-storage times.**

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In human cardiac transplantation the relatively short ischemic tolerance of 4 to 6 hours [1] between explantation and reperfusion of the graft remains a challenging problem. Prolongation of this ischemic time could increase the number of transplantable organs and would provide a greater margin of safety for heart transplant procedures. Therefore, great efforts are made to develop preservation solutions that may extend this time limitation [1, 2]. Recently, a new preservation solution, Celsior, was introduced (see Material and Methods), the ingredients of which are supposed to prevent important causes of ischemic cell damage. The impermeants mannitol and lactobionate are added to reduce cell swelling. Reduced glutathione, histidine, and mannitol minimize oxygen-derived free radical injury. Glutamate is applied as a substrate to enhance energy production and thus prevent contracture. High magnesium content and a slight degree of acidosis are assumed to prevent calcium overload [3]. The efficacy of the new solution has primarily been validated in experimental studies using rabbit and rat hearts [3, 4]. There are experimental studies in which Celsior is compared with University of Wisconsin solution (ViaSpan [DuPont, Bad Homburg, Germany]) in dog hearts [5–7]. Clinical studies were published in which Celsior was used for human heart preservation [8–11].

A well established and widely used preservation solution in heart transplantation is Bretschneider's histidine-tryptophan-ketoglutarate solution (Custodiol). It is based on another major principle of organ preservation and contains, besides other ingredients, a particularly large quantity of histidine, a strong biological buffer, that counteracts acidosis caused by metabolites accumulating in the heart during ischemia. This enhances anaerobic energy production and thus stabilizes the content of energy-rich phosphates [12, 13] on which successful reperfusion highly depends [14].

An experimental study that compares Celsior with Custodiol is still missing. For this reason we examined Celsior and Custodiol with regard to their protective efficiency during ischemia and reperfusion in canine hearts. Because the most important mark of quality in myocardial protection is the postischemic recovery of myocardial function, we set the main focus on this subject. However, as recovery of function depends on the availability of energy at the beginning of reperfusion, a second focus was set on the myocardial content of energy-rich phosphates during ischemia and reperfusion. The aim of our study was to answer the question whether the application of Custodiol or Celsior, under the given conditions, leads to differences in intraischemic protec-

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Table 1. Composition of the Cardioplegic Solutions

Component	Celsior®	Custodiol®
Na^+ (mmol/L)	100	15
K^+ (mmol/L)	15	10
Mg^{2+} (mmol/L)	13	4
Ca^{2+} (mmol/L)	0.25	0.01
Cl^- (mmol/L)	41.5	50
Lactobionate (mmol/L)	80	0
Mannitol (mmol/L)	60	30
Histidine (mmol/L)	30	198
Glutamate (mmol/L)	20	0
Glutathione (mmol/L)	3	0
Tryptophan (mmol/L)	0	2
Ketoglutarate (mmol/L)	0	1
pH (5°C)	7.3	6.8
Osmolarity (mosm/L)	320	314

tion and/or to a different early postischemic recovery of myocardial function.

Material and Methods

Experimental Protocol

Hearts were flushed at 5°C with Custodiol (n = 19) or Celsior (n = 19). The compositions of the preservation solutions Custodiol (Koehler Chemie, Alsbach-Haenlein, Germany) and Celsior (IMTX Sangstat, Lyon, France) are shown in Table 1. After preserving perfusion, hearts were examined with two different experimental models. In the ischemia model (n = 5 for each preservation solution), hearts were rapidly excised and immediately immersed in the respective preservation solution at 5°C. From these hearts, tissue samples for biochemical analysis were taken instantly after perfusion and after 8 or 12 hours of cold storage. In the reperfusion model, hearts were stored for 8 and 12 hours at 5°C (n = 7 for each preservation solution and each ischemic time). Thereafter, they were reperfused in Langendorff technique with modified Tyrode's solution at 37°C. During reperfusion, biochemical and functional variables were monitored.

Animals

Experiments were carried out in 28 to 35 kg adult fox hound dogs (n = 38) from the University of Heidelberg animal farm. All dogs were treated humanely in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985) and the German Animal Protection Law (Tierschutzgesetz 1998). All experiments were approved by the animal protection committee.

