GENE DELETION AND PARTIAL DEFICIENCY OF THE GLUTATHIONE *S*-TRANSFERASE (LIGANDIN) SYSTEM IN MAN

P. G. BOARD

Department of Human Biology, John Curtin School of Medical Research, P.O. Box 334, Canberra, ACT 2601, Australia

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1. Introduction

The glutathione *S*-transferases are a family of enzymes that catalyse the conjugation of reduced glutathione (GSH) with a wide variety of electrophilic compounds [1]. In addition, the glutathione *S*-transferases can act as non-specific binding proteins which facilitate the transport of a variety of ligands of both exogenous (e.g., bromosulphothalein) and endogenous (e.g., bilirubin) origin. Investigators studying hepatic binding proteins have termed the major binding component as ligandin which subsequent studies have identified as a glutathione *S*-transferase [2]. Thus the glutathione *S*-transferase/ligandin system makes a substantial contribution to the metabolism and elimination of a wide variety of reactive and toxic metabolites, mutagens and carcinogens.

Deficiency of hepatic organic anion-binding protein (ligandin/glutathione *S*-transferase) has been proposed as a possible cause of non-haemolytic unconjugated hyperbilirubinaemia in the new born [3]. That study suggested that relative deficiency of these acceptor proteins may be rate limiting in a transfer process. More recently, ligandinaemia has been reported in a number of inflammatory and neoplastic liver diseases, including viral and chronic hepatitis, cirrhosis and primary hepatocarcinoma [4–7]. These observations have prompted the suggestion that ligandinaemia may well provide a further means of evaluating liver damage and be of diagnostic value in cases of primary liver cell cancer [8,9].

Recent biochemical genetic studies utilizing electrophoretic techniques developed in this laboratory [10] have shown that the glutathione *S*-transferases in human liver are the product of 3 distinct structural loci [11]. Two of these loci, known as GST_1 and GST_2, are possibly the result of a gene duplication and are both highly polymorphic. The high degree of polymorphism and heterozygosity at these loci explains the multiple forms identified by ion-exchange chromatographic techniques [12]. Our investigations have demonstrated the existence of a null allele at the GST_1 locus in some individuals [11]. This allele may result from gene deletion, as there is no evidence for the formation of an inactive product [11]. The physiological consequence of this null allele remains to be fully investigated, however, the data presented here demonstrate that the total level of liver glutathione *S*-transferase activity is significantly diminished in individuals homozygous for this allele.

3. Results and discussion

Fig.1 shows schematically the relative electrophoretic mobility of the products of the GST_1 and GST_2 loci and the observed allelic variation [11]. The products of these loci account for almost all the glutathione *S*-transferase activity in human liver. Homozygotes for the null allele at the GST_1 locus (GST_1^0/GST_1^0) fail to show any activity at the anodal end of the gel where GST_1 products are normally observed. Products GST_2 locus are always present and migrate towards the cathode under these conditions. The total glutathione *S*-transferase activity in liver extracts was determined spectrophotometrically using 1-chloro-2,4-dinitrobenzene and GSH as substrates [1] and is given in table 1. Individuals homozygous for the null allele at the GST_1 locus had a significantly lower ($P < 0.01$) total glutathione *S*-transferase activity than a pooled group of individuals who expressed products of the GST_1^1 or GST_1^2

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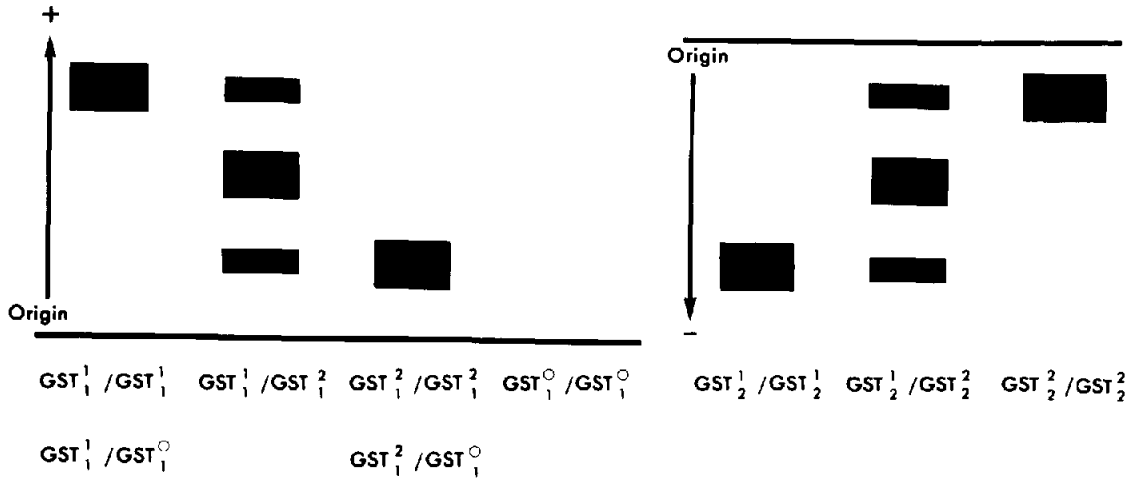

Fig.1. Schematic representation of the glutathione *S*-transferase isozyme phenotypes observed in human liver. The possible genotypes are indicated beneath each phenotype.

alleles. In addition, the variability of the homozygous null group was less than the variability of the pooled group, which would be expected to contain a proportion of individuals heterozygous for the null allele (GST_1^1/GST_1^0, GST_1^2/GST_1^0) in addition to individuals heterozygous and homozygous for the electrophoretically detectable alleles (GST_1^1/GST_1^1, GST_1^2/GST_1^2, GST_1^1/GST_1^2). Although there is residual activity in samples with the GST null phenotype, due largely to the action of GST_2 products and to a minor extent the GST_3 products, the diminished total activity has several significant implications.

Table 1
Glutathione *S*-transferase activity in human liver

GST_1 Phenotype	*N*	Total activity[a] (mean ± SD)
Phenotype null (GST_1^0/GST_1^0)	18	0.66 ± 0.279
Electrophoretically detectable (GST_1^1/GST_1^1, GST_1^1/GST_1^2, GST_1^2/GST_1^2, GST_1^1/GST_1^0, GST_1^2/GST_1^0)	18	1.01 ± 0.415

[a] Activity is expressed as μM conjugate formed . mg protein^{-1} . min^{-1} at 25°C

All liver samples were obtained at post-mortem examination and stored frozen at −20°C until electrophoretic phenotype and total glutathione *S*-transferase activity could be determined

If, as suggested [3], a relative deficiency of glutathione *S*-transferase (ligandin) is a significant factor in the aetiology of non-haemolytic unconjugated hyperbilirubinaemia in the new born, then it is likely that individuals inheriting the null allele may be more likely to suffer this disorder during foetal development and in the neonatal period when production of ligandin is naturally at a low level [3].

These results also have significance in the evaluation of ligandinaemia associated with liver disease [4–9]. The presence of a common null allele which significantly affects the level of glutathione *S*-transferase/ligandin in liver is likely to influence the degree of ligandinaemia which occurs in various hepatic disorders. This genetic factor, therefore, needs to be considered in further studies aimed at determining the clinical value of estimates in ligandinaemia.

In addition, the partial deficiency of glutathione *S*-transferase occurring in individuals inheriting the null allele may place them at a relatively greater risk if they are exposed to elevated levels of certain electrophilic carcinogens. Further studies will be necessary to determine if the occurrence of the null allele and the diminished levels of glutathione *S*-transferase in some individuals are associated with an increased incidence of neoplastic disease.

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