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Minimal amounts of hyaluronidase in HTK or UW solution substantially improve the recovery of preserved hearts

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Abstract Rat hearts were preserved by simple storage for 18 h at 0–1 °C and reperfused parabiotically with whole blood from a host rat. The preservation solutions used for flush perfusion and storage were the commercial solutions EuroCollins, HTK, or UW with or without adding 40 mg/l hyaluronidase or Euro-Flush-Glutathione (EFG) solution, especially designed for prolonged heart storage. All solutions were filtered (0.45 μm) before use. The functional recovery was measured using a latex balloon in the left ventricle for LVP, dp/dt, and isotonic stroke volume. The metabolic recovery as well as the edema formation was determined from freeze-clamped myocardium at the end of reperfusion. In hearts preserved with hyaluronidase-containing solutions, the edema formation during reperfusion was reduced combined with an improvement in the coronary flow. Functional and metabolic recovery were improved in these hearts with significant increase in the stroke volume and ECP in all groups versus hearts preserved in the hyaluronidase-free basic solutions. The effectiveness of HTK preservation was significantly improved by hyaluronidase in all parameters measured in our study. The best functional and metabolic recovery was found in hearts preserved by HTK + H- or EFG-solution. Thus, preservation solutions containing hyaluronidase, especially HTK + H and EFG, seem best suited for the prolonged storage preservation of the heart.

Key words Heart preservation · Hyaluronidase · Hypothermic storage · Parabiotic perfusion · Transplant recovery

Introduction

EuroCollins, Bretschneider's HTK, or the University of Wisconsin organ preservation (UW) solution have been used clinically for up to 4 h storage preservation of hearts [1]. Successful prolonged storage (from 4.2 h [2] to 8 h [3]) has been reported only in a few isolated single cases. The problems resulting from prolonged heart storage are mainly contracture and reduced coronary flow which inhibit sufficient recovery of cardiac function in a reasonable time to guarantee the survival of the recipient. Many attempts have been made in animal experiments to improve the outcome of prolonged-stored hearts including continuous perfusion systems [4], modifications of the storage solution [5], "refreshment" of ageing commercial solutions [6], or development of new preservation solutions [7]. The newly developed solutions "EuroFlush" (EF) [7] or "Euro-Flush-Glutathione" (EFG) [6] contain a component, hyaluronidase, which is well known for preventing ischemically induced increase in vascular resistance [8], but has never been used in preservation solutions before.

Hyaluronidase acts by specifically reducing the hyaluronate content of tissues, which is the principal glycosaminoglycan of the interstitium, has a high water-binding capacity and exerts a strong osmotic force [9]. Application of hyaluronidase decreases the interstitial volume of edematous tissues and prevents ischemically

induced increase in vascular resistance, but does not change the microvascular permeability. It, thus, reduces edema formation and improves the reflow in ischemically damaged organs. Applied intravenously for the treatment of acute myocardial infarction, the cardioprotective effect of hyaluronidase could be shown in many clinical and experimental studies [10–15], demonstrating reduction of edema and vascular resistance or better maintenance of myocardial lymph flow. Hyaluronidase was shown to improve the poststorage reflow in hearts preserved for 24 h at 0–1 °C in our previous asanguinous electrolyte reperfusions. Hearts preserved in EC or EF solution with hyaluronidase [7], also resulted in a higher functional recovery than HTK- or UW-preserved hearts. Experiments with parabiotic reperfusions comfirmed this excellent effect in 18-h-preserved hearts using EFG solution, but the effectiveness of combining hyaluronidase with the commercial solutions was not evaluated in these investigations.

Here we present results from rat hearts stored for 18 h in commercial solutions, the same solutions with hyaluronidase added or EFG solution, developed for prolonged heart preservation and which also contains hyaluronidase. The organs were reperfused with whole blood in a parabiotic system – a technique, which combines the reperfusional effects of a transplantation with the possibility of continuous functional testing.

