Glutathione S-transferases in alcoholic liver disease

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Abstract

There is already evidence in alcoholic liver disease, mostly from studies of morphology and cytokeratin distribution, that hepatocytes can undergo a variety of phenotypic changes. This study reports findings of immunohistochemistry using antibodies against members of the glutathione S-transferase supergene family of detoxification enzymes. Hepatocytes in severe alcoholic liver disease coexpressed both alpha and pi class glutathione S-transferase. This coexpression has been previously described only in human fetal liver and in chemically-induced preneoplastic foci in rat liver. The use of function associated markers should provide additional information in the investigation of liver disease.

Changes in drug metabolism, and in particular the expression of detoxification enzymes, often accompany liver disease, probably as a consequence of chemical stress. The best known instance is the induction of γ -glutamyltranspeptidase in alcoholic liver disease. Other enzyme systems may also be involved. For example, in the mouse, chronic feeding with ethanol results in a significant increase in hepatic glutathione S-transferase activity.¹ These enzymes represent a multigene family of enzymes which catalyse the conjugation of reduced glutathione with many electrophilic substances including drugs, herbicides, and carcinogens.²⁻⁵ Three classes of cytosolic glutathione S-transferase exist in mammals – these are called alpha, mu, and pi,³ and in man they were previously designated by their isoelectric values as basic, neutral, and acidic type glutathione S-transferase respectively. Each of these classes is distinct as judged by structural and functional criteria; they are believed to be encoded by different clusters of genes.⁶ An additional isoenzyme, which is membrane bound and called microsomal glutathione S-transferase, has recently been isolated from human liver.⁷

The different classes of glutathione S-transferase show a noticeable tissue specific expression and although this aspect has not been rigorously studied in man, these enzymes are potentially valuable as immunohistochemical markers.

Quantitatively, the alpha class glutathione S-transferase represents the predominant isoenzyme in adult liver, and this enzyme is present only in hepatocytes and in some cells in large bile ducts. By contrast, pi class glutathione S-transferase is only present in bile ducts.⁸ Little is known about the localisation of mu class glutathione S-transferase but its expression is variable, being absent in 45% of human livers.⁹ The distribution of the microsomal glutathione S-transferase in human tissues is unknown.¹⁰

Another feature of alcoholic liver disease is the acquisition by hepatocytes of a bile duct phenotype, as determined by the pattern of cytokeratin reactivity and the expression of tissue polypeptide antigen.¹¹⁻¹³ This may be associated with the morphological appearance of 'ductular metaplasia' of hepatocytes.

In this study the expression of glutathione S-transferase isoenzymes in alcoholic liver disease has been undertaken for several reasons. Firstly, to establish whether there is a change in hepatocyte phenotype, as defined by glutathione S-transferase isoenzyme expression. Secondly, to assess immunohistochemically whether differential induction of the various glutathione S-transferase classes is observed in alcoholic liver disease. Thirdly, to investigate whether people who lack mu class glutathione S-transferase are more likely to have developed liver disease.

Materials and methods

PATIENTS

Percutaneous needle liver biopsy specimens from 40 patients with a history of alcohol abuse were studied. Specimens showed a spectrum of alcoholic liver disease changes, ranging from steatosis through alcoholic hepatitis with perivenular fibrosis to micronodular cirrhosis.

Figure 1: Normal liver stained for alpha class glutathione S-transferase. Bile ducts did not stain (original magnification $\times 160$).

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Figure 2: Hepatocytes stained for mu class glutathione S-transferase in cirrhotic liver (original magnification $\times 160$).

Twenty six of the patients were men and 14 women; their ages ranged from 30–71 years. Six histologically normal needle biopsy specimens were also studied.

ANTIBODIES AGAINST GLUTATHIONE S-TRANSFERASE

The cytosolic alpha, mu, and pi class glutathione S-transferases and the microsomal isoenzyme were purified as previously described.^{6,7,9,14} The polyclonal antibodies that were used in the present study were raised in rabbits and found to be specific as assessed by Western blot analysis. The antibodies against the individual classes of cytosolic and microsomal glutathione S-transferase did not cross-react with any isoenzyme of a different class.

IMMUNOSTAINING

The method used has been described previously.¹⁵ Briefly, paraffin sections were dewaxed and incubated with rabbit antibody for one hour at a dilution of 1:200 after blocking endogenous

Figure 3: Liver with steatosis stained for microsomal glutathione S-transferase showing endothelial and periportal hepatocyte expression (original magnification $\times 320$).

Expression of pi class glutathione S-transferase (GST) in each morphological group

Group	Bile duct	Hepatocytes adjacent to fibrous tissue	Sinusoidal cells
Steatosis (n=9)	8*	2	8
Alcoholic hepatitis and fibrosis (n=15)	15	15	10
Cirrhosis (n=16)	16	14	4

*One specimen did not stain at all for GST pi.

peroxidase with methanol/hydrogen peroxide. Antibody binding was visualised using biotinylated anti-rabbit IgG (Dako) and an avidin-biotin detection system. This is a more sensitive and specific method than that used in our previous study.⁸ Slides were assessed by two independent observers.