Anesthesia

After subcutaneous premedication with 90 mg piritramide (Dipidolor, Janssen-Cilag, Neuss, Germany) and 0.5 mg atropine (Braun, Melsungen, Germany), anesthesia was introduced intravenously with 15 mg/kg thiopental (Trapanal, Byk Gulden, Konstanz, Germany) and 0.5 mg/kg midazolam (Dormicum, Roche, Grenzach-Wyhlen, Germany) and maintained with approximately 0.015 $mg \cdot kg^{-1} \cdot h^{-1}$ fentanyl (Fentanyl-Janssen, Janssen-Cilag, Neuss, Germany), approximately 5 $mg \cdot kg^{-1} \cdot h^{-1}$ piritramide, and N_2O. Controlled ventilation was performed with N_2O/O_2 (1/1) using a respirator (Stephan, Gackenbach, Germany). The animal's end-expiratory P_{CO_2} was continuously monitored with a Capnomac Ultima analyzer (Datex, Helsinki, Finland). Systemic arterial pressure was measured in the left arteria carotis communis and continuously monitored together with the electrocardiogram (ECG) using an eight-channel recorder (Biobit, Goettingen, Germany) and Variobit software (Dr. Langer, Waldkirch, Germany).

Surgical Preparation and Perfusion Procedure

After median sternotomy, a catheter was introduced into the sinus coronarius through the right vena jugularis externa. A double-lumen perfusion catheter was inserted in the left arteria subclavia and edged until the tip lay in the aortic bulb, immediately distal to the aortic valve. One lumen served as perfusion-line, the other permitted the measurement of perfusion pressure. After these preparations, the dogs were given 10,000 units heparin intravenously. The cardioplegic perfusion was commenced by inflow occlusion of the inferior and superior vena cava. The aorta ascendens was ligated distal of the aortic bulb, simultaneously perfusion with the preservation solution (30 to 50 mL/kg, previously cooled to 5°C) was started at a pressure head of 80 cm H_2O. Both auricles and the left ventricular apex were incised to allow drainage of the effluent. During perfusion, samples of arterial and coronary venous perfusate were taken every minute to determine arteriovenous differences of P_{O_2}, K^+, and lactate. The ECG and aortic perfusion pressure were monitored using an eight-channel recorder and Variobit software. The infusion of the solution was completed after 3 to 5 min. During this time, perfusion pressure descended to approximately 55 cm H_2O.

Experimental Groups

In the ischemia model, immediately after the end of cardioplegic perfusion a transmural tissue sample for biochemical analysis was taken from the left ventricular wall. Then hearts were rapidly excised and stored in a beaker containing the respective preservation solution, and tempered in a refrigerated bath at 5°C. Additional transmural tissue samples were taken after 8 and 12 hours of ischemia. All tissue samples were immediately processed.

In the reperfusion model, after cardioplegic perfusion, hearts were left in position, enveloped in a plastic bag containing the respective cardioplegic solution, and tempered at 5°C. After 8 or 12 hours of ischemia, they were reperfused in Langendorff technique at 37°C with an oxygenized ($P_{O_2} > 700$ mm Hg) modified Tyrode's solution [15] (mmol/L: NaCl 136, Na-lactate 5, KCl 4, $MgCl_2$ 0.5, $CaCl_2$ 2.5, NaH_2PO_4 0.5, glucose 5, HEPES 10, HEPES-Na 13.5, tryptophan 0.1, insulin 0.1 UI/L, gluca-

