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Thomas Minor
Tetsuya Yamaguchi
Wolf Isselhard

Effects of taurine on liver preservation in UW solution with consecutive ischemic rewarming in the isolated perfused rat liver

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T. Minor (✉) · T. Yamaguchi · W. Isselhard
Institut für Experimentelle Medizin,
Universität Köln,
Robert-Koch-Str. 10,
D-50931 Köln, Germany
Fax: +49 221 478 62 64

Abstract Taurine (2-aminoethane sulfonic acid) is a physiologic amino acid involved in cellular osmoregulation in various species including man. This study was intended to compare the respective effects of cold storage and consecutive ischemic rewarming of the liver on postischemic hepatic flow and hepatocellular outcome upon reperfusion with or without the addition of taurine to the preservation medium. Livers from male Wistar rats were rinsed free of blood via the portal vein and stored ischemically at 4 °C in UW solution. Livers from group 1 were then rinsed again with 10 ml Ringer's solution and reperfused with Krebs-Henseleit buffer at a constant pressure of 10 mmHg for 45 min at 37 °C in a nonrecirculating manner. Livers from groups 2 and 3 were subjected to 30 min of warm ischemia subsequent to cold storage and prior to reperfusion with 10 mM taurine added to the UW solution in group 3. While there were only very few signs of hepatic injury in group 1, the additional period of warm ischemia (group 2) led to a significant reduction in early perfusate flow and enhanced enzyme leakage from the livers during postischemic rinse and reperfusion. Livers in group 3 exhibited an amelioration in hepatic circulation and significantly reduced enzyme release as compared to group 2. The results clearly demonstrate a remarkable impact of postischemic rewarming on graft viability. Furthermore, the addition of taurine to the preservation medium was shown to improve hepatic circulation and enhance viability of the liver upon reperfusion.

Key words Taurine, liver preservation, rat · Liver preservation, taurine, rat · Preservation, taurine, liver

Introduction

Along with the rapid increase in clinical transplantation of solid organs like the kidney, heart, or liver, much effort has been put into improving the conditions of ischemic storage. Alterations of the graft during ischemic preservation and/or subsequent reoxygenation may still limit the acceptable time of hypothermic preservation or affect the viability of the organ to be transplanted after a given ischemia time.

Despite new organ preservation solutions like the University of Wisconsin (UW) solution, which has proven to be superior to the previously used Euro-Collins (EC) solution [2, 9] and which has allowed for significant prolongation of the ischemic period, early graft dysfunction attributed to preservation injuries is still a clinically observed event [13].

Taurine (2-aminoethane sulfonic acid) is a phylogenetically ancient compound and one of the most abundant low-molecular weight organic constituents in many animals [10]. Recently, it was shown that taurine

improves the viability of isolated kidney tubular cells during hypoxic preservation and reoxygenation in vitro [23].

This study examined the effects of the addition of taurine to the UW preservation solution on an isolated rat liver preparation. The model includes cold preservation as well as a short, consecutive period of warm ischemia, simulating the effects of ischemic rewarming, which occurs during surgical implantation of the graft in vivo.

Materials and methods

All experiments were performed in accordance with the federal law regarding the protection of animals. The principles of laboratory animal care (NIH Publication No. 85-23, revised 1985) were followed.

Male Wistar rats weighing 200–300 g were used. The animals had free access to food and water until the operation. Under ether anesthesia the abdomen was opened via a midline incision with transversal extensions. The liver was then skeletonized and freed from its retroperitoneal attachments. The hepatic artery was ligated and divided, and the infrahepatic caval vein ligated. The portal vein was cannulated with a 16-gauge cannula (total length 2 cm; inner diameter 1.3 mm) and the livers were flushed in situ via the portal vein with 10 ml of Ringer's solution and 10 ml of UW preservation solution. The organs were excised and stored for 24 h in 125 ml UW solution at 1–4 °C immediately after the portal flush.

A short 14-gauge polyethylene splint (inner diameter 1.7 mm) was telescoped into the suprahepatic vena cava and secured. Prior to oxygenated reperfusion, the livers were rinsed with exactly 10 ml of Ringer's solution at room temperature and the eluate was collected for enzyme analysis. Reperfusion was carried out over 45 min with Krebs-Henseleit bicarbonate buffer, oxygenated with a mixture of 95 % O_2 and 5 % CO_2, in a nonrecirculating manner. The livers were placed in a bath of 37 °C Krebs-Henseleit buffer to assure homogenous perfusion in vitro, and the perfusate entered the portal vein at a pressure adjusted to 10 mmHg.

