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α-glutathione s-transferase (α-GST) release, an early indicator of carbon tetrachloride hepatotoxicity in the rat

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1 The use of the cytoplasmic enzyme, alpha glutathione s-transferase (α-GST) as an early index of carbon tetrachloride (CCl_4) toxicity in the rat was investigated and compared with a standard enzyme marker, aspartate aminotransferase (AST). The hepatotoxic effects of CCl_4 in the rat were determined in a time and dose-response study.

2 Following CCl_4 exposure, α-GST release was shown to be an earlier and more sensitive biomarker of hepatotoxicity than AST.

3 Significant increases in α-GST were detected 2 h after CCl_4 exposure. Using the enzyme marker AST, this early hepatotoxic injury went undetected. At 6 and 16 h, α-GST was also a more sensitive indicator of hepatotoxicity than AST.

4 α-GST release was significantly increased at a dose of 5 μl/kg, the lowest concentration of CCl_4 administered and clearly responded in a dose-dependent manner with increasing doses of CCl_4. In contrast, release of AST did not reach statistical significance until a dose of 25 μl/kg.

5 Thus, these findings indicate that α-GST is a more sensitive and more accurate reflector of CCl_4 induced hepatotoxicity than AST.

Keywords: α glutathione s-transferase; α-GST; aspartate aminotransferase; AST; hepatotoxicity; carbon tetrachloride; biomarker

Introduction

Glutathione S-transferases (GSTs) are a major group of phase II detoxification enzymes found in all eukaryotic organisms.[1] They are composed of a complex super-gene family that collectively metabolise a broad range of compounds, including chemotherapeutic drugs, carcinogens, and environmental pollutants, among others.[2] There are four classes of cytosolic GST; namely alpha, mu, pi and theta.[1] In addition, a membrane-bound form called microsomal GST also exists.[1] In humans, 80% of all α-GST is found in the liver, the rest being localised in the testis, kidney and adrenal glands.[3–6] In the rat, α-GST consists of two sub-units designated Ya and Yc, each with a molecular weight of approximately 25 kDa.[7]

Cytosolic enzymes released into the circulation as a result of hepatocellular damage are regularly used in the assessment of liver function. The most commonly chosen serum or plasma enzyme markers include aspartate aminotransferase (AST) and alanine aminotransferase (ALT). However, the utility of measurement of the aminotransferases for monitoring hepatocyte damage has been questioned since activity levels may be normal in patients with chronic liver disease.[8–10] The uneven distribution of aminotransferases within the liver may explain their poor sensitivity as indicators of certain types of liver damage. Periportal hepatocytes contain the highest concentrations of the aminotransferases but centrilobular hepatocytes, which are relatively deficient in aminotransferases, are most susceptible to damage from hypoxia and various toxins.[11]

Recently, the measurement of α-GST has been advocated as a superior marker of hepatocellular damage to either AST or ALT in humans for a variety of clinical conditions including halothane hepatotoxicity,[12] paracetamol poisoning,[13,14] and liver rejection post transplantation.[15] In contrast to the aminotransferases, α-GST is found uniformly throughout the liver.[7] Other advantages of using α-GST as a marker of hepatocellular damage are the high cytosolic concentration (4–5% of total hepatocellular protein) and the small molecular weight (51 kDa as opposed to 95 kDa for AST).[2] Consequently α-GST is readily and rapidly released in quantity into the circulation following hepatic damage.

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A further benefit of monitoring α-GST release is the relatively short half-life of the enzyme in the circulation. In man, the reported *in vivo* half life is approximately 1 h, whereas the reported *in vivo* half-life of ALT is approximately 47 h.[15] Consequently, serum α-GST levels may give a better reflection of changes in hepatocellular integrity, due to the rapid clearance from the circulation of α-GST.

In this study a commercial enzyme-immunoassay (EIA) for the quantitation of total rat serum α-GST (Ya and Yc) was used. Previously published methods of quantifying enzymatic activity of α-GST have usually been non-specific and susceptible to interference from endogenous substances.[1] Radioimmunoassays are considered to be too time-consuming.[2] Using an EIA ensures specificity while reducing assay time to approximately 3 h.

Carbon tetrachloride (CCl_4) is a well characterised hepatotoxin which leads to acute hepatic injury upon the formation of free radicals during its biotransformation. CCl_4 is metabolised in the endoplasmic reticulum where it causes accumulation of lipid, depression of protein synthesis and depression of mixed-function oxidase activity.[16] In this study, rats were treated with CCl_4 in a time and dose-response study and hepatocellular injury was assessed by the measurement of serum α-GST and AST levels.

