The metabolism of 7-ethoxycoumarin in human liver microsomes and the effect of primary biliary cirrhosis: implications for studies of drug metabolism in liver disease

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Using 7-ethoxycoumarin as a probe substrate, microsomal monooxygenase activity has been measured in liver tissue from patients with primary biliary cirrhosis (PBC) of varying histological severity, and in histologically normal control tissue. Interindividual variation in enzyme activity was considerable, and in no histological category was the activity significantly different to control. We conclude that: (a) in PBC, hepatic microsomal monooxygenase activity is determined primarily by factors other than the histological severity of the liver disease, and (b) studies of xenobiotic metabolism in patients with liver disease should specify the nature of the underlying disease process.

Keywords liver microsomes 7-ethoxycoumarin primary biliary cirrhosis

Introduction

The hepatic cytochrome P450 dependent microsomal monooxygenases are responsible for the biotransformation of a wide variety of drugs and other xenobiotics. Using probe substrates, the *in vitro* activities of these enzymes have been shown to be impaired in liver tissue from patients with alcoholic cirrhosis (Woodhouse *et al.*, 1983, 1984) and in heterogeneous groups of subjects with liver disease of mixed aetiology (Brodie *et al.*, 1981; Farrell *et al.*, 1979). The interpretation of such findings is often rendered difficult by the variable nature of the liver disease, and by concomitant ethanol exposure and drug intake.

Primary biliary cirrhosis (PBC) is an autoimmune liver disorder in which liver histology (see MacSween, 1979) may reveal no fibrosis (stages I and II), fibrosis not amounting to cirrhosis (stage III) or established cirrhosis (stage IV). At this stage the histological appearances of

the liver may be indistinguishable from cirrhosis secondary to other causes including ethanol. Patients with PBC frequently avoid ethanol and many receive no drugs which are known to modify xenobiotic metabolism *in vitro*. In an attempt to evaluate the effect of disease severity (judged histologically), alcohol exposure, and the underlying disease process, on monooxygenase activity in patients with liver disease, we have examined microsomal monooxygenase activity in liver from patients with PBC of varying histological stage, and in control tissue, using the *O*-de-ethylation of 7-ethoxycoumarin as a biochemical probe. This compound was chosen as a measure of monooxygenase activity firstly because the metabolite, 7-hydroxycoumarin is highly fluorescent, and the assay is sufficiently sensitive for use with tiny liver samples, and secondly because we have previously under-

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taken extensive studies of the metabolism of this agent in alcoholic cirrhosis (Woodhouse *et al.*, 1984).

Methods

Patients

Liver was obtained from patients undergoing diagnostic percutaneous Menghini needle biopsy, using tissue surplus to histological requirements. Control tissue was obtained either from patients who underwent liver biopsy during diagnostic workup and who were subsequently shown to have normal histology, or from liver biopsy taken at cholecystectomy in individuals with normal liver function tests and stones only in the gallbladder. Ethical approval for this procedure had been granted.

No patient in the control group was receiving drugs known to interfere with microsomal monooxygenase activity, and only two PBC subjects (both in the stage IV subgroup) were receiving agents which may modify xenobiotic metabolism as measured *in vitro*; both were taking spironolactone. No patient was encephalopathic or had signs or symptoms of gastrointestinal bleeding at the time of biopsy. Details of patients studied are given in Table 1.

Storage of specimens

Biopsy specimens were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Assay of 7-ethoxycoumarin O-de-ethylation

Biopsies were homogenised and microsomal fractions prepared as previously described (Woodhouse *et al.*, 1983). Enzyme activity was determined by measuring the rate of formation of 7-hydroxycoumarin from the substrate, 7-ethoxycoumarin (Greenlee & Poland, 1978). A substrate concentration of $1000\text{ }\mu\text{M}$ was used. Activity (EOC) was expressed as pmol 7-hydroxycoumarin formed/mg microsomal protein/minute ($\text{pmol mg}^{-1}\text{ min}^{-1}$).

Protein assay

Microsomal protein concentrations were measured by the method of Lowry *et al.* (1951).

Statistical analysis

Results are expressed as mean $\pm$ s.e. mean. The significance of differences between means was assessed by both Student's *t*-test for unpaired values and the Mann-Whitney U-test.

Results

Increasing severity of liver disease was reflected by more marked derangement of liver function tests (Table 1). However, mean serum albumin was significantly less than control only in the Stage IV PBC group ($P < 0.01$).