Portal venous pressure was controlled using a Statham P23 Db pressure transducer, and transhepatic flow was measured at given intervals by collecting the effluate. Effluate was collected during the in vitro reperfusion by means of a short tube telescoped over the splint in the caval vein, making it possible to take perfusate samples for the determination of oxygen partial pressure under the exclusion of room air contamination.

There were three experimental groups in the present study. In group 1, livers were cold-stored in UW solution for 24 h, rinsed, and immediately reperfused as described. In group 2, livers were cold-stored for 24 h and then incubated in 37° Krebs-Henseleit buffer for an additional 30 min (rewarming) prior to rinsing and reperfusion. This protocol should account for the period of slow rewarming of the organ during surgical implantation in vivo. Finally, in group 3, livers were subjected to the same protocol as in group 2, but taurine (purchased from Sigma Chemicals, St. Louis, Mo., USA) was admixed to the UW solution during flush and preservation at a concentration of 1 mmol/l.

At the end of the experiment, livers were freeze-clamped according to the Wollenberger technique [24] for the determination of the tissue content of adenine nucleotides. Frozen liver tissues were weighed and preserved for at least 5 days in a vacuum freezer (−45 °C, < 0.001 atm) to evaporate the tissue water.

Freeze-dried tissue samples were homogenized and deproteinized with 0.33 M percloric acid. The extract was centrifuged and the supernatant neutralized with 2 N KOH and frozen to remove $KC10_4$. Adenine nucleotides were determined enzymatically in the supernatant as described elsewhere, using the hexokinase and glucose-6-phosphate dehydrogenase reactions [17]. Total adenine nucleotides (TAN) were calculated as ATP + ADP + AMP. The energy charge potential was calculated according to Atkinson [1] as: ECP = (ATP + 1/2 ADP) / TAN.

As for the assay of enzyme activities in the effluate, glutamate dehydrogenase (GLDH) and alanine aminotransferase (ALT) were determined photometrically using commercialized standard kits (Fa. Boehringer, Mannheim, Germany). Purine nucleoside phosphorylase (PNP), which is indicative of vascular endothelial lesions [19], was measured spectrophotometrically using the method of Dwenger and Trautschold [5].

In order to calculate the hepatic oxygen consumption, perfusate samples were taken from the inflow and from the effluent in a glass syringe and oxygen partial pressure was immediately analyzed using a pH-blood gas meter (ABL 2 Acid-Base-Laboratory, Radiometer, Copenhague). From given flow rates (Q), temperature (t), and atmospheric pressure (P), oxygen uptake of the liver was calculated according to the formula:

$$VO_2 = \frac{Q \times (P02_i - P02_e)}{wt} \times a$$

where $PO2_i$ represents the oxygen partial pressure at the inflow, $PO2_e$ the oxygen partial pressure at the effluent, a the coefficient, and wt the liver weight (assumed to be 4 % of body weight). The results were expressed as mean ± standard deviation (SD) of five to six experiments per group.

Stochastical significance of differences between the groups was determined after one-way analysis of variance and multiple comparison of the means with the Bonferroni *t*-test.

Results

During postischemic reperfusion in vitro there was a quick and complete restoration of transhepatic flow to normal values around 3 ml/g liver per minute in the livers subjected only to cold preservation (group 1; Fig. 1). Normal flow values of nonischemic livers under the described experimental conditions averaged between 3.48 ± 0.55 and 3.58 ± 0.15 at minute 2 and minute 45 of perfusion, respectively (data not shown).

In contrast to that, the additional period of rewarming (group 2) resulted in an increased portal vascular resistance (PVR), as seen from the impaired postischemic recovery of the transhepatic flow with significantly reduced values during the first 10 min of reperfusion. These circulatory disturbances were obviously attenuated in the taurine-treated group, though no complete recovery was observed in this group either.

