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Alpha Glutathione S-Transferase as Novel Parameter for Hepatocellular Damage in the Isolated Perfused Rat Liver

B.A. van Wagenveld, J.J.G. Scheepers, T.M. van Gulik, W.M. Frederiks, W.K. Bleeker, H. Obertop, and D.J. Gouma

ALPHA glutathione S-transferase (alpha-GST) is a cytosolic enzyme predominantly located in hepatocytes with a uniform distribution in the liver.¹ Several clinical and experimental studies have shown that alpha-GST is an early and sensitive parameter for hepatocellular membrane damage.²⁻⁴ Liver damage during hypothermic ischemia and reperfusion has been demonstrated to occur first at the microvascular level, especially in the sinusoidal endothelial cells (SEC).⁵ In a previous study from our laboratory it was shown that uptake capacity of exogenous hyaluronic acid (HA) is a sensitive parameter to detect SEC damage during reperfusion of isolated rat livers.⁶ Parameters of hepatocellular damage, such as release of transaminases, were increased later on in the process of ischemia and reperfusion (I/R).^{7,8} The aim of this study was to compare release of alpha-GST with the release of conventional enzymes that serve as parameters of hepatocellular injury in isolated perfused rat livers. In literature, a beneficial effect of preflush with an albumin-containing solution of cold-preserved livers prior to reperfusion is suggested.⁹⁻¹¹ The effect of albumin preflush was therefore studied in reperfusion-induced liver injury.

MATERIALS AND METHODS

Rat livers (12.6 ± 0.4 g, $n = 6$ in each group) were washed out in situ via the portal vein with cold (4°C) University of Wisconsin preservation solution. Immediately after hepatectomy (0 hour) or after 8- and 24-hour cold (4°C) ischemia time (CIT), livers were reperfused during 90 minutes with oxygenated, 0.5% bovine albumin containing Krebs-Henseleit (KH) buffer at 37°C to which exogenous HA (obtained from rooster comb, Sigma, St Louis, Mo) was added. Prior to reperfusion, livers were flushed with either 15 mL of a 40 g/L human albumin solution (ALB) with a purity of more than 95%, containing 140 mmol/L NaCl and 4 mmol/L caprylate (GPO, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam), or 15 mL Ringer's lactate (RL). Levels of alpha-GST, AST, ALT, LDH, and HA were assessed in the perfusate at the start (t1) and end (t90) of reperfusion, along with bile production during reperfusion. Alpha-GST was measured by a quantitative enzyme immunoassay (HEPKIT-Alpha, Biotrin, Dublin, Ireland). Uptake of exogenous HA or release of endogenous HA was measured by a radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) and was expressed as percentage of the initial HA concentration in the perfusate.

RESULTS

Release of alpha-GST ($\mu\text{g/L}$ per gram liver) during reperfusion (concentration at t90 vs concentration at t1) significantly increased with longer CIT in the ALB group (0 vs 24 hours' CIT; $P = .02$), and in the RL group (0 vs 24 hours' CIT: $P = .01$ and 8 vs 24 hours' CIT: $P = .02$; Fig 1). After 8 hours' CIT, concentrations of alpha-GST at t1 and t90 in the ALB group were significantly higher than in the RL group. However, the increase of alpha-GST during reperfusion (t90 - t1) was not significantly different between the two groups. Release of AST (U/L per gram liver) during reperfusion was only significantly increased between 8 and 24 hours' CIT in the ALB group ($P = .05$). Increase of ALT or LDH (U/L per gram liver) was not significantly different between any period of CIT. Uptake of exogenous HA was significantly decreased after 8 hours' CIT, compared with 0 hours' CIT (ANOVA: $P < .001$), indicating SEC damage (Fig 2). After 24 hours' CIT, release of endogenous HA occurred, indicating progressive SEC necrosis. There was no significant difference in HA kinetics between ALB and RL preflush. Bile production during reperfusion, in both the ALB and RL preflush groups, was significantly reduced after 24 hours' CIT compared to 0 hours' CIT (ANOVA: $P = .02$ and $P = .003$ in ALB and RL, respectively), however, was not different between the two groups (0 hours' CIT: 0.44 ± 0.06 $\mu\text{L/g}$ liver per minute and 0.45 ± 0.07 $\mu\text{L/g}$ liver per minute, respectively; 8 hours' CIT: 0.32 ± 0.09 $\mu\text{L/g}$ liver per minute and 0.32 ± 0.07 $\mu\text{L/g}$ liver per minute, respectively; 24 hours' CIT: 0.23 ± 0.05 $\mu\text{L/g}$ liver per minute and 0.40 ± 0.06 $\mu\text{L/g}$ liver per minute in ALB and RL, respectively).