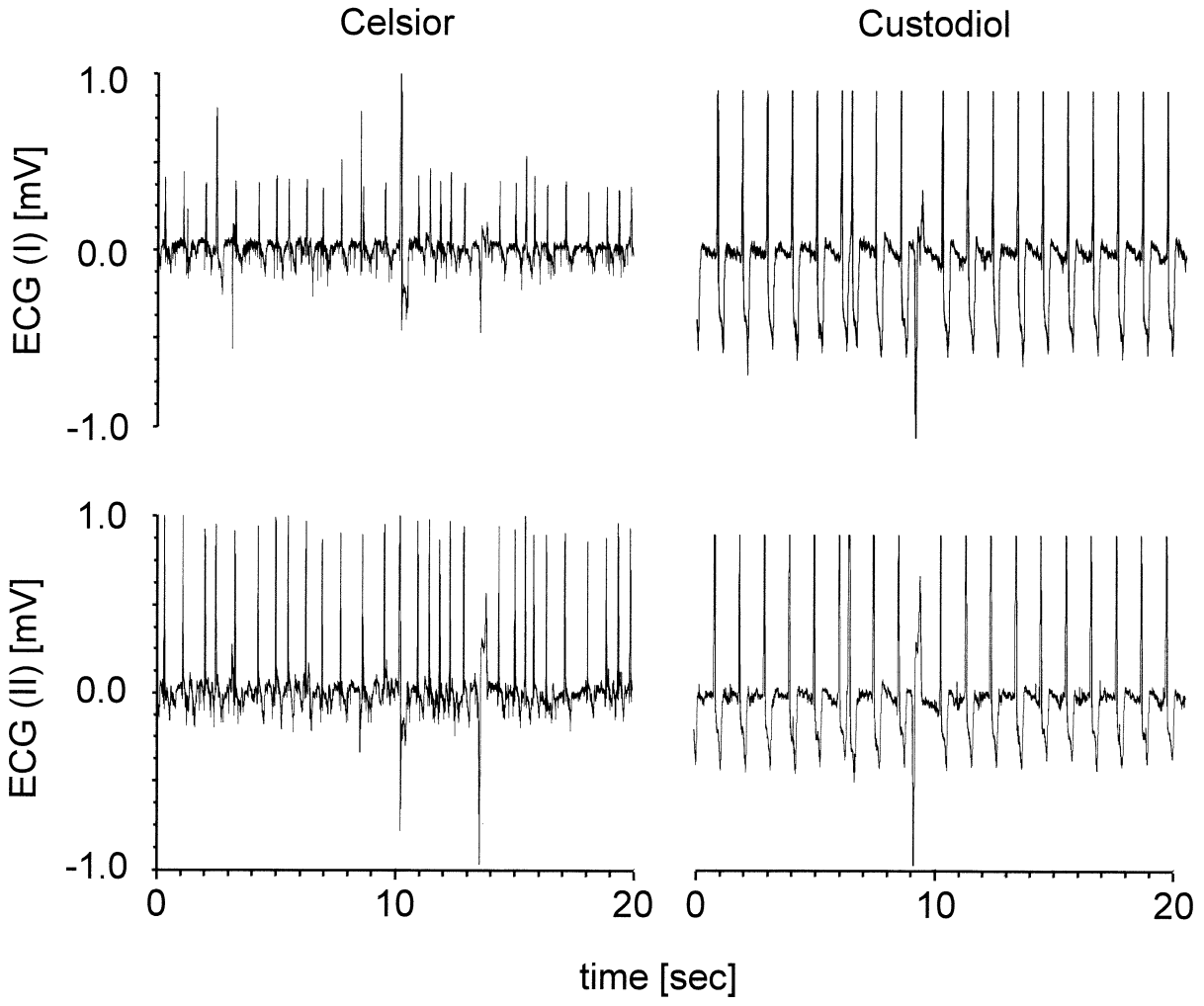

*Fig 1. Two representative examples of the original tracing of electrocardiogram (*ECG*) during reperfusion. Hearts were preserved with Celsior or Custodiol and stored at 5°C for 8 hours. The incidence of arrhythmias was estimated by dividing the number of arrhythmic beats per minute by the heart rate.*

gon 0.1 μg/L). During the first 4 minutes of reperfusion, hearts were allowed to warm to at least 35°C; then they were defibrillated. Defibrillation was performed with 30 J using a Codemaster XL intrathoracal defibrillator (Hewlett-Packard, Böblingen, Germany); after three ineffective defibrillations, further attempts were made with 50 J. To meet the tissue's need for oxygen, the flow was, depending on the rise of heart rate, successively raised from 176 ± 5 $mL \cdot min^{-1} \cdot 100g^{-1}$ wet weight at the beginning to 300 ± 12 $mL \cdot min^{-1} \cdot 100g^{-1}$ wet weight at the end of reperfusion. During the first 11 minutes of reperfusion, the left ventricle was vented with a suction catheter that was introduced through the incision at the apex cordis. Samples of arterial and coronary venous perfusate were taken every minute to determine arteriovenous differences in Po_2, K^+, and lactate. The ECG, aortic perfusion pressure, left ventricular pressure, and left ventricular dP/dt were monitored using an eight-channel recorder and Variobit software. Measurements of left ventricular pressure and its first derivative dP/dt were performed using a SPC-471A Micro-Tip pressure transducer catheter with lumen (Millar Instruments, Houston, TX), which also was inserted into the left ventricle through the incision in the apex cordis. The tip of the catheter was provided with a latex balloon (Hugo Sachs Elektronik, March-Hugstetten, Germany), which was filled with saline to prevent dislocation. The incidence of arrhythmias in percent of heart rate was calculated by dividing the number of arrhythmic beats per minute by heart rate (Fig 1). The reperfusion ended after 20 minutes. Immediately after the end of reperfusion, three tissue samples were taken from the left ventricular wall for biochemical analysis.

