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SHORT COMMUNICATIONS

The polymorphic expression of neutral glutathione *S*-transferase in human mononuclear leucocytes as measured by specific radioimmunoassay

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Drug-metabolising enzyme systems, catalysing either the activation or detoxification of various xenobiotics, exist in most human tissues [1]. The activation of potentially toxic or carcinogenic substances occurs via the Phase I mixed-function oxygenases and the reactive intermediates formed by this oxidative metabolism may then be detoxified by Phase II enzymes such as epoxide hydrolase and the glutathione *S*-transferases (GST) (EC 2.5.1.18). The study of these enzyme systems in man usually relies on post-mortem tissue. However, mononuclear leucocytes have been used for the investigation of certain drug-metabolising enzymes, including aryl hydrocarbon hydroxylase [2], epoxide hydrolase [3] and GST [4].

The GST are multi-functional detoxification enzymes which catalyse the conjugation of glutathione to a wide range of harmful electrophiles [5]. In man three groups of cytosolic enzymes have been identified on the basis of their isoelectric points; the basic, neutral and acidic forms [6]. The isoenzymes from each group are thought to be encoded by three separate loci [5]. The three types of GST possess distinct catalytic properties and the neutral GST has a higher catalytic activity towards the mutagenic epoxides benzo(α)pyrene 4,5-oxide and styrene 7,8-oxide, than do the basic and acidic forms of GST [5, 7]. Marked inter-individual differences in the hepatic neutral-type GST (locus 1) content have been established and about 50% of liver specimens have been reported to lack neutral-type GST [8]. Two hepatic neutral-type GST have been isolated: GST μ of pI 6.1 and GST ψ of pI 5.5 [9, 10]. Although various GST isoenzymes are active with substrates which have been implicated in carcinogenesis [1], the evidence suggests that individuals who express the neutral GST have some degree of protection from chemical carcinogens found in cigarette smoke [11].

In this paper we describe the first specific radioimmunoassay (RIA) to human netural-type GST. We have used the RIA to investigate the polymorphism of neutral GST in human mononuclear leucocytes.

Materials, methods and results

Reagent preparation. GST μ and GST ψ were purified and iodinated as described previously [9, 12]. Standards were prepared in assay diluent consisting of 0.25 M phosphate buffer pH 7.4, containing 0.1% (w/v) bovine serum albumin and 0.02% (w/v) sodium azide.

Antisera to GST μ and GST ψ were raised in 4 and 6 New Zealand White rabbits, respectively, using standard immunisation techniques [12]. Pre-precipitated second antibody (donkey anti-rabbit serum) was prepared as described previously [13].

Assay protocol. All assay constituents were made up in assay diluent. Sample or standard (100 μl) was incubated with antisera (100 μl of a 1:1000 dilution) for 48 h at 4°. Radiolabelled GST was then added (30,000 dpm in 50 μl) and incubated at 4° for a further 24 hr. Pre-precipitated second antibody reagent (100 μl) was then added followed by a 1 hr incubation at room temperature. Wash solution (2 ml 0.15% Brij solution in water) was added and the tubes centrifuged at 3000 *g* for 20 min at 4°. The supernatant was decanted and the bound fraction counted in a Nuclear Enterprises 1600 multi-well gamma counter.

Specificity and precision. Adequate titres of antibody were only obtained in one of the four antisera to GST μ and three of the six antisera to GST ψ. All four of these antisera exhibited 100% cross-reactivity with GST μ and GST ψ but less than 0.2% cross-reactivities with the basic GST ε (B_1 B_1) and γ (B_2B_2) and the acidic GST λ.

The best assay precision and sensitivity obtained were achieved with one of the GST ψ antisera and ^{125}I-GST ψ but it was found that GST ψ was unstable on storage. Identical precision, specificity and sensitivity were achieved with the GST ψ antiserum and ^{125}I-GST μ. In all subsequent assays the combination of anti-GST ψ and ^{125}I-GST μ was used.

The mean precision profile obtained from three consecutive assays and a standard curve are shown in Fig. 1. A coefficient of variation of less than 10% was obtained for GST μ concentrations from 9.0 μg/l to 110 μg/l.

Subjects. Blood (10 ml) was obtained from 42 healthy laboratory volunteers (ages ranging from 17 to 53; 22 females, 20 males). The blood was placed in a lithium heparin tube and processed within 1 hr of collection.