Table 1 Components (mmol/l) of preservation solutions used in this study

	EC	EFG	HTK	UW
Na^+	10	15	15	30
K^+	115	90	10	125
Ca^{++}	–	0.05	–	–
Mg^{++}	–	35	4	5
Cl^-	15	0.1	50	–
HCO_3^-	10	15	–	–
SO_4^{--}	–	80	–	5
Phosphate	57.5	–	–	25
Glutarate	–	–	1	–
Histidine	–	–	198	–
Lactobionate	–	–	–	100
Tryptophane	–	–	2	–
Glucose	194.3	–	–	–
Mannitol	–	–	30	–
Sucrose	–	100	–	–
Raffinose	–	–	–	30
Adenosine	–	–	–	5
Allopuriol	–	0.5	–	1
Glutathione	–	–	–	3
Hepes	–	1	–	–
Pentastarch	–	–	–	50 g/l
Fresh additives before use:				
Dexamethasone	–	–	–	16 mg/l
Glutathione red.	–	3 mmol/l	–	–
Hyaluronidase	–	40 mg/l	–	–
Insulin	–	–	–	40 U/l
Penicillin	–	–	–	2×10^5 U/l
Verapamil	–	2 mg/l	–	–

Materials and methods

All animals were housed, fed, and handled in compliance with German legislation on protection of animals and the "Guide for the Care and Use of Laboratory Animals" published by the NIH (Publication number 86–23, 1985).

Hearts of male inbred Lewis rats (220–260 g body weight) were grafted under ether anesthesia. Pressure-controlled flush perfusion with cold (0–1 °C) preservation solution started in situ via an aortic catheter without ischemic damage to the heart. It was performed at 75 mm Hg for 5 min in all groups except the HTK groups, where it lasted for 10 min with reduction of the perfusion pressure to 50 mm Hg after 1 min with respect to the special HTK application rules [16].

The preservation solutions were as follows ($n = 6$ in all groups):
A. Three commercially available original solutions, EuroCollins solution (EC, Fresenius AG, Bad Homburg, Germany), HTK solution (Custodiol, Dr. F. Köhler Chemie, Alsbach-Hähnlein, Germany), or UW solution (ViaSpan, Du Pont Pharma, manufactured by NPBI, The Netherlands)
B. Hyaluronidase-containing solutions, i.e., the original solutions with hyaluronidase (40 mg/l) added (EC + H, HTK + H, UW + H) or EuroFlush-Glutathione solution (EFG) prepared in our laboratory (Table 1)
All perfusates were filtered (0.45 μm) before use. This pore size guaranteed the extraction of all damaging particles without having any influence on the crystalloid or colloidal components of the solutions.

After flushing, the organs were stored for 18 h in 5 ml of the respective preservation solution at 0–1 °C in small, safely sealed vessels. The vessels were continuously gassed with purest nitrogen (purity > 99.9993 vol%), to guarentee real ischemia of the organs during the storage period without oxygenation from the surface. Control hearts were arrested by surface cooling and immediately reperfused similar to the preserved hearts.

Reperfusion started after a 5 min warm reflush (37 °C) using oxygenated modified Krebs-Henseleit solution (only 50 μmol/l calcium, but containing 15 μmol/l adenosine and 1 mmol/l uric acid) at a pressure of 50 mm Hg (non-recirculating Langendorff mode). The reperfusion was performed for 30 min parabiotically using whole blood from the carotid artery of an anesthetized host rat (pentobarbital anesthesia) of the same inbred Lewis strain with the graft maintained at 37 °C in a water-jacketed constant temperature chamber. The blood retured from the pulmonary artery into the jugular vein of the host. Adenosine was infused up to the 20th min of reperfusion in declining concentrations, resulting in an initial blood pressure of about 50 mm Hg, similar to the warm reflush, with continuous increase to normal values (> 80 mm Hg) within a few minutes.

The coronary effluent was determined by timed collection. Functional recovery was measured with the aid of a latex balloon in the left ventricle and continuously recorded on a thermo-oscillographic recorder. Isometric left ventricular pressure amplitude (LVP) at end-diastolic pressures of 10 mm Hg with or without pacing to a heart rate of 300/min, dp/dt, and serial measurements of isotonic stroke volume were recorded.

At the end of reperfusion the hearts were immediately frozen in liquid nitrogen using the freeze-stop technique. The energy metabolism was determined in the lyophilized myocardium using enzymatic [17] and HPLC techniques [18].

Table 2 Myocardial water content (ml/g dry weight) at the end of storage or after 18 h storage and 30 min parabiotic reperfusion, determined from freeze-clamped rat heart ventricles. Mean ± SD, $n = 6$ per group, C: hearts in situ or after control reperfusion without storage

	After storage	After reperfusion
Control	3.42 ± 0.08	4.04 ± 0.23
EC	3.97 ± 0.16	4.36 ± 0.49
EC + H		3.80 ± 0.28
HTK	3.48 ± 0.04	4.61 ± 0.47
HTK + H		4.44 ± 0.10
UW	3.15 ± 0.13	5.14 ± 0.88
UW + H		4.07 ± 0.27
EFG	3.68 ± 0.08	4.15 ± 0.42