Sample Preparation and Metabolic Assays

Tissue samples were homogenized in cold 3.5% perchloric acid using a Miccra D-8 homogenizing system (Art, Muellheim, Germany). Protein in the homogenate was removed by centrifugation for 10 minutes at 5°C and 20,000 rpm in a F-28/50-rotor of a Sorvall RC 28S centri-

Table 2. Myocardial Energy Metabolism During Ischemia

	8 Hours Ischemia at 5°C		12 Hours Ischemia at 5°C	
	Custodiol®	Celsior®	Custodiol®	Celsior®
Phosphocreatine	5.1 ± 0.3	4.0 ± 0.2[a]	3.2 ± 0.5	2.4 ± 0.5
ATP	20.1 ± 0.6	17.7 ± 1.3	14.1 ± 1.6	11.3 ± 1.0[a]
ADP	6.4 ± 0.3	7.0 ± 0.3	6.3 ± 0.4	6.9 ± 0.5
AMP	1.4 ± 0.1	1.7 ± 0.1	2.1 ± 0.3	2.4 ± 0.2
ATP/ADP ratio	3.2 ± 0.2	2.5 ± 0.1[b]	2.3 ± 0.0	1.6 ± 0.1[a]
Lactate	99.3 ± 3.7	81.5 ± 8.1[a]	134.4 ± 6.3	116.2 ± 11.3
Glycogen	142.3 ± 18.7	155.8 ± 11.3	111.2 ± 16.8	119.6 ± 23.8

Hearts were perfused with Custodiol or Celsior and stored at 5°C for 8 or 12 hours. Values are means ± SEM of five experiments (given as μmol/g dry weight).

[a] Significantly different from corresponding ischemic time of the Custodiol group; $p < 0.05$. [b] Significantly different from corresponding ischemic time of the Custodiol group; $p < 0.01$.

ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate.

fuge (DuPont, Bad Homburg, Germany). The protein pellet remaining was used for determination of wet-to-dry weight ratios. The acid extracts were neutralized with 1.63 mol/L K_2CO_3 in 0.054 mol/L triethanolamine hydrochloride and filtered. An aliquot of the neutralized extracts was used subsequently for assays of phosphocreatine, ATP, ADP, AMP, glycogen, and lactate. The metabolites were assayed enzymatically based on the absorbance of NADH at 340 nm (Roche, Mannheim, Germany). Chemicals were analytic reagent grade and purchased from Merck (Darmstadt, Germany), J. C Baker (Deventer, The Netherlands), Sigma (St. Louis, MO), Hoechst Marion Russel GmbH (Frankfurt, Germany), Novo Nordisk (Bagsvaerd, Denmark), or Fluka (Buchs, Switzerland).

Statistical Analysis

All data are given as mean ± standard error of the mean (SEM). Statistical significance between pairs of values was evaluated by applying the *U* tests of Wilcoxon, Mann, and Whitney; differences among groups were tested using analysis of variance (ANOVA). Statistical significance was assumed when the *p* value was less than 0.05.

Results

Energy Metabolism During Ischemia

At the onset of ischemia immediately after cardioplegic perfusion, there was no significant difference between Custodiol- and Celsior-treated hearts concerning the myocardial content of phosphocreatine, ATP, ADP, AMP, lactate, and glycogen. Values for Custodiol and Celsior, respectively, were: phosphocreatine 46.2 ± 2.9 and 40.4 ± 3.8, ATP 26.7 ± 0.3 and 27.3 ± 1.1, ADP 4.4 ± 0.2 and 4.6 ± 0.3, AMP 0.7 ± 0.0 and 0.6 ± 0.1, lactate 3.5 ± 1.4 and 5.3 ± 1.4, glycogen 230.6 ± 15.0 and 237.7 ± 14.7 μmol/g dry weight. In the course of ischemia, the myocardial content of phosphocreatine, ATP, and glycogen decreased while the content of ADP, AMP, and lactate increased. The results after 8 and 12 hours of ischemia are shown in Table 2. There was no significant difference in myocardial ATP content between preservation solutions after 8 hours. After 12 hours, hearts of the Custodiol group contained significantly more ATP compared with Celsior-treated hearts ($p < 0.05$). The ATP/ADP ratio was significantly higher in Custodiol-treated hearts after both ischemic times (8 hours $p < 0.01$, 12 hours $p < 0.05$). Custodiol-preserved hearts metabolized more glycogen than Celsior-preserved hearts (8 and 12 hours, p = ns) reaching approximately 50% of preischemic values in both groups after 12 hours of ischemia. This shows that, even after this ischemic time, the myocardial glycogen content still is no limiting factor for glycolysis. At the same time, Custodiol-treated hearts accumulated more lactate than Celsior-treated hearts (8 hours $p < 0.05$, 12 hours, p = ns).

