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Value of α glutathione S-transferase for in vitro evaluation of preservation injury in normal and steatotic livers

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Abstract Liver steatosis is frequently encountered at organ harvest and, although functionally inapparent in the donor, may seriously affect the functional recovery of the graft after ischemic preservation. The present study was aimed to investigate the diagnostic value of alpha-glutathione S-transferase (GST) in non-schemic and ischemic livers with or without compensated steatosis. A histologically documented mild to moderate steatosis was induced in livers of male Wistar rats by fasting for 2 days and subsequent feeding of a fat-free diet enriched in carbohydrates. Fatty livers (FL) were retrieved and perfused in vitro for 45 min either immediately or after ischemic preservation at 4 °C in HTK solution. Effluate was collected during isolated perfusion and later analysed for liver specific enzymes, including GST. Normal livers (NL) were excised from healthy rats and underwent the same protocol. Non-ischemic livers showed similar enzyme release (FL versus NL) for ALT or GLDH but significant differences in GST. After ischemic preservation of NL, enzyme release increased mildly with respect to the non-ischemic reference values for ALT, remained unchanged for GLDH and rose substantially for GST. In FL, there was a more than 10-fold increase in all parameters, being most pronounced for GLDH as a marker of mitochondrial damage. It is concluded that GST may discriminate between healthy and suboptimal steatotic livers prior to ischemia and that the release of GST upon postischemic reperfusion of normal livers proves to be the most sensitive indicator for hepatocellular injury. However, GST turned out to be less useful for the detection of postischemic reperfusion injury in steatotic grafts.

Key words Alpha GST · Glutathione S-transferase · Fatty liver · Marginal donor · Suboptimal donor · Quality assessment

Introduction

Liver steatosis is frequently encountered at organ harvest and, although functionally inapparent in the donor, may seriously affect the functional recovery of the graft after ischemic preservation [4, 6, 11].

Due to the perplexing shortage of donor organs [2], many of these "marginal" livers are nonetheless accepted for clinical transplantation [8] and it has been reported that about 17% of all transplanted livers were actually steatotic grafts [1].

However, the decision whether to accept or reject a marginal graft retrieved for transplantation remains a delicate clinical challenge to the transplant physician. Any means to alleviate this problem would be of pivotal interest to improve the availability of liver grafts while identifying those organs, which are unsuitable for ischemic preservation.

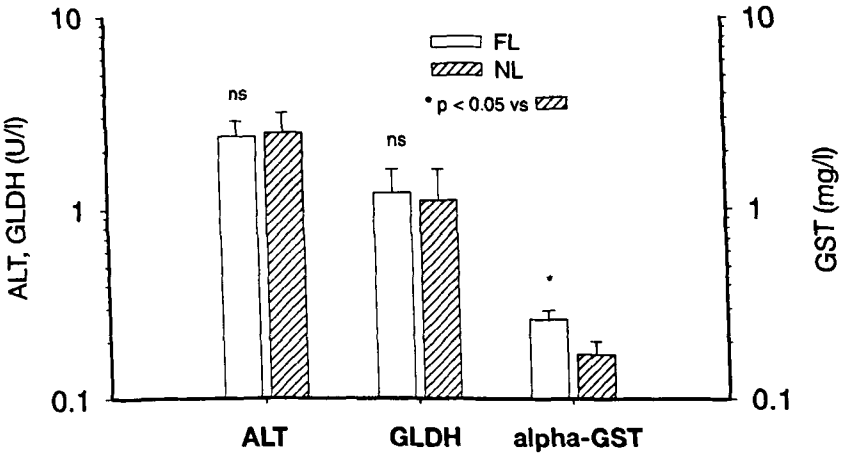

Fig. 1 Enzyme release of fatty livers (*FL*) and normal livers (*NL*) upon isolated perfusion in vitro without ischemic insult. Values are given as mean ± SE of $n \geq 5$ experiments per group

The present study was aimed to investigate the diagnostic value of α glutathione S-transferase (GST) in non-ischemic and ischemic livers with or without compensated steatosis.

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 between 200 and 300 g were used in this study. In half of the animals a histologically documented mild to moderate liver steatosis was induced by fasting for 2 days and subsequent feeding of a fat-free diet enriched in carbohydrates as detailed previously [3].

Experimental results were compared between fatty livers (FL) and normal livers (NL) from healthy rats ($n \geq 5$/group).

All rats were anestethized by intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg). Atropine (0.05 mg/kg) was given to reduce salivation. The abdomen was opened by midline incision, the liver skeletonized and freed from all ligamentous attachments. The common bile duct was cannulated with 27G polyethylene tubing, allowing for collection of total bile outflow during isolated perfusion in vitro. The hepatic artery was doubly ligated and divided. Finally, the portal vein was cannulated and the livers were retrieved and perfused in vitro either immediately or after ischemic preservation at 4 °C for 24 h in HTK solution.

Isolated perfusion was performed for 45 min at 37 °C with oxygenated (95% O_2-5% CO_2; $pO_2 > 500$ mmHg) Krebs-Henseleit buffer at a constant flow of approximately 3 ml/g × min, using a Masterflex-easy load roller pump head, connected to an Ismatec BVP precision pump drive (ISM 736; Ismatec Labortechnik Wertheim-Mondfeld, Germany).

Effluate was collected at the end of the experiment and later analysed for liver specific enzymes, including alpha-GST.

Enzyme activities of alanine aminotransferase (ALT) and glutamate dehydrogenase (GLDH) were assessed photometrically using commercialized standard kits (Boehringer, Mannheim, Germany). Activities of alpha-GST were determined by a quantitative enzyme immunoassay (Hepkit-alpha, Biotrin, Dublin, Ireland).

