Gut, 1991, **32**, 813–818

Glutathione S-transferase in humans in health and disease

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Most biological processes are catalysed by means of enzymes and study of these in health and disease is important for our understanding of both biology and disturbed function. The glutathione S-transferases (GST:E.C.2.5.1.18) are a complex multigene family of enzymes that are widely distributed in the animal kingdom.[1] These enzymes possess many biological functions, the most important of which is detoxification, conjugating reduced glutathione with a large number of electrophiles.[2] Such conjugation reactions result in the synthesis of mercapturic acids and represent an important excretory route for xenobiotics including carcinogens, toxins, and drugs.[1] As well as being concerned in the metabolism of xenobiotics, GST are involved in the metabolism of endogenous substances such as leukotriene A4 and prostaglandin A1.[3 4] GST are also involved in non-substrate binding to such substances as bilirubin and bile acids and have an important role in hepatic anion transport. Several GST isoenzymes possess selenium-independent glutathione peroxidase activity with certain organic hydroperoxides such as linoleic and arachidonic hydroperoxide but not with hydrogen peroxide.[5]

In humans the cytosolic GST isoenzymes are dimeric and can be subdivided on the basis of their physicochemical and immunological properties into three major groups, namely pi, alpha, and mu class GST (Table[6]). In humans two immunologically distinct subunits of the alpha class GST have been named B_1 and B_2. A fourth GST isoenzyme has recently been identified in the liver and has been purified from the microsomal subcellular fraction. By contrast with the cytosolic enzymes, the microsomal GST is trimeric containing subunits with a molecular mass of 17 000 kDa.[7 8]

In this review we concentrate on the distribution of these GST isoenzymes in various human tissues in both health and disease. We also discuss the value of measuring GST isoenzymes in plasma as a means of identifying tissue damage and neoplasia.

Tissue distribution of GST isoenzymes

Alpha class GST have been shown to be the predominant isoenzymes in human hepatocytes with the microsomal and mu class GST present in small quantities.[9] Mu class GST is subject to polymorphism and, in the Scottish population only about 55% of individuals express this GST class.[10] Pi class GST in the adult human liver is only expressed in the biliary epithelium. Why pi class GST is expressed so strongly in biliary epithelium including the gall bladder is unclear, but large quantities of this isoenzyme can be identified in human bile.[11] In most hepatocellular carcinomas alpha class GST are expressed, whereas pi class GST is the predominant enzyme in cholangiocarcinoma.[12]

The heterogeneity of GST isoenzyme distribution has also been shown in small bowel epithelium. Pi and mu class GST are present in cells lining both the villi and crypts, whereas alpha class GST has only been identified in the epithelium of villi and microsomal GST only in cells lining the intestinal crypts.[9] In the colon, both pi and mu class GST (the latter in 50% only) are present in the epithelium, whereas alpha class and microsomal GST are absent. Overexpression of pi class GST in carcinoma of the colon has been reported while expression of the other isoenzymes is variable.[13]

In the pancreas, pi class GST has been identified in the centroacinar cells and ducts but not in the exocrine acini nor in the islets of Langerhans.[14] Alpha class GST are the predominant isoenzymes in the acini and are also present in pancreatic ducts and mu class GST is predominantly present in the islets of Langerhans. Microsomal GST is variably present and shows no constant pattern of distribution. Pancreatic adenocarcinoma strongly expresses pi class GST but the other isoenzymes are generally absent.[14]

The explanation for the heterogeneity of distribution of the various GST isoenzymes within and between tissues is unclear, but one possible reason is that they subserve different functions in different tissues, sometimes acting as carrier proteins rather than being metabolically active. The overexpression of certain isoenzymes, principally pi class GST in intestinal and pancreatic tumours and alpha class GST in hepatocellular carcinoma, may simply represent clonal expansion of those cells expressing these isoenzymes. It is particularly noteworthy that in pancreatic carcinoma, which is believed to arise either from ductular or acinar cells,[15] the pi class and not alpha class GST is strongly expressed. Recent evidence by Pour[16] suggests that the centroacinar cells may give rise to the pseudoductular complexes which are believed to be preneoplastic lesions. This distribution of GST isoenzymes would support such a hypothesis.