Table 3 Coronary flow (ml/min) during initial 5 min warm reflush (mean of 2–5 min) or at the end of 30 min parabiotic reperfusion after 18 h storage preservation. Mean ± SD, $n = 6$ per group

	2–5 min KH	30 min blood
Control	11.7 ± 1.4	6.1 ± 0.8
EC	3.6 ± 0.6*	1.4 ± 0.5
EC + H	5.4 ± 0.5*	1.6 ± 0.3
HTK	4.4 ± 0.8*	1.8 ± 1.2
HTK + H	7.3 ± 1.0*	2.6 ± 0.7
UW	4.2 ± 0.3*	1.3 ± 0.3
UW + H	5.5 ± 1.4*	2.2 ± 1.1
EFG	7.0 ± 0.5	2.3 ± 0.7

* $P < 0.05$

Energy charge potential (ECP) was calculated according to Atkinson [19] from the formula ECP = (ATP + $^1/_2$ ADP)/(ATP + ADP + AMP).

Mean values and standard deviations of the parameters are given in Fig. 3. The Mann-Whitney test was used for the comparison of two groups and differences were considered significant when $P < 0.05$. For multiple comparison, adjustment of the Mann-Whitney test was by the Bonferroni method.

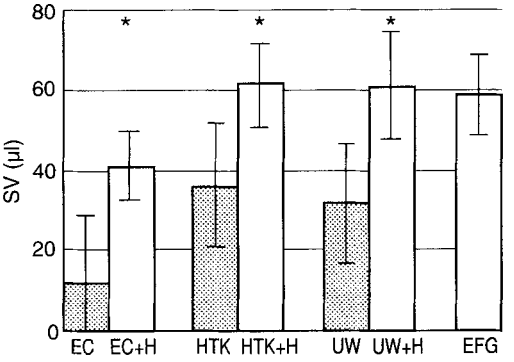

Fig. 1 Left ventricular isotonic stroke volume *(SV)* of 18-h-preserved rat hearts, ejected from the intraventricular balloon. Highest values after 20–30 min of parabiotic reperfusion. Mean ± SD, $n = 6$ per group. (* $P < 0.05$ versus basic solution without hyaluronidase)

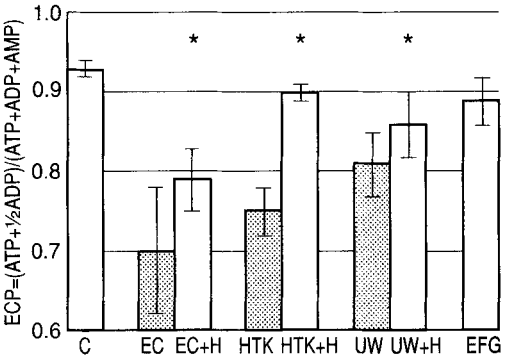

Fig. 2 Energy charge potential after 30 min of parabiotic reperfusion of 18-h-preserved rat hearts. Mean ± SD, $n = 6$ per group. (*C* control reperfusion, * $P < 0.05$ versus basic solution without hyaluronidase)

Results

After 18 h preservation of rat hearts in one of the commercially available solutions, EC, HTK, or UW solution, parabiotic whole blood reperfusion resulted in edema formation and impaired recovery. The amount of fluid accumulation during reperfusion as well as the total water content at the end of reperfusion was highest in the group with the lowest poststorage edema (UW).

In combination with each of these solutions, hyaluronidase improved the recovery of the hearts. In the hyaluronidase-containing solutions the reperfusion edema was reduced, reaching the level of the control group after EC + H, UW + H, and EFG preservation (Table 2). The coronary flow was already significantly increased during the warm reflush (Table 3), resulting in an increase of several functional and metabolic parameters during further reperfusion compared to hearts preserved in solutions without hyaluronidase. The ventricular isotonic stroke volume increased significantly in all groups (Fig. 1), as well as the myocardial energy charge potential at the end of reperfusion (Fig. 2). For most other functional and metabolic parameters of the hearts, hyaluronidase content of the storage solution resulted in a more or less pronounced improvement. This improvement was always significant in HTK + H- versus HTK-preserved hearts, but for EC + H- or UW + H-preserved hearts it did not reach the level of significance versus the basic solution in all of the remaining parameters (see Fig. 3, Tables 4, 5).