Statistics

Results are expressed as mean ± SE of 5 or more measurements per observation point. Stochastical significance of differences was assessed using the non-parametric U-test according to Mann and Whitney. *P*-values < 0.05 were considered to be significant.

Results

Non-ischemic livers showed similar enzyme release for steatotic and healthy grafts with respect to ALT or GLDH (Fig. 1). However, significant differences were found for alpha-GST; using a cutoff point of 220 µg/l, this parameter allowed for complete discrimination between fatty and normal livers in our study ($P < 0.01$ by Fisher's Exact test).

After ischemic preservation of normal livers, the enzyme release increased mildly compared to the respective non-ischemic references for ALT, remained unchanged for GLDH and rose substantially for alpha-GST (Fig. 2).

In contrast to the results obtained from normal livers, fatty livers responded to the cold preservation period by a more than 10-fold increase in all parameters, being most pronounced for the GLDH as a marker of mitochondrial damage (Fig. 3).

Discussion

Alpha glutathione S-transferase is found in high concentrations in the hepatocytes and is known to be readily released in response to parenchymal injury to the liver [5, 12].

Due to its short half-life in plasma of about 90 min, alpha-GST may be used as a sensitive indicator of hepatocellular injury after ischemia [9] or circulatory shock [10], allowing for close monitoring of post-traumatic he-

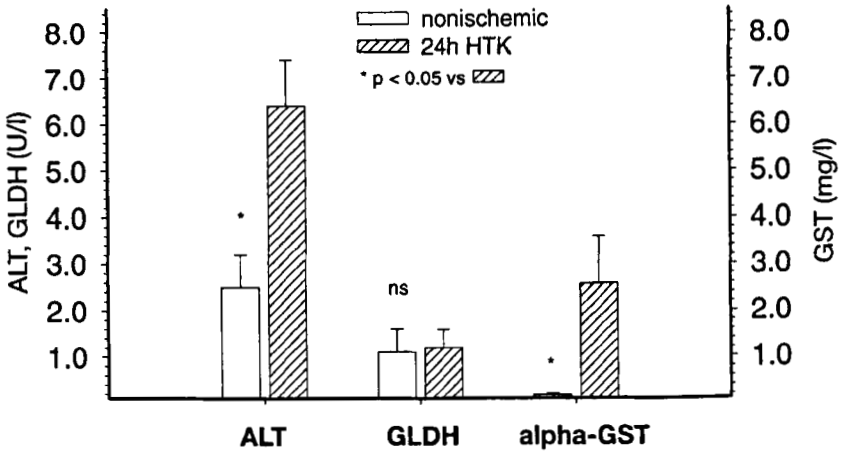

Fig. 2 Changes in enzyme release after 24 h of cold ischemic preservation in normal livers. Values are given as mean ± SE of $n \geq 5$ experiments per group

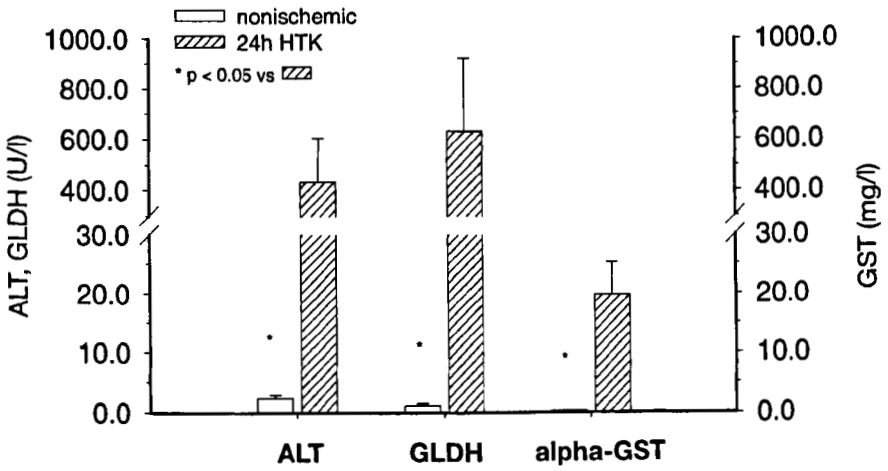

Fig. 3 Changes in enzyme release after 24 h of cold ischemic preservation in steatotic livers. Values are given as mean ± SE of $n \geq 5$ experiments per group

patic recovery which will be directly reflected by declining concentrations of the enzyme.

Recently, the release of alpha-GST has also been found to be a valuable parameter of liver damage in response to cold ischemic preservation even in an isolated perfused rat liver model, where alpha-GST turned out to be more sensitive than was the determination of conventional liver enzymes [13]. Our findings in normal livers corroborated this observation in so far that the relative increase of alpha-GST activities in the perfusate after ischemic preservation was more than 1 order of magnitude higher than that of the other conventional parameters. However, we also investigated the respective parameters in steatotic livers and it was striking that alpha-GST turned out to be less useful for the detection of postischemic reperfusion injury of these grafts than was ALT or GLDH. From the data of our study, we cannot yet explain this difference between normal and steatotic livers. Nonetheless, it has previously been pointed out that fatty liver grafts are particularly prone to mitochondrial alterations upon cold ischemia and subsequent reperfusion [4, 7], and thus the enzyme release from the mitochondria (as GLDH) may be notably increased after ischemic challenge in steatotic with respect to normal livers.

Another interesting finding of our study was the fact that alpha-GST, unlike ALT or GLDH, was able to discriminate between healthy and suboptimal/steatotic livers prior to ischemia. At that time, even functional integrity as evaluated by hepatic bile production upon isolated perfusion showed no significant differences between normal and steatotic livers (data not shown).

It is thus concluded that the determination of alpha-GST may be a useful additional parameter for evaluation of the quality of a questionable donor graft.

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