Metabolism and Function After Reperfusion

During reperfusion high energy phosphates were noticeably rephosphorylated in all hearts (Table 3). However, except for phosphocreatine, preischemic values were not reached and the differences between Custodiol- and Celsior-preserved hearts were not significant.

There were no significant differences in heart rate between Custodiol- and Celsior-preserved hearts after 8 hours or 12 hours of ischemia (beats per minute in the 20th minute of reperfusion: 8 hours 71 ± 6 and 90 ± 9, 12 hours 86 ± 7 and 97 ± 3, respectively).

Myocardial O_2-consumption during reperfusion after 8 hours as well as after 12 hours of ischemia was higher in Custodiol-treated hearts than in Celsior-treated hearts. After 8 hours, myocardial O_2-consumption per heartbeat in the 20th minute of reperfusion was 3.7 ± 0.5 and 3.0 ± 0.5 μL/g dry weight (ns), after 12 hours 2.6 ± 0.3 and 2.1 ± 0.1 μL/g dry weight ($p < 0.05$) for Custodiol- and Celsior-preserved hearts, respectively.

The balance of potassium during reperfusion showed a marked difference between Custodiol- and Celsior-treated hearts. Although all hearts showed a K^+ uptake, it was nearly twice as high in the Celsior group compared with the Custodiol group after 8 hours (51.9 ± 4.6 and 29.7 ± 5.9 $\mu mol \cdot g\ dry\ weight^{-1} \cdot 20\ min^{-1}$, respectively, $p < 0.005$) as well as after 12 hours of ischemia (60.3 ± 3.7

Table 3. Myocardial Energy Metabolism After 20 Minutes of Reperfusion

	8 Hours Ischemia at 5°C		12 Hours Ischemia at 5°C	
	Custodiol®	Celsior®	Custodiol®	Celsior®
Phosphocreatine	37.3 ± 5.9	34.8 ± 6.3	30.5 ± 4.4	43.2 ± 6.2[a]
ATP	19.9 ± 1.6	20.3 ± 1.4	16.4 ± 1.2	15.7 ± 1.0
ADP	3.9 ± 0.2	4.0 ± 0.2	3.9 ± 0.1	4.0 ± 0.2
AMP	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	0.8 ± 0.1
ATP/ADP ratio	5.1 ± 0.5	5.2 ± 0.4	4.2 ± 0.2	3.9 ± 0.2
Lactate	40.6 ± 7.4	47.4 ± 8.1	53.3 ± 8.6	41.5 ± 6.2
Glycogen	149.8 ± 26.4	154.0 ± 26.6	116.4 ± 15.0	98.3 ± 10.4

Hearts were perfused with Custodiol or Celsior, stored at 5°C for 8 or 12 hours, and reperfused with Tyrode's solution for 20 minutes. Values are means ± SEM of seven experiments (given as μmol/g dry weight).

[a] Significantly different from corresponding ischemic time of the Custodiol group; $p < 0.05$.

ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate.

and 34.4 ± 4.3 $\mu mol \cdot g$ dry $weight^{-1} \cdot 20\ min^{-1}$, respectively, $p < 0.005$).

Left ventricular systolic and diastolic function postischemically recovered in all hearts (Figs 2 and 3). Both dP/dt_{max} and dP/dt_{min}, were rising, but to a different degree. After 8 hours of ischemia in Custodiol and Celsior cardioplegia, dP/dt_{max} and dP/dt_{min} reached approximately the same absolute value within 20 minutes. After 12 hours of ischemia and 20 minutes reperfusion, Custodiol-preserved hearts nearly recovered to the same absolute degree as after 8 hours of ischemia, whereas Celsior-preserved hearts reached significantly lower values (ANOVA, $p < 0.005$).