Results

HISTOPATHOLOGY

Biopsy specimens were divided into three groups according to the nature of the alcohol induced injury. Nine patients showed steatosis with no appreciable fibrosis; 15 patients showed alcoholic hepatitis with varying degrees of pericellular and perivenular fibrosis; and 16 patients showed established micronodular cirrhosis.

IMMUNOHISTOCHEMISTRY

Alpha class. Each of the normal biopsy specimens showed strong staining of hepatocyte nuclei and cytoplasm (Fig 1). For all patients with alcoholic liver disease, antibodies to alpha

Figure 4: Liver with steatosis showing pi class glutathione S-transferase in bile ducts portal tract, inflammatory cells, and sinusoidal lining cells (Kupffer cells) (original magnification $\times 320$).

Figure 5: Strong expression of pi class glutathione S-transferase in hepatocytes at the edge of a cirrhotic nodule and hepatocytes entrapped within fibrous tissue. Sinusoidal cells are also stained (original magnification $\times 160$).

class glutathione S-transferase showed uniform strong staining of hepatocytes; both cytoplasm and some nuclei. Bile ducts and sinusoidal cells were not stained.

Mu class. The anti-mu class glutathione S-transferase antisera gave variable and often weak staining of hepatocytes (Fig 2) similar to that seen in normal liver.⁸ In 14 specimens no definite staining was identifiable – six of nine with steatosis, six of 15 with alcoholic hepatitis and fibrosis, and two of 16 with cirrhosis. The distribution of nulled cases was not significant ($p > 0.1$, χ^2 test).

Microsomal glutathione S-transferase. Normal liver gave weak granular cytoplasmic staining of hepatocytes. Biliary epithelium was not significantly stained. Hepatocyte staining was weak and variable with nine specimens of the 40 studied showing no identifiable staining at all. In seven specimens an increase in periportal staining was observed and endothelial staining was noted (Fig 3).

Pi class. In normal biopsy specimens there was uniform staining of bile ducts but hepatocytes were not stained. Results categorised according to morphological groups of alcoholic liver disease are summarised in the Table. All specimens but one showed bile duct staining (Fig 4). In most patients with steatosis only or alcoholic hepatitis, sinusoidal lining cells (probably Kupffer cells) stained for pi class glutathione S-transferase (Fig 4) but normal sinusoidal lining cells did not express this.

Where there was appreciable fibrosis or cirrhosis, hepatocytes were stained for both alpha and pi class glutathione S-transferases (Fig 5). Mallory's hyaline did not contain detectable amounts of any glutathione S-transferase isoenzyme. In some cirrhotic nodules all hepatocytes were found to express pi class glutathione S-transferase.

Discussion

In this study we have shown that hepatocytes in alcoholic liver disease may express both alpha and pi glutathione S-transferase. This is of interest as pi class glutathione S-transferase in the adult liver is restricted to bile ducts.^{8, 16} This is

consistent with reported changes which occur in the hepatocyte cytoskeleton.¹¹⁻¹³ Coexpression of glutathione S-transferases pi and alpha is found in fetal human liver before 24 weeks' gestation¹⁶ and is recognised as a step in the development of drug resistant preneoplastic nodules in chemically induced liver carcinoma in rats.¹⁷⁻¹⁹

The glutathione S-transferase enzymes have several roles besides that of drug detoxification, which may include nuclear RNA processing²⁰ and DNA repair after peroxide injury.²¹ Some of these functions may be compromised during alcoholic liver disease. In the plasma of patients with alcoholic cirrhosis, alpha class glutathione S-transferase activities are often raised, even when transaminases are normal,²² indicating chronic injurious stimulus of hepatocytes. This and an associated fall in glutathione available for conjugation reactions, can lead to defective detoxification of drugs such as paracetamol⁵ and a failure of the normal handling of a variety of carcinogens.²³ This may be relevant to the later development of hepatocellular carcinoma in a small proportion of patients with alcohol-induced cirrhosis.

The results of staining for mu class glutathione S-transferase are consistent with the finding that almost half the normal population fails to express this isoenzyme because of a genetic polymorphism.^{23, 24} Harada *et al*²⁵ have noted that liver biopsy samples from a Japanese population of patients with hepatitis and carcinoma were less likely to express mu class glutathione S-transferase. In our small series there was no statistically significant relation between severity of alcohol injury and mu class glutathione S-transferase status. To detect minor differences in mu expressor status when around half of individuals are nulled would require a very much larger study group. People who lack a glutathione S-transferase with high activity towards trans-stilbene oxide, now known to be a mu class enzyme, have an increased susceptibility to lung cancer.²⁶ These people possibly possess a more general susceptibility to chemical insult that may result in a proneness towards developing several types of liver disease.

Our study provides preliminary evidence that microsomal glutathione S-transferase is polymorphic in man. The basis of this and its biological as well as pathological consequences merit further study. We are now undertaking this work.

It is not clear how the de novo expression of pi class glutathione S-transferase in sinusoidal lining cells, presumably Kupffer cells, relates to other studies which have shown a decrease in lysozyme content, phagocytic ability, and absolute number of these cells.^{27, 28} Activated alveolar macrophages, however, also express glutathione S-transferase pi (unpublished observations). The assessment of hepatocyte differentiation by both cytoskeletal and functional associated phenotypic markers provides a valuable approach to the investigation of the liver's response to injury.

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