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Value of Alpha-Glutathione Sulfotransferase in the Assessment of Hepatic Injury After Experimental Long-Term Preservation

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ALPHA-glutathione sulfotransferase (α-GST) is a more specific and sensitive marker for hepatocellular damage in comparison to the traditional liver parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH).[1] This high sensibility is probably due to the elevated concentration of this enzyme in the liver. In addition, the short half-life (less than 90 minutes) and its wide and homogeneous distribution in liver parenchyma make α-GST a good parameter to detect any kind of hepatocellular injury.[2]

The purpose of this study is to assess α-GST regarding ischemic liver damage after cold extended preservation with University of Wisconsin (UW) solution,[3] using a nonrecirculating isolated liver perfusion model.

MATERIALS AND METHODS

For this study, 23 rat livers (15 ± 1.2 g) were used. After laparotomy, the main bile duct was cannulated and the liver was in situ cold flushed via the portal vein with 50 mL of UW solution for 2 minutes. Once the explantation was made, the infrahepatic cava vein was clamped and the suprahepatic cava vein was cannulated. Rat livers were preserved at 4°C for 0 hours (control group, n = 5), 12 hours (group 2, n = 6), 24 hours (group 3, n = 6), and 48 hours (group 4, n = 6). After the preservation period, livers were washed with 20 mL. Ringer lactate and introduced in a nonrecirculating isolated perfusion system for 120 minutes at 37°C.[4] Krebs-Henseleit-bicarbonate (KHB) buffer containing 2% bovine albumin (Sigma-Aldrich, Chemie Gmbh, Steinhelm, Germany) were circulated at 0.8 mL/min/gr and oxygenated with a membrane oxygenator (CAPIOX-SX, Terumo Corporation, Japan).[5,6] Samples for α-GST, AST, ALT, and LDH were taken via the suprahepatic cava vein at 30, 60, 90, and 120 minutes of isolated perfusion. Levels of α-GST were measured by quantitative enzyme immunoassay (Biotrin HEPKIT™-Alpha Rat GST-Alpha, Biotrin Ltd, Dublin, Ireland).[7] Samples were also taken at 30-minute intervals from the portal inflow and cava outflow for estimation of perfusate pH and oxygen consumption. Bile production was registered during the complete perfusion period.[4] Biopsies for optic and electron microscopy were taken in the left lobule after in situ perfusion, after the preservation period (another group was developed for these biopsies), and after isolated perfusion. For the comparison of the experimental groups (groups 2, 3, and 4) with the control group, simple lineal regression lines were made with each parameter in all groups. Increment, represented as media slope of this line, was calculated and compared with control by hypothesis contrast. To compare bile production between groups, the Kruskal-Wallis test was used. Regarding oxygen consumption, analysis of variance (ANOVA) was applied to compare the experimental groups with the control group. A P value of less than .05 was considered to be significant.

Table 1. Simple Linear Regression Analysis Showing Media Slopes and Determination Coefficient (R^2) of the Different Parameters in Each Group

Group	Slope	R^2
α-GST		
Group control	0.818	0.409
Group 2	1.247	0.543
Group 3	3.127 ($P < .05$)	0.335
Group 4	8.958 ($P < .002$)	0.783
AST		
Control group	0.021	0.029
Group 2	0.273	0.409
Group 3	0.116	0.233
Group 4	0.696 ($P < .01$)	0.632
ALT		
Control group	0.053	0.240
Group 2	0.446	0.196
Group 3	0.212	0.262
Group 4	1.084 ($P < .01$)	0.700
LDH		
Control group	0.654	0.396
Group 2	0.876	0.241
Group 3	1.597	0.315
Group 4	7.779 ($P < .002$)	0.744

RESULTS

During isolated perfusion, release of α-GST, AST, ALT, and LDH in group 2 (12 hours) showed no significant differences with the control group. Concerning group 3 (24 hours), only α-GST showed a significant increment ($P <$

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.05) with respect to the control group. On the other hand, all parameters showed significant increments comparing group 4 (48 hours) with the control group (Table 1). Bile production was significantly lower in groups 3 ($P < .001$) and 4 ($P < .001$) than in the control group. Oxygen consumption was not significantly different between groups 2, 3, and 4 and the control group.

Lightly swollen and vacuolated hepatocytes were found in livers from group 2 concerning optic microscopy, without cytoplasmic organelle damage in electron microscopy. Livers in group 3 showed a higher degree of injury in centrilobular areas than in periportal ones, with swollen hepatocytes and wide vacuoles. Lightly swollen mitochondria were found. Glycogen deposits were still present in the cells and the granular endoplasmic reticulum retained its lamellar profile. On the other hand, big vacuoles, swollen mitochondria, and condensated nuclei in hepatocytes were found in liver slices from group 4. The granular endoplasmic reticulum was frequently vesiculated and sinusoids with plenty of plasma membrane bleb formations were seen.[8]

DISCUSSION

Hypoxic injury in the liver occurs during cold ischemia preservation. The incidence of hepatocellular damage due to hypoxia, represented as cytoplasmic vacuoles, swollen hepatocytes, condensated nuclei and mitochondria, destructured sinusoids, and plama membrane bleb formations, shows a gradual increase from the portal to the centrilobular zones. Release of α-GST during isolated perfusion correlates with microscopic findings due to homogeneous distribution of this enzyme in portal and centrilobular areas. In this way, slices in group 3 (24 hours) show moderate hepatocellular damage, being more intense in centrilobular areas. In this group, only α-GST increased significantly with respect to the control group. AST, ALT, and LDH increased in this group, but not significantly.

In conclusion, α-GST proves to be a powerful and early parameter of hepatocellular damage. Only bile production presents a similar sensibility than α-GST to detect liver injury.

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