Nomenclatures for human GST[6]

GST isoenzyme	*Family*	*Subunit (mol wt)*	*Isoelectric point*
$B_1 B_1$ ε	alpha	25 900	8·9
$B_1 B_2$ δ	alpha	25 900	8·75
$B_2 B_2$ γ	alpha	25 900	8·4
μ	mu	26 700	6·1
ψ	mu	26 600	5·5
π	pi	24 800	4·8
Microsomal	–	17 300	Not determined

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Accepted for publication
10 September 1990

An alternative explanation for the particular expression of GST in tumours is that selective overexpression of certain GST isoenzymes confers replicative advantage to dedifferentiated cells. Cellular drug resistance is commonly accompanied by increases in GST and in the drug-binding membrane-bound P-glycoprotein, and a decrease in cytochrome P450.[17] Many parallels between acquired drug resistance and preneoplasia exist and support the selective advantage hypothesis.

GST measurements in plasma as markers of disease

The GST isoenzymes catalyse the conjugation of glutathione to a wide range of compounds of which 1-chloro-2-4-dinitrobenzene is most frequently used as the substrate to measure the activity of human GST.[18] There are, however, several problems associated with activity measurements. Firstly, normal plasma activity is low and a precise measurement of activity is difficult to achieve.[19] Secondly, the GST binds a number of anions such as bile salts and bilirubin which inhibit enzymic activity.[20] In liver disease, when high plasma concentrations of these anions occur the activity of the GST released from the hepatocyte into plasma is inhibited thus decreasing the clinical sensitivity of the test. Another major problem with activity measurements is their poor organ specificity because the various substrates do not allow the isoenzymes of GST to be differentiated. Because of these problems assays of plasma or serum GST based on activity measurements have proved to be of little clinical value.[19 21] Immunoassays have been described that allow the precise and specific measurement of each of the three cytosolic classes of GST[22-26] and also the measurement of individual GST subunits within a class such as the B_1 and B_2 subunits which comprise the alpha class GST.[22 27] Immunoassay measurements measure GST concentrations even when they are enzymatically inactive because of the presence of inhibitors such as bilirubin or bile salts. Using these immunoassays it has become apparent that GST measurements may have a clinical role in the diagnosis and monitoring of patients with hepatobiliary disease and malignancy.[28] At present the only methodological problem relates to the speed of the assay which takes at least 24 hours. The use, however, of labelled antibody rather than labelled antigen assays should overcome this problem and enable results to be provided in less than three hours.

GST in liver damage

The alpha class GST, which comprise the immunologically distinct subunits B_1 and B_2, constitute as much as 3% of cytosolic protein in the hepatocyte. The subunits are distributed uniformly across the liver lobule; this contrasts with the aminotransferases which are found predominantly in the periportal hepatocytes.[29] The physicochemical properties and hepatic distribution of the B_1 and B_2 subunits mean that they are released quickly and in large quantities from damaged hepatocytes into the plasma. The plasma half life of the B_1 and B_2 subunits is less than 60 minutes; this contrasts with alanine aminotransferase whch has a half life of approximately 48 hours.[30] These characteristics of the B_1 and B_2 subunits indicate that their measurement in plasma should provide an early and sensitive measure of hepatocellular damage even when only centrilobular hepatocytes are involved. The short half life of the GST should also allow early recognition of when active liver damage has stopped, and measurements of the B_1 and B_2 subunits might therefore provide a useful means of investigating mechanisms of hepatotoxicity.[30 31]

Homogenates of normal human liver obtained at necropsy contain extremely low concentrations of pi class GST[32] and immunohistochemical examination has shown that this GST is confined to the cells of the biliary epithelium.[13 33] In alcoholic liver disease, however, sinusoidal macrophages, Kupffer cells, and hepatocytes express GST-pi.[34] There are also data to suggest that biliary epithelial cells secrete GST-pi into bile possibly as a mechanism to export potentially harmful toxins from the cell.[11]