Cardiac rhythm showed differences concerning the number of necessary defibrillations at the beginning and the incidence of arrhythmias in the further course of reperfusion. All hearts started with fibrillation approximately 10 to 20 seconds after the beginning of reperfusion. After 8 hours of ischemia, Custodiol- and Celsior-treated hearts needed about the same number of defibrillations to start regular cardiac action (1.9 ± 0.6 and 1.7 ± 0.4, respectively). After 12 hours of ischemia, the number of defibrillations remained unchanged for Custodiol-preserved hearts, whereas Celsior-preserved hearts needed more than twice as many defibrillations (1.7 ± 0.4 and 3.9 ± 0.5, $p < 0.01$). Comparing the incidence of arrhythmias during the first 20 minutes of reperfusion, Celsior-treated hearts showed a higher rate of arrhythmic beats than Custodiol-treated hearts after 8 as well as after 12 hours of ischemia (Fig 4). The differ-

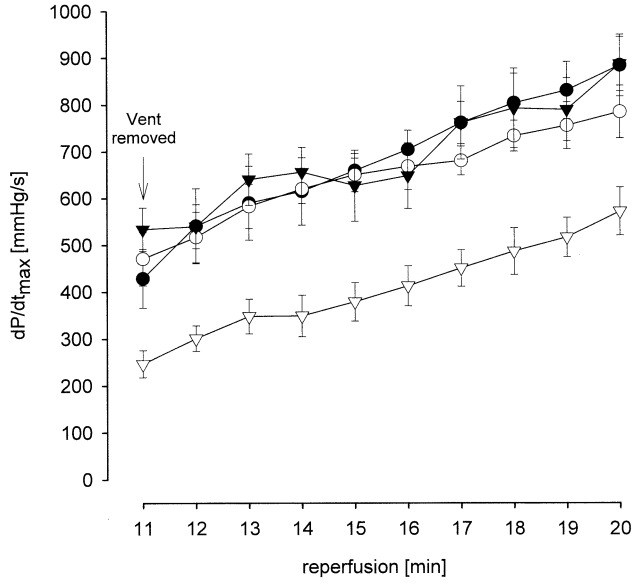

Fig 2. Left ventricular dP/dt_{max} during reperfusion. Hearts were perfused with Custodiol (—●—, —○—) or Celsior (—▼—, —▽—) and stored at 5°C for 8 hours (—●—, —▼—) or 12 hours (—○—, —▽—[a]). Values are means ± SEM, n = 7 for each group. [a]Significantly different from corresponding ischemic time of the Custodiol group; analysis of variance $p < 0.005$.

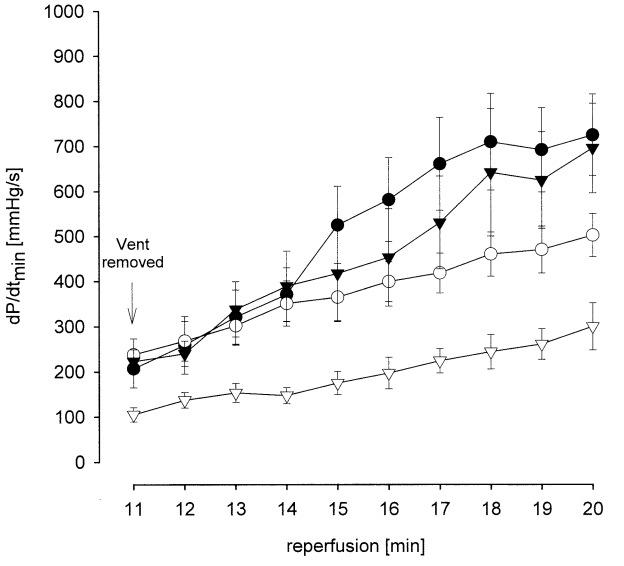

Fig 3. Left ventricular dP/dt_{min} during reperfusion. Hearts were perfused with Custodiol (—●—, —○—) or Celsior (—▼—, —▽—) and stored at 5°C for 8 hours (—●—, —▼—) or 12 hours (—○—, —▽—[a]). Values are means ± SEM, n = 7 for each group. [a]Significantly different from corresponding ischemic time of the Custodiol group; analysis of variance p < 0.005.

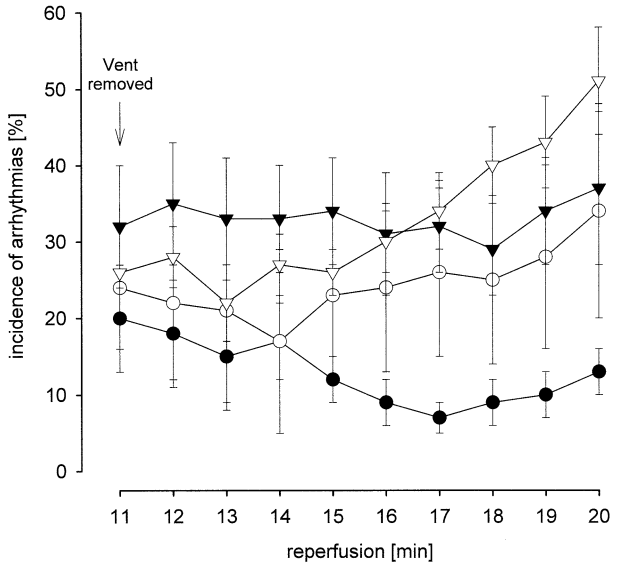

Fig 4. Incidence of arrhythmias during reperfusion. Hearts were perfused with Custodiol (—●—, —○—) or Celsior (—▼—, —▽—) and stored at 5°C for 8 hours (—●—, —▼—[a]) or 12 hours (—○—, —▽—). Values are means ± SEM, n = 7 for each group. [a]Significantly different from corresponding ischemic time of the Custodiol group; analysis of variance $p < 0.01$.