Enzyme leakage into the effluate from the hepatic parenchyma showed significant differences between the three groups (Fig. 2). In group 1 the release of the cytoplasmic ALT as well as of the intramitochondrial GLDH was only slightly elevated at the 1st minute of reperfusion, showing constantly low values during the

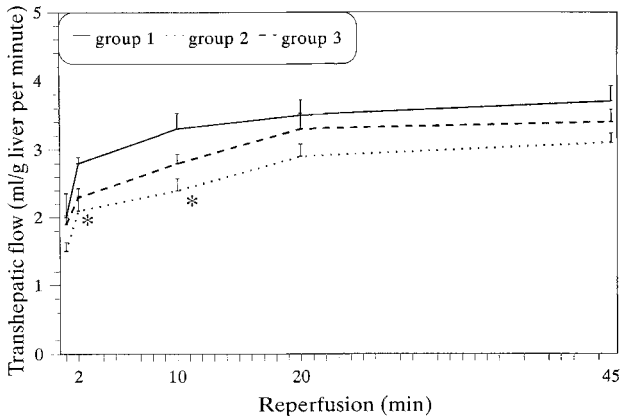

Fig. 1 Total portal flow upon isolated reperfusion at constant pressure in vitro. * $P < 0.05$ vs group 1

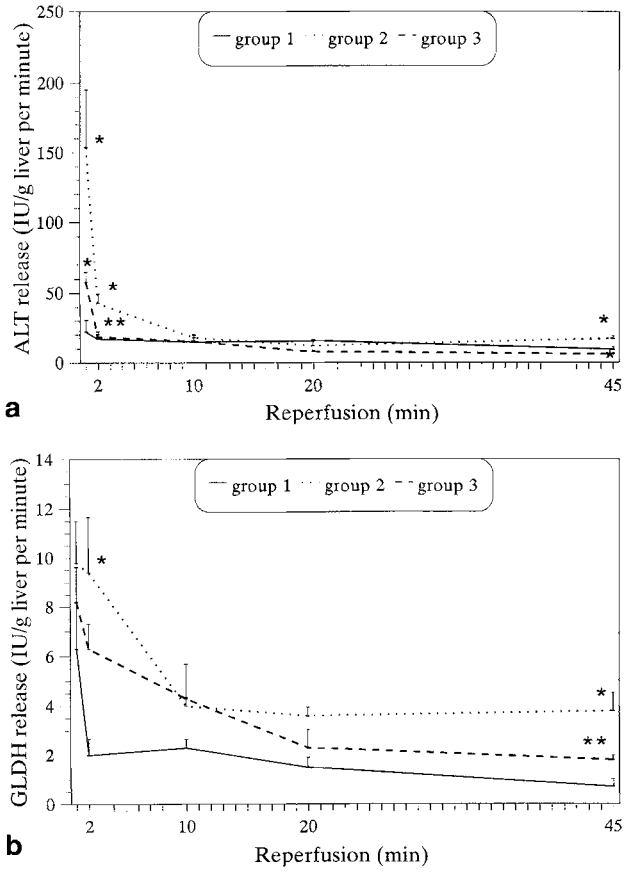

Fig. 2a,b Enzyme release into the perfusate during isolated reperfusion in vitro; **a** ALT; **b** GLDH. * $P < 0.05$ vs group 1; ** $P < 0.05$ vs group 2

Table 1 Adenine nucleotides in liver tissue and dry weight in percent of wet weight (DW) at the end of 45 min of reperfusion after preservation according to the different groups (mean ± SD). Control values, obtained from livers perfused immediately after harvest without ischemic alteration, are given as a reference

	ATP (μmol/g)	TAN (μmol/g)	ECP (1/1)	DW (%)
Control	9.43 ± 1.75	15.98 ± 2.78	0.740 ± 0.077	26.4 ± 0.7
Group 1	5.07 ± 0.44	13.53 ± 1.64	0.575 ± 0.041	25.5 ± 0.4
Group 2	5.08 ± 1.68	11.69 ± 1.08	0.589 ± 0.122	23.1 ± 1.4*
Group 3	4.91 ± 0.58	10.77 ± 0.11	0.633 ± 0.046	25.0 ± 1.4**

* $P < 0.05$ vs group 1; ** $P < 0.05$ vs group 2

later observation period. In group 2, however, a significant rise in the initial cellular loss of ALT and GLDH could be documented during the first 2 min of reperfusion, and at the end of 45 min of reperfusion the leakage of both enzymes was again slightly, but significantly, higher than in group 1. A significant reduction in the initial enzyme loss was observed after the addition of taurine in group 3 in comparison to group 2. Moreover, these livers did not show any differences with regard to group 1 at the later reperfusion period.