ACUTE LIVER DAMAGE

(i) Paracetamol poisoning

After paracetamol poisoning abnormal plasma concentrations of GST B_1 subunits (B_1) are seen in approximately 90% of patients while alanine aminotransferase becomes abnormal in about 40%. In addition, abnormal concentrations of B_1 are observed within four hours of paracetamol ingestion whereas abnormalities in alanine aminotransferase are not recognised for at least 12 hours.[30 35] The results of plasma B_1 measurements show two distinct phases of hepatotoxicity in paracetamol poisoning.[30] The early phase occurs between four and 12 hours after the overdose and is characterised by increasing B_1 concentrations; this phase may be reversed by giving intravenous N-acetylcysteine. The second phase of hepatotoxicity occurs up to 24–36 hours after overdose when concentrations of B_1 plateau at approximately 100 times the upper reference interval. In this second phase of hepatotoxicity the hepatocytes are damaged irreversibly and ultimately hepatic necrosis occurs. The measurement of plasma B_1, on admission, may provide a good indication of which patients will develop subsequent liver damage.[35]

(ii) Birth asphyxia

Early evidence of liver damage after birth asphyxia is provided by plasma B_1 measurements.[36] In a study of 14 neonates with a clinical diagnosis of birth asphyxia abnormal plasma concentrations of B_1 were found within six hours of asphyxia in 11 whereas only seven had abnormal alanine aminotransferase activities at this time. In plasma sampled 24 hours after birth, values for alanine aminotransferase were abnormal in 10 neonates while B_1 remained abnormal in only six. The same study also showed that plasma B_1 concentrations were greater in normal infants than in adults despite

the fact that the concentration of the B_1 subunit in neonatal liver is significantly lower than in the adult. These data suggest that during the first few days of life there may be impaired hepatocellular integrity particularly centrilobular cells.

Although measurement of plasma B_1 or B_2 subunits in the adult seems to be equally sensitive in detecting impaired hepatocellular integrity, in the neonate B_2 measurements are of no value since this subunit seems to be poorly expressed in developing liver.[36]

(iii) Ethanol ingestion

We have recently shown that plasma concentration of B_1 increased in heavy drinkers 60 minutes after 80 g of ethanol had been ingested over a 30 minute period. No increase in B_1 was observed when healthy volunteers, with a modest alcohol intake, were given the same acute alcohol load. Although as a group the heavy drinkers produced a rise in B_1 after alcohol ingestion, some showed no significant change in B_1.[37] These observations suggest that the measurement of plasma B_1 in response to an alcohol load may be a predictor of the sensitivity of an individual to alcohol induced liver damage.

(iv) Halothane anaesthesia

Halothane anaesthesia may be followed by unexplained hepatitis that may be mild or severe. The more severe form (type 2) has a 20–50% mortality but the nature of the mechanism and predisposing factors that relate to type 2 halothane hepatitis remain unclear, although hypersensitivity and familial constitutional susceptibility have been implicated.

The mild type 1 form of halothane hepatitis is associated with moderate increases in the transaminases. Production of toxic metabolites of halothane after a combination of reductive biotransformation of halothane and hepatic hypoxia have been implicated in halothane induced hepatotoxicity. Enflurane and isoflurane are associated with less severe hepatic problems than halothane.[38–40]

Three hours after halothane anaesthesia small but significant increases in plasma B_1 concentration occur in approximately half of all patients which resolve after 12 hours. In approximately 10% of patients a secondary rise in plasma B_1 occurs 24 hours after anaesthesia.[41] In contrast, isoflurane produces no significant change in plasma B_1 concentrations at three hours and the frequency of abnormal plasma B_1 concentrations at 24 hours is lower than for halothane.[42] It seems likely that the increase in plasma B_1 concentrations three hours after halothane results from diminished hepatic blood flow, while it is possible that the secondary rise at 24 hours may be due to production of toxic halothane metabolites.[41 42]

Although changes in plasma B_1 concentrations may be seen after halothane anaesthesia, these studies have not shown any clinical value for measurement of the GST B_1 subunit as a predictor of patients who will subsequently develop clinical hepatic dysfunction.