ence was significant after 8 hours (ANOVA, $p < 0.01$), but not after 12 hours of ischemia.

Comment

There are different approaches to estimate the efficiency of cardioprotective solutions. Before reperfusion, the myocardial content of ATP is one of the most important variables, because lack of energy-rich phosphates may reduce the activity of the contractile apparatus. Functional recovery should progress as efficiently as possible so the recipient can be weaned from cardiopulmonary bypass. Therefore, in the early postischemic period, the most interesting question is, how completely a heart recovers from ischemic stress, ie, regains normal contractility and rhythm. Thus, we compared both the myocardial content of high-energy phosphates during ischemia and after reperfusion, and the recovery of contractility and rhythm during reperfusion. We limited the reperfusion to 20 minutes to avoid any alteration of functional variables through increasing edema, a frequent problem when saline perfusates are used, because of their lack of colloid-osmotic pressure.

In clinical heart transplantation, the assumed limit of ischemic time is 4 to 6 hours [1]. However, possibly damaged hearts of human donors and hearts of healthy dogs are not directly comparable. In the healthy canine heart, resuscitation after cardioplegia with Custodiol and 8 hours of ischemia at 5°C still proceeded smoothly; the first disturbances concerning the incidence of arrhythmias occurred after 12 hours of ischemia. At this ischemic time, near the frontier of resuscitability, differences in protective efficacy should be pronounced. For this reason, we chose these two ischemic times for our comparison.

The investigation of energy metabolism showed that the myocardial content of ATP in Celsior- and Custodiol-treated hearts lay in all of our measurements in the same order of magnitude, although before reperfusion Custodiol-preserved hearts contained more ATP after 8 and 12 hours. The ATP/ADP ratio, which is relevant to the regulation and status of oxidative phosphorylation [13] before reperfusion, was significantly higher in Custodiol-treated hearts than in Celsior-treated hearts after both ischemic times. These findings indicate that the energetic starting point before reperfusion was better in hearts perfused with Custodiol compared to those perfused with Celsior. A possible explanation for these differences is the higher buffering capacity of Custodiol. Both solutions contain the same buffer, histidine, but its concentration in Celsior is only approximately 15% of that in Custodiol. This leads to an approximately sevenfold higher buffering capacity of Custodiol as compared with Celsior. Pulis and colleagues [13] compared differently buffered preservation solutions and showed that increasing buffering capacity enhances anaerobic energy production by relieving the pH inhibition of key enzymes of the glycolytic pathway and by maintaining the phosphorylation state of phosphofructokinase. Consistent with these findings are our data for myocardial lactate and glycogen content. Due to the higher intensity of ATP-producing anaerobic glycolysis in Custodiol-treated hearts, the glycogen values were lower and the lactate values higher compared with Celsior-treated hearts, which does not lead to acidotic damage because of the high buffering capacity.

In our study, all hearts were revivable after 8 hours as well as after 12 hours of ischemia, no matter which preservation solution had been used. Furthermore, during reperfusion all hearts refilled their energy stores to a certain extent. Nevertheless, recovery of contractility and cardiac rhythm during reperfusion were different depending on the preceding ischemic time and the preservation solution used. After 8 hours of ischemia, systolic and diastolic function were almost identical for both preservation solutions; thus, the protective range was at least 8 hours for both solutions. After 12 hours, dP/dt_{max} reached no more than approximately 60% of the 8-hour values in the Celsior group ($p < 0.005$), whereas in the Custodiol group it reached nearly the same values as after 8 hours. In both groups, dP/dt_{min} was reduced after 12 hours compared with 8 hours of ischemia, but the difference was only significant in the Celsior group ($p < 0.005$). The inferior systolic and diastolic function in the Celsior group after 12 hours of ischemia corresponds to a significantly lower oxygen consumption during and a significantly higher myocardial content of phosphocreatine after reperfusion (Table 3), the so called "phosphocreatine rebound" that is known to be correlated with reduced postischemic function [16].