Isolation of mononuclear leucocytes. The mononuclear leucocyte fraction was isolated by centrifugation on a discontinuous gradient of Histopaque® 1077 (Sigma Chemical Co. Ltd., Poole, Dorset, U.K.) using the procedure recommended by the manufacturer. The cells that collected at the opaque liquid interface were washed twice with 10 mM phosphate buffer pH 7.4 containing 120 mM NaCl and 2.7 mM KCl. After the second wash the cells were lysed by resuspending in 20 mM phosphate buffer pH 7.4 and freezing at −20°. The samples were thawed, centrifuged at 3000 *g* for 15 min at 4°, and the protein content [14] and neutral GST were then measured. Phase contrast microscopy showed that the freeze/thaw procedure resulted in a complete lysis of the cells.

Distribution of neutral GST in a control population. Neutral GST was detectable in the cell lysates from 23 of the 42 individuals tested (55%). A mean level of 229 ng neutral GST/mg of protein was obtained (Table 1). In the remaining 19 individuals GST could not be detected, i.e. there was less than 20 ng neutral GST/mg protein.

Discussion

We have developed a specific RIA that measures total (μ + ψ) human neutral GST and have shown that, in a healthy control population, neutral GST is only expressed in the mononuclear leucocytes of approximately 55% of individuals. This agrees with the findings of Warholm *et al.* [7] who identified human neutral GST μ in 60% of human livers obtained at post mortem.

Previous studies using human mononuclear leucocytes have been limited to the measurement of GST enzyme activities [4, 11]. Seidegard *et al.* [4] observed a marked inter-individual variation in the activity of leucocyte GST towards *trans*-stilbene oxide. The identity of the GST isoenzyme responsible for this activity has not been fully established, however, given the available evidence, it seems highly probably that the neutral GST can be implicated. Family studies and the distribution of *trans*-stilbene oxide activity in a large control population provide evidence that either the genes coding for the isoenzyme(s) or a regulatory

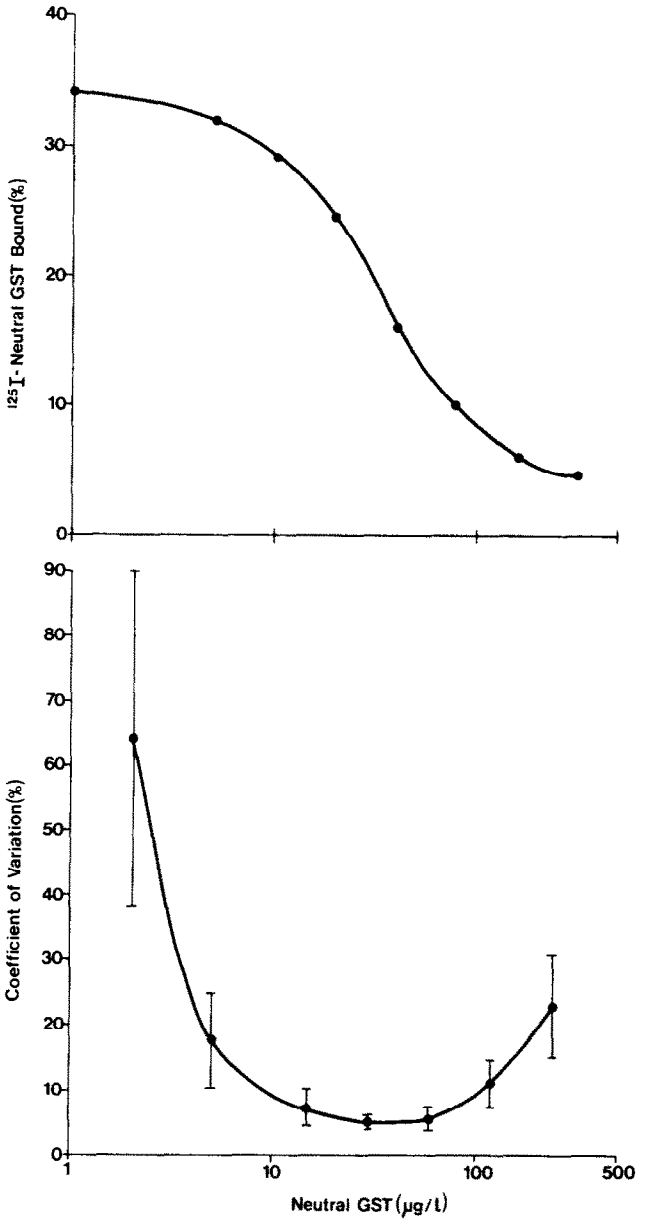

Fig. 1. A standard curve and the mean inter-assay precision profile (± SD) for three consecutive RIA.