CHRONIC LIVER DISEASE

(i) Chronic active hepatitis

An early study showed that all patients with chronic active hepatitis had raised serum concentrations of alpha class GST; serum GST concentrations correlated significantly with the activity of the disease assessed by histology, whereas aminotransferase activities showed no such correlation.[31] We have recently produced evidence to suggest that serum B_1 may be of value in monitoring the effectiveness of treatment in patients receiving immunosuppressive drugs for autoimmune chronic active hepatitis. We studied 22 patients and found that serum B_1 concentrations were raised in 16 patients, whereas aspartate aminotransferase activities were abnormal in only six of the patients. This work led us to suggest that perhaps the aim of immunosuppressive treatment should be to normalise serum B_1 rather than aminotransferase levels.[43]

(ii) Cirrhosis

Raised serum concentrations of B_1 occur in more than half of patients with cirrhosis.[31 44] In a study of 54 patients with biopsy proved alcoholic cirrhosis, abnormal levels of the aminotransferases and B_1 subunits were found in 28 patients, but these two measurements seemed to identify different populations.[44]

An explanation for the observation of an abnormal B_1 concentration in the presence of a normal aspartate aminotransferase is that B_1 measurements are probably more sensitive at detecting alcohol induced centrolobular damage. The explanation for the converse pattern of results – that is, normal B_1 with raised aspartate aminotransferase – found in nine patients is less clear but this may be due to a change in GST expression from alpha class to pi class GST in the hepatocyte.[34]

(iii) Thyrotoxicosis and thyroxine replacement therapy

Patients with thyrotoxicosis often have abnormal plasma B_1 concentrations.[45] This is not surprising as before effective treatment for hyperthyroidism was introduced serious hepatobiliary complications were commonly associated with the disease.[46] The observation, however, that many patients who are receiving longterm thyroxine replacement therapy for hypothyroidism have raised plasma B_1 concentrations[47] has fuelled the controversy regarding the necessity to monitor the dose of thyroxine by biochemical tests.[48]

Patients who most commonly have abnormal plasma B_1 concentrations are those in whom plasma free thyroxine is raised and thyroid stimulating hormone suppressed to undetectable levels.[47] These observations have led to the suggestion that the aim of thyroxine replacement therapy should be to normalise plasma thyroid stimulating hormone as this reduces the prevalence of abnormal plasma B_1 concentrations.[49] Recent observations have complicated the issue for it seems that patients with spontaneous

hypothyroidism are more prone to develop impaired hepatocellular integrity when starting thyroxine replacement therapy than patients with radioiodine induced hypothyroidism.[50] The evidence for this is attributable to the observation that plasma B_1 concentrations increase significantly when the dosage of thyroxine is increased above 100 μg per day in patients with spontaneous hypothyroidism, while no significant change is found in patients with radioiodine induced hypothyroidism.

At present it is unknown whether overreplacement with thyroxine has any longterm deleterious effects on the liver because no retrospective or prospective studies have been reported. In animals, however, thyroid hormones increase the hepatotoxicity associated with administration of halogenated hydrocarbons such as halothane or carbon tetrachloride.[51 52] In part, the increased hepatotoxicity of these compounds in thyroid hormone treated animals may be the result of a diminished hepatic GST content because the GST may be involved in the metabolism and detoxification of these compounds.[53]

GST-mu and susceptibility to liver disease

The mu class GST have different substrate specificities than the alpha and pi class isoenzymes. A number of mu class GST occur but GST-mu is of particular interest as it is polymorphic and expressed in only approximately half of the normal population.[23 28 54 55] Harada *et al* showed that only 25% and 20%, respectively, of patients with alcoholic liver disease and liver carcinoma expressed GST-mu, whereas the enzyme was expressed in 94% of patients with chronic hepatitis.[56] This study, however, comprised a very small number of patients; a much larger study is necessary to confirm these findings.