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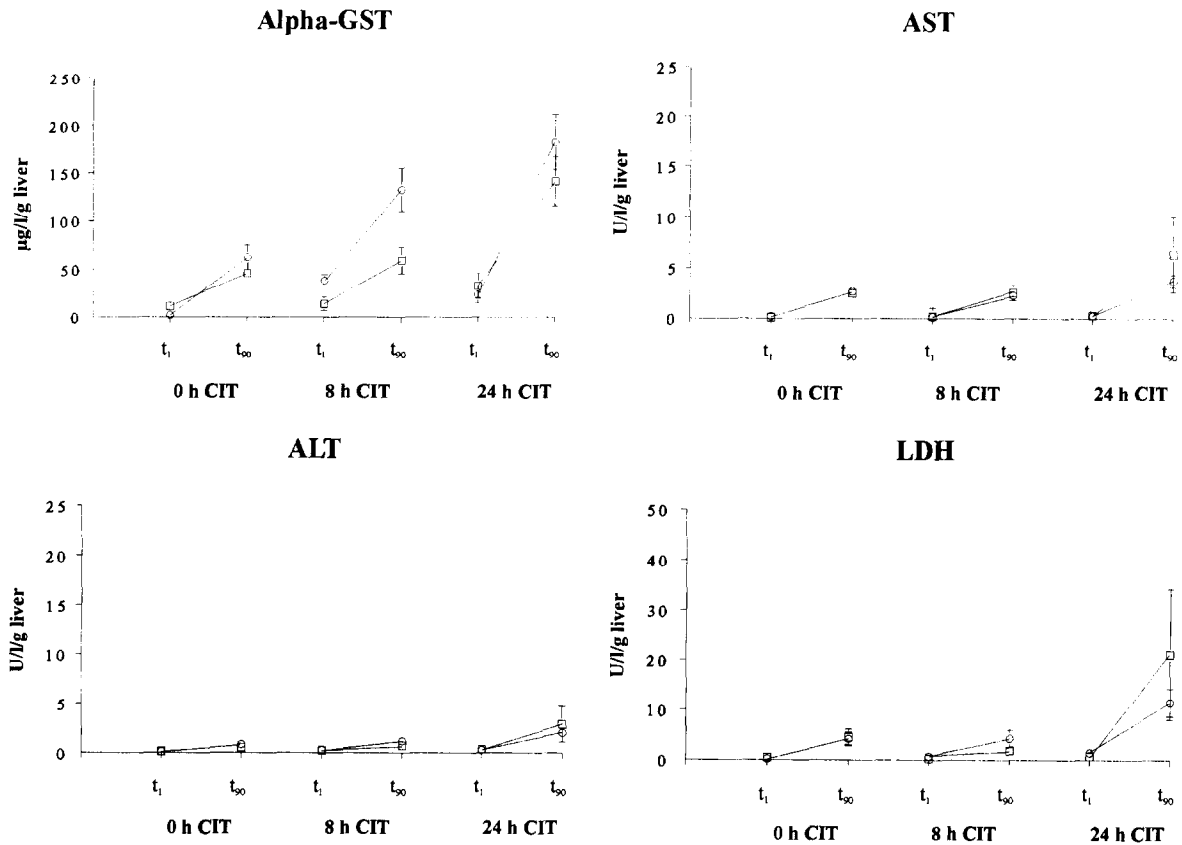


Fig 1. Release of alpha glutathione S-transferase (alpha-GST), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) at the start (t_1) and after 90 minutes of reperfusion (t_{90}) in cold (4°C)-preserved rat livers (8 and 24 hours' cold ischemia time; CIT), and control livers (0 hour CIT). Livers were flushed prior to reperfusion with 15 mL albumin-containing solution (\circ — \circ) or Ringer's lactate (\square — \square).