Mean EOC activity (Figure 1) was $111 \pm 9\text{ pmol mg}^{-1}\text{ min}^{-1}$ in controls ($n = 10$); $125 \pm 16\text{ pmol mg}^{-1}\text{ min}^{-1}$ in subjects with Stage I and II

Table 1 Details of the patients studied

Histology (Number)	Male: Female	Mean age (years)	Smokers: non- smokers	Mean ethanol consumption (units/day)	Liver function tests			
					Serum albumin (g/l)	Serum bilirubin ($\mu\text{mol/l}$)	Serum AST (iu/l)	Serum alkaline phosphatase (iu/l)
Control ($n = 10$)	3:7	50 ± 4	4:6	0.6 ± 0.3	42.3 ± 0.8	12.6 ± 3.4	27.6 ± 2.0	103.5 ± 13.9
PBC								
Stage I and II ($n = 10$)	2:8	56 ± 4	4:6	0.2 ± 0.1	42.1 ± 0.6	16.8 ± 5.3	42.3 ± 5.7	406.4 ± 136.9
PBC								
Stage III ($n = 10$)	0:10	59 ± 3	7:3	Nil	40.4 ± 2.2	18.4 ± 4.3	69.5 ± 8.0	876.0 ± 91.2
PBC								
Stage IV ($n = 9$)	2:7	60 ± 1	2:7	0.2 ± 0.2	37.7 ± 1.2	21.0 ± 4.7	69.0 ± 10.6	558.8 ± 92.9

1 unit of ethanol = 1 measure of spirits or equivalent
AST = aspartate aminotransferase

Figure 1 7-ethoxycoumarin *O*-de-ethylase activity (EOC) in liver microsomes from subjects with primary biliary cirrhosis (PBC) of differing histological grade.

Patients marked * were receiving potential enzyme inducers.

disease ($n = 10$); 94 ± 9 pmol mg⁻¹ min⁻¹ in patients with Stage III PBC ($n = 10$); and 86 ± 16 in those with fully established cirrhosis (Stage IV) ($n = 9$). In no group was EOC activity significantly different to control. Variation between individuals within groups was considerable.

Discussion

Several features of the results presented here are of particular interest. There was a three fold variation in enzyme activity in control subjects, and this was even greater in some of the disease groups. We have previously noted similar variation in both normal liver and in alcoholic cirrhotics, using both 7-ethoxycoumarin and aldrin as probe substrates (Woodhouse *et al.*, 1984, 1983), and Boobis *et al.* (1980) found that aryl hydrocarbon hydroxylase activity in human liver biopsies varied more than three fold. The main finding of the study, however, is that the histologically graded severity of the liver disease appears to be a relatively minor determinant of 7-ethoxycoumarin *O*-de-ethylation in PBC. There was a trend for activity to be lower in the Grade IV patients, but this was not statistically significant. It seems, therefore, that whilst it is not possible to exclude a small effect of disease status, judged histologically, on monooxygenase

activity in liver from these patients (because of wide interindividual variation within groups), it is likely that other factors (genetic or environmental) predominate.

These findings contrast with our earlier study in alcoholic cirrhotic liver, where activity was considerably lower than controls (61 ± 12 pmol mg⁻¹ min⁻¹ vs 111 ± 9 pmol mg⁻¹ min⁻¹) (Woodhouse *et al.*, 1984). These apparent differences between PBC and alcoholic cirrhosis may be related to a number of factors:

First, the ethanol consumption of the PBC patients was minimal (see Table 1), whereas the alcoholic cirrhotics in our earlier investigations were consuming significant quantities of ethanol up to the time of biopsy; in most instances 60 g ethanol/day or more. Chronic ethanol exposure has been shown in man to cause a reduction in the activity of at least one monooxygenase, aryl hydrocarbon hydroxylase, and this may be unrelated to the degree of liver damage (Brodie *et al.*, 1981). Acute ethanol consumption has also been shown to impair microsomal oxidation both in normal subjects and people with liver disease (McKay *et al.*, 1982). It is therefore possible that in alcoholic cirrhotics, lower monooxygenase activity may be partly related to continued alcoholism rather than liver disease alone. This effect would not be seen in the abstaining PBC patients studied in the investigations described here.

Second, despite damage to portal tracts and intrahepatic bile ducts, the majority of hepatocytes in PBC patients frequently look relatively normal histologically even in severe disease, whereas in alcoholic cirrhosis cells often appear 'sick' and this damage is more homogeneous within liver lobules (MacSween, 1983). It may be postulated that cell necrosis or continuing immunological attack are important determinants of monooxygenase activity in diseased liver.

The findings presented here may be of importance since studies of *in vivo* and *in vitro* drug

and xenobiotic metabolism in patients with hepatic disorders are frequently in very heterogeneous groups of patients (Farrell *et al.*, 1979; Boobis *et al.*, 1980; Brodie *et al.*, 1981). In some other studies the underlying pathological process is not even specified. Our investigations suggest that it would be appropriate to specify the underlying disease process in order to facilitate complete interpretation of results.

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