Table 1. Neutral GST levels in a control population

Expression of neutral GST	No. of individuals (%)	GST level; Mean and range (ng/mg of protein)
Undetectable	19 (45)	<20
Detectable	23 (55)	229 110–330

gene are inherited in an autosomal dominant manner [15].

Seidegard and co-workers also showed significant differences between the distribution of *trans*-stilbene oxide activity in a population of control smokers and a population of lung cancer patients with similar smoking histories [11]. GST activity towards *trans*-stilbene oxide, therefore, has been proposed as a marker for susceptibility to lung cancer.

The study of polymorphism in drug-metabolising enzymes can yield useful information as to individual and tissue responses to toxic and carcinogenic compounds [2, 16, 17]. In the present study, we have described an RIA method that allows the specific measurement of levels of

neutral-type GST in human mononuclear leucocytes and demonstrates the existence of polymorphism. This method should prove valuable during further investigations into the expression of neutral GST in populations that are at risk from chemical carcinogenesis.

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Effect of age on the sinusoidal release of hepatic glutathione from the perfused rat liver*†

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GSH plays a critical role in cellular detoxification and defense. Recently, a complex interorgan process of regulation of GSH has been proposed [see, for example, Ref. 1]. According to this view, the liver releases GSH into the circulation by a carrier-mediated process [2, 3], accounting for nearly all release of GSH into plasma [4]. The circulating GSH is then cleared by the kidneys and other organs [5]. Thus, the liver plays a central role in the homeostasis of GSH.

The rate of hepatic GSH turnover has been reported to decrease with age [6]. However, while it has been estimated that the sum of sinusoidal and biliary release accounts for total hepatic GSH turnover [7], this relationship has not been explored over the whole range of ages used in the turnover studies. Besides, in these studies, similar to all earlier ones [1, 8], sinusoidal release was estimated to account for 80–90% and the biliary release for 10–20% of the total hepatic release. This contention is now under major revision due to findings in two recent independent studies [9, 10]. These studies, using retrograde biliary infusions of AT-125, a potent inhibitor of γ-glutamyltransferase, have documented substantial hydrolysis of GSH in bile. Thus, all previous studies, not using this agent, have underestimated biliary GSH release, which may actually account for up to 50% of total hepatic GSH release. In addition, one study [9] has shown that, while biliary GSH release remains constant in immature rats, it increases linearly (by about 6-fold) in the range of 180–300 g body weight. Thus, for total hepatic turnover to decline with age, the sinusoidal release will have to drop sharply to compensate for the rising biliary GSH.

The evidence reviewed above points to the fact that the sinusoidal GSH release may not quantitatively account as the major (i.e. ≈ 80%) component of total hepatic GSH turnover. Instead, it may account for no more than 50% of total turnover. Nevertheless, it still remains a significant component of hepatic GSH release and turnover. Therefore, it is important to determine its relationship to age. Our studies were designed to delineate this relationship.

Methods

Livers of male, Sprague–Dawley rats, 85–350 g body weight, fed *ad lib.*, were perfused *in situ*, single-pass with Krebs bicarbonate buffer gassed with 95% O_2/5% CO_2, pH 7.4 at 37°, as described earlier [2]. After an initial 10- to 15-min equilibration period, perfusates were sampled at 5-min intervals for 30–60 min. The rate of sinusoidal GSH release [nmol · min^{-1} · (g liver)$^{-1}$], measured as the product of perfusate GSH concentration, determined by the method of Tietze [11], and the perfusion rate (kept constant in each perfusion) remained at steady state throughout each and every perfusion (coefficient of variation ≤ 10%). As before

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† In our previous publications, we used efflux for the process we refer to as release here. The two terms refer to the same process in our papers.