Materials and methods

Animals
Adult male Sprague Dawley rats (240–280 g body weight) were obtained from the Biomedical Facility, University College Dublin, Ireland. Animals were housed in a temperature controlled environment and allowed free access to food (standard rat pellet) and water.

Time-response study
CCl_4 (BDH, analar grade) at a dose of 25 μl/kg diluted with an equal volume of corn oil was administered to groups of six animals by gavage. Each animal received the same final volume of 50 μl/kg. The animals were anaesthetised and serum collected at 2, 6, 16, 24 and 48 h. Separate animals were used for each time point.

Dose-response study
Four groups of six animals received a single oral dose of CCl_4 by gavage. The concentrations studied were 5, 12.5, 25 and 50 μl/kg and the animals were anaesthetised and serum collected after 16 h. All doses given in a 50 μl/kg final volume mixed in corn oil where appropriate. A control group of three animals received equivalent volumes of corn oil (50 μl/kg).

Collection of samples
Animals were bled under anaesthesia [(hypnorm fentanyl citrate, 0.315 mg/ml and fluanisone 10 mg/ml,) 0.3 ml/kg intra-muscular and diazepam 2.3 mg/kg, intra peritoneal] from the abdominal aorta. Blood was collected and allowed to clot at room temperature for 1 h prior to centrifugation. Serum was collected and stored at 4°C for no longer than 5 days prior to assay. Results are expressed as mean ± the standard error of the mean (s.e.m).

Enzyme assays
α-GST concentration in serum was measured using the EIA Hepkit™-Rt kit (Biotrin, Dublin, Ireland). The test procedure of this quantitative solid-phase enzyme immunoassay is based upon the sequential addition of sample, antibody-enzyme conjugate and substrate to microtiter wells coated with rat α-GST antibody. The resultant colour intensity is proportional to the amount of α-GST in the sample. The assay range is 0–50 ng/ml. AST activity in serum was determined on a Beckman SYNCHON CX system.[17]

Statistics
The data obtained for CCl_4 treated animals at each time point, was compared to its corresponding control group using Student's Unpaired *t* test (Apple Macintosh Statsworks™ software). Results were deemed statistically significant when $P \leqslant 0.05$.

Results

Time-response study
The results obtained in the time-response study are presented in Figure 1. α-GST concentration is expressed as ng/ml, while AST concentration is expressed as IU/L. A control group (n=3) was compared to the CCl_4 treated group (n=6) at each of the time points. A significant increase ($P \leqslant 0.05$) in the serum concentration of α-GST in treated animals was observed at the 2 h first time point studied. Serum AST levels only reached statistical significance 6 h after dosing. Both enzymes were released into the serum in a time-dependent manner. Levels of both enzymes reached a peak at 24 h and subsequently declined by 48 h.

Dose-response study
The results obtained in the dose-response study are presented in Figure 2. An increase in the release of α-GST was statistically significant at the lowest dose studied, 5 μl/kg. Release of this enzyme into the serum continued to occur in a dose-dependent manner over the dose range studied. In contrast, release of AST only became statistically significant at the higher dose of 25 μl/kg.

Discussion

The value of α-GST as an early detector of hepatotoxicity is evident from this study. Using α-GST, CCl_4 induced hepatocellular toxicity was detectable as early as 2 h following exposure. Using the enzyme marker AST, this early hepatotoxic injury went undetected.

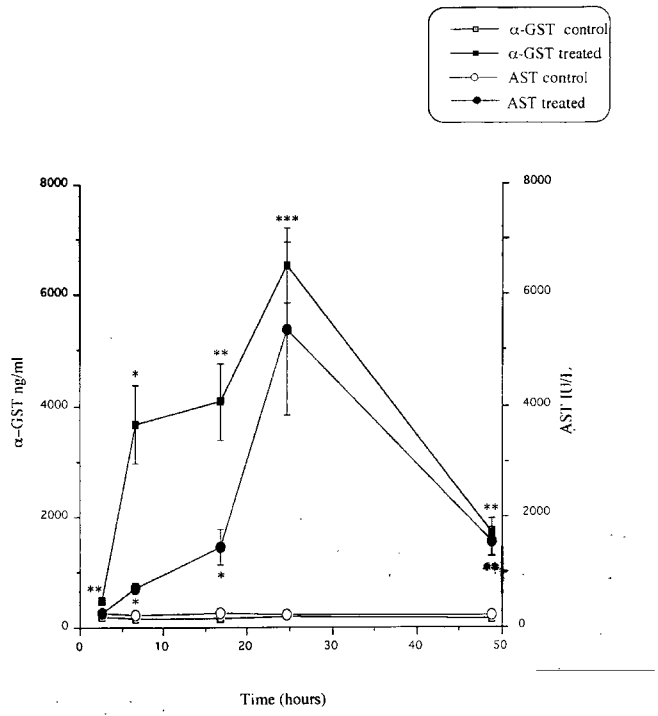

Figure 1 Release of α-GST and AST into serum following administration of CCl_4 at 25 μl/kg. Each point represents the mean ± s.e.m. of 3–6 animals. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