Oxygen consumption at the end of the reperfusion period averaged 3.2 ± 0.4 ml/g per minute in group 1, thus reaching values comparable to those of non-ischemic livers after 45 min of isolated perfusion (3.2 ± 0.29 ml/g per minute). It recovered only to 2.2 ± 0.3 ml/g per minute ($P < 0.05$ vs group 1) in group 2 and to 2.7 ± 0.3 ml/g per minute ($P < 0.05$ vs group 2) in group 3.

Hepatic bile production during the 45 min of reperfusion was 25.0 ± 8.8, 10.5 ± 4.1, and 24.3 ± 3.1 μl/g per 45 min in groups 1, 2, and 3, respectively, revealing a significantly ($P < 0.05$) reduced performance in group 2 as compared to either group 1 or group 3.

The postischemic recovery of hepatic energy metabolism is shown in Table 1. Tissue levels of ATP after reperfusion reached about 50 % of control values, with no differences among the three experimental groups. Total adenine nucleotides were slightly reduced in groups 2 and 3; the differences, however, were not significant. The energy charge potential (ECP) showed no statistically relevant differences between the experimental groups. Tissue edema was expressed as percentage of dry weight to wet weight of the livers. While dry weight content was significantly reduced in group 2 as compared to group 1, the addition of taurine in group 3 resulted in a significant increase in the dry weight/wet weight ratio to values comparable to those in group 1.

The enzyme activities in the primary eluate of the postischemic rinse prior to reperfusion are depicted in Fig. 3. While the activities of GLDH were more or less comparable in all experimental groups, significant differences were observed with ALT and PNP, with lower

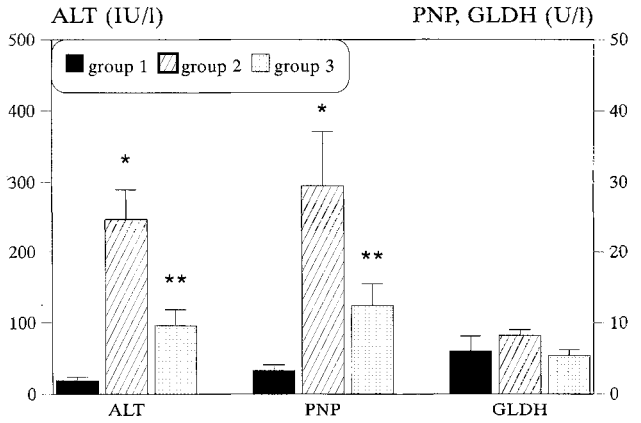

Fig. 3 Enzyme activities in the eluate after postischemic rinse with 10 ml of Ringer's solution. (*ALT* alanine aminotransferase, *PNP* purine nucleoside phosphorylase, *GLDH* glutamate dehydrogenase) * $P < 0.05$ vs group 1; ** $P < 0.05$ vs group 2

values in the groups containing the more viable livers. Finally, despite the significant differences between the mean enzyme activities in the different groups, only fair correlations of bile production and the eluate's enzyme activities could be found for the individual livers, showing coefficients of only $r^2 = 0.30$ for PNP and $r^2 = 0.42$ for ALT.

Discussion

In the course of clinical or experimental liver transplantation in vivo, the liver is subjected both to an extensive time of cold ischemic storage during transport and preservation and to a shorter period of slow rewarming of the organ under ischemic conditions during surgical implantation into the recipient. The latter period may easily exceed 1 h in human liver transplantation [4, 21], and the effect of rewarming has been ignored in most studies up to now [4]. This is especially relevant when in vitro experiments are performed, where rewarming is not automatically included in the experimental procedure. Therefore, studies on isolated organs are likely to underestimate the presumable tissue injury upon transplantation in vivo if they do not account for this rewarming period.

Our results demonstrate the impact of a 30-min warm ischemia period consecutive to cold preservation of the liver on functional outcome and enzyme release upon reperfusion. It has to be kept in mind, however, that due to the small size of the rat liver compared to larger animals or to humans, the isolated rat liver will reach elevated core temperatures much more quickly during rewarming and, thus, this model only allows for qualitative conclusions with regard to the human situation.