To our knowledge, no experimental comparison between Celsior and Custodiol has been published, and the clinical studies concerning the recovery of heart function

after preservation with Celsior are partly contradictory. Wieselthaler and colleagues [8] published preliminary data of a randomized prospective study with 48 human heart transplantations and reported an increased requirement of postoperative inotropic support after Custodiol (7 of 24) compared with Celsior (2 of 24). In a recently published, randomized multicenter study with 131 heart transplantations [11], Celsior was claimed to be as safe and effective as a variety of not exactly specified conventional solutions for flush and cold storage of cardiac allografts. In contrast, Garlicki and colleagues [9] reported, in a trial with 224 patients, a trend toward decreased need for inotropic agents after preservation with Custodiol compared with Celsior and ViaSpan. In a comparison between ViaSpan and Celsior in 41 cardiac transplantations, Wildhirt and colleagues [10, 17] found that Celsior-preserved hearts required more inotropic support in the early phase after transplantation, and these investigators assumed a reversible dysfunction (stunning) as the underlying phenomenon.

The second functional variable that we investigated was cardiac rhythm. Reperfusion arrhythmias are commonly observed during postischemic reperfusion after open heart surgery and transplantation [18]. Many different mechanisms are believed to be responsible for triggering reperfusion arrhythmias, eg, heterogeneous reperfusion, free oxygen radicals, imbalance of electrolytes, and low levels of glycolysis and ATP during ischemia (for review, see [19]). In our study, we found differences in cardiac rhythm between both groups of hearts. First, the number of necessary defibrillations at the beginning of reperfusion was twice as high in Celsior-preserved hearts after 12 hours of ischemia compared with Custodiol-preserved hearts. However, the most striking difference was the pronounced heterogeneity in cardiac rhythm in the Celsior group after both ischemic times. The difference was significant after 8 hours of ischemia, when Celsior-treated hearts showed a nearly constant incidence of arrhythmias of about 35% over the period of reperfusion, whereas Custodiol-treated hearts started with about 20% and improved to about 15% until the end of reperfused period. After 8 hours of ischemia, there was still no dramatic decrease in energy-rich phosphates, and energy levels were replenished during reperfusion. On that account, energy status cannot be the reason for the rhythm disturbances in Celsior-preserved hearts, because cardiac rhythm during reperfusion did not improve whereas energy status did. Dysrhythmias, however, can also be caused by potassium imbalances between extra- and intracellular space. After 8 and 12 hours of ischemia, the myocardial K^+ uptake during reperfusion was twice as high in Celsior-treated hearts than in Custodiol-treated hearts. This implicates two possible mechanisms that may have induced the observed arrhythmias. Either dysfunction of the ATP-dependent Na^+-K^+ pump during 5°C ischemia caused an intracellular lack of potassium that had to be replenished, or there was an additional K^+ uptake during reperfusion that increased the intracellular concentration of potassium to above-normal values. In the first case, the preceding ischemic injury must have been more severe in Celsior-treated hearts. In the second case, a higher K^+ gradient across the membrane, triggering arrhythmias as in hypokalemia, would be the consequence in Celsior-treated hearts.

Only two of the published studies comparing Celsior or Custodiol with other preservation solutions provide information concerning cardiac rhythm. In a heart transplantation study by Wieselthaler and colleagues [8], more hearts in the Celsior group developed spontaneous sinus rhythm than in the Custodiol group (19/24 vs 9/24). In contrast, in the study of Vega and associates [11], the incidence of arrhythmias in Celsior-preserved hearts was twice as high as in a control group with conventional preservation solutions, but the difference was reported to be nonsignificant.

The data presented here cannot be immediately extrapolated to clinical conditions. We investigated the early reperfusion period. Therefore, no statements can be made about problems developing later. Furthermore, we reperfused hearts with a saline solution instead of blood. Reperfusion with blood may, on one hand, facilitate recovery, eg, through more homogeneous oxygen supply, less edema, and the ability of erythrocytes to scavenge oxygen free radicals [20], but, on the other hand, may cause additional problems such as release of oxygen free radicals from activated neutrophils [21]. Nevertheless, our study shows that cardioplegia with Celsior seems to have no advantage over cardioplegia with Custodiol. In Celsior-preserved hearts energy values were lower, and the hearts showed a higher rate of arrhythmias and, after 12 hours of cold storage, a lower contractility during reperfusion. The differences may be of clinical relevance, especially in the case of cold-storage times greater than the usual 4 hours.

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