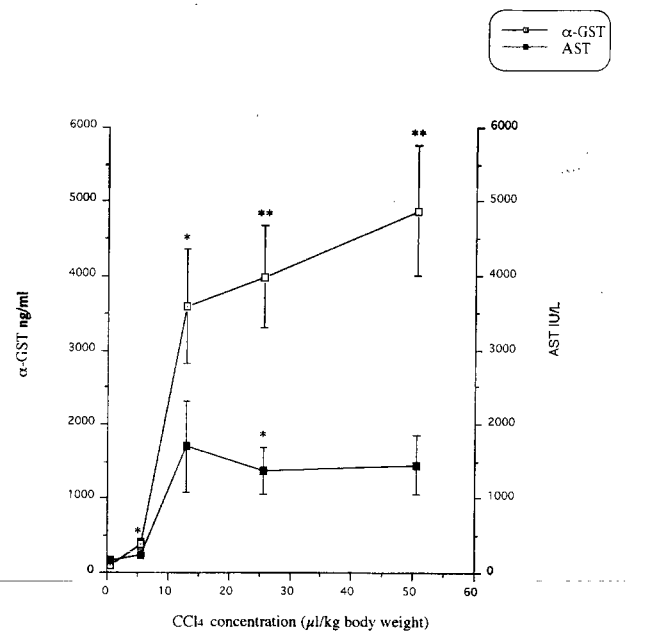

Figure 2 Release of α-GST and AST into serum 16 h following administration of CCl_4 at doses ranging from 0–50 μl/kg. Each point represents the mean ± s.e.m. of 3–6 animals. $^*P<0.05$, $^{**}P<0.01$

α-GST release was significantly increased at a dose of 5 μl/kg, the lowest concentration of CCl_4 administered in the dose-response study and clearly responded in a dose-dependent manner with increasing doses of CCl_4. In contrast, release of AST did not reach statistical significance until a dose of 25 μl/kg. Thus, it would appear α-GST is a more sensitive and more accurate reflector of CCl_4 induced hepatotoxicity than AST.

The studies described in this paper demonstrate the benefits of using serum α-GST rather than a conventional marker, AST, as a marker of hepatocellular injury in the rat. CCl_4 was chosen because it is known to be a potent hepatotoxin and therefore, likely to result in the gross release of intracellular contents. The results obtained are not unexpected since, it has previously been established that the concentration of the aminotransferases are lower than glutathione-S transferases in centrilobular hepatocytes, an area known to be sensitive to toxic damage.[11] In addition, the high concentration of GSTs in the liver coupled with their small molecular weight means that when hepatotoxic injury occurs to these cells, α-GST is readily released in large amounts.

The relatively restricted distribution of α-GST in tissues could also offer advantages over aminotransferases as markers of liver function. Numerous studies in both animals[20,21] and humans[22,23] suggest that elevated aminotransferase levels can be due to release of muscle isoforms from damaged fibres, leading to an inaccurate reflection of hepatocellular integrity. α-GST release, however, is in no way influenced by muscle tissue damage.[24]

Many of the enzyme markers such as AST are quantified on the basis of their enzymatic activity. However, in this study α-GST was determined quantitatively by the newly developed solid phase enzyme immunoassay, the Hepkit™-Rt Kit. This assay is extremely sensitive since it can be used to measure concentrations of α-GST as low as 1 ng/ml. In addition as this is an immunoassay, the capturing antibodies for α-GST are highly specific, thus guaranteeing little or no interference. This is not the case with enzymatic activity assays for α-GST, as no isoenzyme-specific substrate exists and thus susceptibility to interference from endogenous substances is a possibility.

A further benefit of monitoring α-GST release is the relatively short half-life of the enzyme in the circulation. In man the reported *in vivo* half life is approximately 1 h.[15] Consequently, serum levels of this enzyme may give a better reflection of hepatocellular integrity and response to therapy in cases of hepatotoxicity, because once released into the bloodstream α-GST is rapidly degraded.

Therefore, based upon this evidence α-GST is a more suitable indicator of hepatotoxicity in rats than AST. It could play a role in early identification of toxicity in screening drug candidates and also in toxicity monitoring in man.

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