GST measurements in malignancy

There is considerable interest in the association between pi class GST and malignancy after the discovery that increased expression of this enzyme occurs in many tumours.[32 57-59] In neoplasms of the lung, colon, and stomach the expression of GST-pi is increased approximately twofold when compared with matched normal tissue, but in kidney and liver no significant change in the expression of this isoenzyme occurs.[32]

The expression of GST-pi may vary with the oestrogen receptor status in breast cancer; the levels of both GST-pi RNA and the protein are significantly higher in oestrogen receptor positive tumours when compared with oestrogen receptor negative tumours.[60]

In contrast to GST-pi the expression of B_1 and B_2 subunits is suppressed in some tumours including stomach and kidney. In other tumour types, however, concentrations are unchanged with the exception of lung in which B_1 seems to show an increased expression.[32]

The polymorphic GST-mu and its relation to malignancy is important. It has been claimed that smokers who lack GST-mu are more susceptible to developing carcinoma of the lung compared with smokers who express the enzyme. This relation, however, only seems to hold true for adenocarcinoma of the lung.[55]

The changes in GST expression that occur in malignancy have resulted in the use of GST measurements as tumour markers.

GST-pi measurements in malignancy

PLASMA GST MEASUREMENTS

Two studies suggest that the serum concentrations of GST-pi are increased in a wide range of malignant growths including gastric, oesophageal, colonic, pancreatic, and hepatobiliary cancers. In contrast, few abnormalities in serum GST-pi are found in patients with lung or breast cancer.[25 26] Recently, however, a serious methodological problem has been identified with serum GST-pi measurements following the observation in healthy subjects that, in the clotting process, large quantities of GST-pi are released from platelets.[61] These findings cast doubt on the results from previous studies that used serum and show that only plasma should be used.

Using plasma obtained under strict sampling conditions, we have shown that plasma GST-pi concentrations are raised in patients with lung cancer, particularly in adenocarcinoma of the lung.[61] We have found that plasma GST-pi is raised in a high proportion of patients with gastrointestinal malignant growths, particularly if there are metastases (unpublished observation).

We conclude that serological measurements of GST-pi show promise as a tumour marker for hepatobiliary and gastrointestinal malignant tumours but earlier studies must be repeated using plasma. It is unlikely, however, that even with strict sampling conditions GST-pi measurements will find a wide role in diagnosis of malignant disease because the ubiquitous nature of the enzyme suggests that it may be raised in a variety of diseases and, indeed, raised concentrations are often found in benign liver disease.[25 26]

GST MEASUREMENTS IN BILE

Using radioimmunoassay it has been shown that GST-B_1 and GST-pi are present in bile. Increased concentrations of GST-pi have been reported in two patients with cholangiocarcinoma but not in one patient with pancreatic carcinoma.[11] Further studies are needed to show whether biliary GST measurements have any value in diagnosing biliary tract cancers.

Conclusion

Study of the distribution of GST isoenzymes in healthy tissue has shown pronounced heterogeneity. The reasons for this are unclear but probably relate to different biological functions of GST isoenzymes in different tissues. Changes in the tissue expression in diseased states may

relate to metabolic changes which occur with disease progression. Overexpression of GST in neoplasia may be causal, allowing replicative advantage, or casual, accompanying clonal expansion.

The use of radioimmunoassay has enabled different GST isoenzymes to be measured in body fluids. Particularly, in situations where acute liver damage occurs, assays for GST-B_1 show high sensitivity compared with transaminase estimation. The major limitation to its widespread use is the length of time needed for performance of the assay and until this is overcome it will remain primarily a research tool.

We thank Mrs Vanessa Campbell for typing this manuscript.

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