It has recently been pointed out that cold and warm ischemic injury divergently affect the various cell populations of the liver [3, 11, 14]. Isolated warm ischemia predominantly impairs parenchymal cell integrity while nonparenchymal cells are primarily involved in cold ischemic tissue damage. In this context it is of interest that the eluate activity of PNP, reflecting morphological alterations of the vascular endothelium, was merely altered after simple storage for 24 h in UW solution. This is in keeping with histological evidence of only minor endothelial injuries up to 24 h in UW solution reported by other investigators [8]. Even upon oxygenated reperfusion, these livers did not show elevated PNP activities in the perfusate from minute 2 to minute 45 (data not shown). Despite this, the additional warm ischemia resulted in a great increase in endothelial damage is judged from the PNP activities in the primary eluate, suggesting a potentially superadditive impact of the rewarming after cold preservation on the vascular endothelium. The relevance of the rewarming period was further documented by the significant impairment of the hepatic circulation in group 2, a putative explanation of which may be endothelial cell swelling or reduced endothelial vasodilative function [15].

The measurement of PNP activities in the primary eluate prior to reperfusion has been proposed as an early marker of organ viability after warm ischemia [19] or ischemic storage [20]. Indeed, we were able to reproduce significant differences between different experimental groups, representing livers of varying viability, similar to those reported by Reinders and coworkers [20]. Yet, the potential prognostic value of these eluate enzyme activities with respect to the later functional outcome of the livers can be better defined by calculating the correlation between bile production upon reperfusion and the respective enzyme activities in the eluate of ALT or PNP.

Only fair correlations were observed between PNP activities in the primary eluate and bile production upon reperfusion using all individual livers, irrespective of the experimental protocol. Therefore, from our data, this parameter does not seem to be suitable for clinical use. Moreover, the activities of PNP did not prove to be superior to the data on ALT, the latter being much more feasible for use in the clinical routine.

The addition of taurine to the flush and storage solution resulted in a net reduction in hepatic preservation injury. The benefit of taurine was manifest with functional parameters such as oxygen comsumption as well as with morphological ones such as enzyme loss from the cytoplasm or the mitochondria. In contrast to that, the recovery of hepatic energy metabolism did not improve after taurine treatment. Although the ability of the liver to restore the tissue pool of high-energy phosphates during postischemic reperfusion has been described as a good indicator of hepatic viability in vari-

ous models using warm ischemic alterations [12, 21], tissue levels of adenine nucleotides proved to be insensitive in differentiating between simple cold storage and additional rewarming in our model. It comes thus as little surprise that they did not respond to the treatment with taurine either. This observation is similar to that from a recent 31-P nuclear magnetic resonance study [6] in which a quick restoration of hepatic ATP levels was found after cold ischemia up to 60 % of control values, remaining constant during 3 h of isolated reperfusion in vitro despite ongoing cellular alterations in the liver as evidenced by electron microscopy.

Taurine is known to exert osmoregulatory functions in a variety of experimental settings [10]. Cells seem to be able to regulate their volume via a modulation of transmembrane flux of osmoprotective molecules like taurine [22], although the precise mechanism behind taurine-mediated osmoprotection is not fully elucidated. Since cell swelling during cold storage has to be considered a major determinant of circulatory disturbances upon reperfusion, the reduction of tissue edema by taurine may have contributed to the improvement in hepatic flow conditions in the taurine-treated group, which also exhibited slightly, but significantly, higher dry weight percentage at the end of the experiments.

Other possible explanations for the beneficial effect exerted by taurine may include membrane-stabilizing properties [18] and the inhibition of free radical-mediated lipid peroxidation. Furthermore, taurine can act as an indirect modulator of cellular calcium homeostasis [10]. In the isolated heart, taurine protected against intracellular calcium overload associated with hypoxia and reoxygenation, resulting in an amelioration in cardiac integrity [7]. Postischemic calcium influx into the cells has also been shown to be involved in oxidative tissue damage upon reperfusion of the rat liver [16]. The action of taurine is a modulator of cellular calcium homeostasis may, therefore, account for the positive effects on liver preservation seen in this study.

In sum, this study demonstrated a beneficial effect of taurine on hepatic recovery from cold ischemic storage with consecutive rewarming and reperfusion, the precise mechanism of which should be further investigated to eventually improve the preservation modalities in transplantation surgery.

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