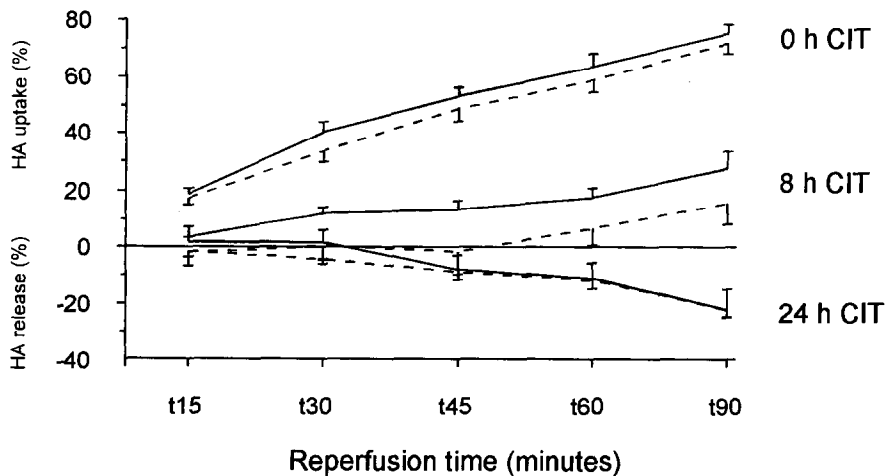


Fig 2. Uptake of exogenous hyaluronic acid (HA) and release of endogenous HA during 90 minutes of reperfusion in cold-preserved rat livers (8 and 24 hours' cold ischemia time; CIT), and in control livers (0 hour CIT). Livers were flushed prior to reperfusion with 15 mL albumin-containing solution (ALB; solid line) or Ringer's lactate (RL; dotted line). HA uptake was significantly decreased after 8 hours' CIT, compared to 0 hour CIT (ANOVA: $P < .001$). After 24 hours' CIT, release of endogenous HA occurred. There was no significant difference in HA kinetics between ALB and RL preflush.

DISCUSSION

The isolated perfused rat liver is a widely used model to study I/R injury in the liver. Parenchymal and nonparenchymal damage during asanguinous reperfusion of cold-preserved livers results from production of reactive oxygen metabolites and microcirculatory disturbances sustained during reperfusion.¹² The definition of sensitive parameters of microvascular and hepatocellular damage is therefore important for the localization and quantification of I/R injury. Alpha-GST may offer a valuable extension of the parameters commonly used in the assessment of hepatocellular damage. In the present study, HA kinetics in both the ALB and RL groups correlated well with the period of CIT, as was demonstrated by decreased HA uptake after 8 hours' CIT and release of endogenous HA after 24 hours' CIT, indicating progressive SEC necrosis. Release of alpha-GST after 24 hours' CIT was significantly increased in both preflush groups, in contrast to the release of conventional liver enzymes. Tiainen et al⁴ demonstrated in human liver grafts that release of serum alpha-GST in the early phase of reperfusion correlated well with duration of CIT. Release of alpha-GST preceded release of transaminases and, similar to the results of the present study, the increase of alpha-GST concentrations was much higher than that of serum transaminases. Thus, alpha-GST proves to be a powerful and early parameter of hepatocellular damage.

Addition of albumin to the preflush solution did not attenuate reperfusion injury, which is in contrast to other reports dealing with albumin preflush of both in vitro (asanguinous) and in situ (whole blood) reperfused livers.⁹⁻¹¹ In the present study, reperfusion with a bovine albumin containing KH-buffer might have masked any beneficial effect of an albumin preflush. Therefore, in a next study we reperfused the livers with KH-buffer without addition of bovine albumin (study in progress). A significant improvement of portal venous flow (obtained with Transonic Systems, Ithaca, NY) was found in the ALB preflush

group during the entire period of 90 minutes' reperfusion, as compared to the RL group (21.4 ± 1.2 mL/min and 15.5 ± 1.8 mL/min at t90, respectively; $P < .001$, ANOVA). This indicates an improvement of microcirculatory flow during reperfusion after ALB preflush, which is likely the result of reduced cell swelling.^{9,11} Albumin-containing solutions, therefore, seem appropriate as a basis to develop preflush solutions further.

In conclusion, release of alpha-GST in the perfusate of isolated perfused rat livers was a more sensitive parameter than was the release of conventionally used liver enzymes (AST, ALT, and LDH) in the assessment of preservation-induced, early hepatocellular damage. Preflush with an albumin-containing solution did not reduce hepatocellular damage during reperfusion of rat livers up to 24 hours of hypothermic ischemia.

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