Optimal recovery – substantially higher than after storage in any of the commercial solutions – was reached with the preservation solutions HTK + H and EFG and, in some parameters (stroke volume, ATP content, and total adenine nucleotide content), for UW + H also. The recovery after storage in the commer-

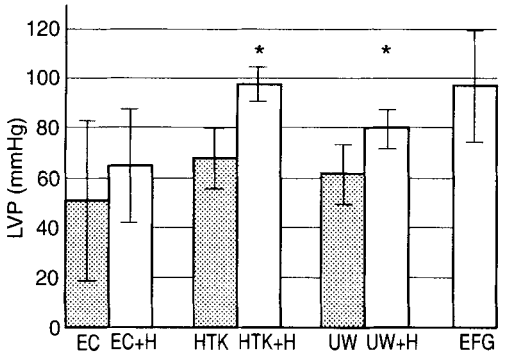

Fig. 3 Left ventricular pressure amplitudes (LVP) (isovolumetric) of 18-h-preserved rat hearts at the end of parabiotic reperfusion. Spontaneously beating hearts at heart rates between 74 and 135/min, except HTK (176/min) and HTK + H (226/min). Mean ± SD, $n = 6$ per group. (* $P < 0.05$ versus basic solution without hyaluronidase)

cial solutions EC, HTK, or UW without hyaluronidase was insufficient. Damage by the particles, which have been shown to be typical for UW solution [6], could be prevented by the filtering procedure used in our experiments for all solutions and, thus, did not influence these results. Under these conditions the effectiveness of the commercial solutions was for functional parameters UW = HTK > EC, for metabolic parameters UW > HTK > EC, while the use of unfiltered UW solution did not allow recovery of 18 h preserved rat hearts.

Table 4 ATP, total adenine nucleotide (TAN = ATP + ADP + AMP) or creatine phosphate (CP) content of ventricular myocardium (μmol/g dry weight) after 18 h storage and 30 min parabiotic reperfusion. Mean ± SD, $n = 6$ per group.

	ATP	TAN	CP
Control	21.9 ± 1.1	25.1 ± 1.5	39.4 ± 3.4
EC	4.7 ± 1.6*	8.0 ± 1.7*	4.2 ± 2.3
EC + H	6.9 ± 1.6*	10.1 ± 1.9*	6.5 ± 2.1
HTK	6.2 ± 1.0*	10.2 ± 1.5*	5.4 ± 1.3*
HTK + H	12.5 ± 1.1*	15.2 ± 1.1*	20.3 ± 3.6*
UW	9.2 ± 3.0	13.1 ± 3.1	10.9 ± 6.5
UW + H	11.9 ± 2.6	15.2 ± 2.8	15.2 ± 4.5
EFG	11.7 ± 2.7	14.2 ± 2.6	19.4 ± 6.9

* $P < 0.05$ for difference between basic solutions and modification with hyaluronidase

Table 5 Left ventricular pressure amplitude, contractility (+ dp/dt_{max}) and relaxation velocity (− dp/dt_{max}) at a paced heart rate of 300/min after 25–30 min parabiotic reperfusion. Mean ± SD, $n = 6$ per group.

At 5 Hz pacing	LVP	+ dp/dt_{max}	− dp/dt_{max}
Control	139 ± 39	3293 ± 976	2187 ± 658
EC	24 ± 30	527 ± 668	393 ± 506
EC + H	41 ± 18	920 ± 419	670 ± 275
HTK	58 ± 15*	1355 ± 310*	980 ± 231*
HTK + H	97 ± 13*	2153 ± 297*	1600 ± 209*
UW	56 ± 15	1253 ± 339	900 ± 222
UW + H	72 ± 19	1607 ± 454	1163 ± 299
EFG	87 ± 22	1999 ± 492	1398 ± 382

* $P < 0.05$ for difference between basic solutions and modification with hyaluronidase

Discussion

Eighteen hour storage preservation using commercial EC, HTK, or UW solution, did not allow acceptable recovery of rat hearts in our experiments. However, the addition of 40 mg/l hyaluronidase to these solutions enhanced the functional recovery in rat hearts with increase in the energy charge potential, obviously caused by reduced fluid accumulation and hereby improved coronary flow. Thus, the effectiveness of HTK solution could be significantly improved by the addition of hyaluronidase. Hearts preserved in HTK + H solution had an optimal recovery during parabiotic reperfusion similar to those stored in EFG. EC + H-preserved hearts did not reach a comparable level and UW + H-hearts reached an optimal recovery only in a few parameters. Here, especially for UW and UW + H solution, a better improvement seems to be hindered by the oxidation of GSH in commercial UW solution (as shown in our previous study [6]).

Thus, for prolonged 18 h heart storage preservation, the commercial solutions EC, HTK, or UW should not be used without the addition of hyaluronidase and, in the case of UW solution, in combination with filtration and refreshing procedures.

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