THE ACUTE EFFECTS OF ETHANOL ON ACETANILIDE DISPOSITION IN NORMAL SUBJECTS, AND IN PATIENTS WITH LIVER DISEASE

J. McKAY, M.D. RAWLINGS, I. COBDEN & O.F.W. JAMES

Wolfson Unit of Clinical Pharmacology, and Department of Medicine (Geriatrics), The University, Newcastle upon Tyne.

- 1 The effects of single doses (25 g and 50 g) oral ethanol on the disposition of acetanilide (50 mg/kg metabolic active mass) has been studied in normal subjects, and in patients with chronic non-alcoholic liver disease.
- 2 In normal subjects, ethanol produced a dose-dependent increase in acetanilide half-life, and a decrease in acetanilide clearance. There was a significant correlation ($r_s = 0.71$, $P < 0.01$) between the 90 min blood ethanol concentration and the reduction in acetanilide clearance.
- 3 In patients with liver disease, ethanol produced a similar proportional change in acetanilide half-life and clearance, but these were less consistent. Moreover, liver disease itself was associated with an increase in acetanilide half-life, and a reduction in clearance.
- 4 It is concluded that single oral doses of ethanol, comparable to those consumed during social drinking, may inhibit some forms of microsomal oxidation and thus have important clinical implications.

Introduction

Chronic consumption of ethanol in both animals and man, is known to induce microsomal monooxygenase activity in liver (Misra *et al.*, 1971; Lieber, 1973; Iber, 1977) as well as in lung and small intestine (Seitz *et al.*, 1981). In the absence of alcoholic liver disease, this leads to accelerated clearance of some drugs whose elimination is dependent on microsomal oxidation. By contrast, the acute administration of ethanol in rats inhibits the microsomal oxidation of a variety of substances both *in vitro* and *in vivo* (Rubin *et al.*, 1970; Powis, 1975; Powis & Grant, 1976), whilst in man the sustained administration of ethanol over 24 h is associated with the increased half-lives of meprobamate and pentobarbitone (Rubin *et al.*, 1970). It is less certain, however, whether single doses of ethanol, comparable to those consumed during social drinking, influence microsomal drug oxidation in normal subjects and in individuals with liver disease.

Methods

(a) Subjects and patients

Ten healthy volunteers (aged 20–62 years) were studied. All had normal conventional liver function

tests (serum bilirubin, alkaline phosphatase, aspartate aminotransferase, albumin); none had a history of liver disease or were taking drugs.

Ten patients with liver disease (aged 21–64 years) were also studied. All had chronic, compensated, non-alcoholic liver disease confirmed by conventional liver function tests and liver biopsy. The diagnoses were primary biliary cirrhosis (four patients), chronic active hepatitis with cirrhosis (two patients), secondary biliary cirrhosis, chronic persistent hepatitis, cryptogenic cirrhosis, and arsenical hepatic fibrosis (one patient each). No patient was taking drugs known to influence microsomal oxidation. Informed consent was obtained from each patient before the study, which had achieved prior approval from the Newcastle AHA (T) Ethical Committee.

(b) Procedure

Each subject or patient received a single oral dose (50 mg/kg metabolically active mass) of acetanilide, prepared in a gelatin capsule without excipient, on two or three occasions after an overnight fast. All subjects received acetanilide alone (as a control), and also 1.5 h prior to the ingestion of 75 ml or 150 ml vodka (in orange cordial) containing 25 g or 50 g

ethanol respectively. The order in which studies were carried out was randomised, and at least 3 days were allowed to elapse between investigations. Some subjects and patients received both ethanol doses, as well as acetanilide alone.

Venous blood was withdrawn, using an indwelling catheter inserted into an antecubital vein, at hourly intervals, for 8 h after the administration of acetanilide. Plasma was separated by centrifugation and stored at -20°C until analysis, in duplicate, for acetanilide concentrations by gas liquid chromatography (Buchanan *et al.*, 1980). Additional blood samples were obtained at 30, 60, 90 and 120 min after the administration of ethanol and ethanol concentrations in these were measured by gas liquid chromatography. For both plasma acetanilide and blood ethanol concentration measurements, the variation between duplicate samples was less than 5%.

(c) Calculations

Acetanilide half-lives were calculated from the terminal mono-exponential decay of plasma acetanilide concentration by least squares regression analysis. The area under the plasma acetanilide concentration-time curve (AUC) was determined by the trapezoidal rule and extrapolated to infinity. Clearance was calculated thus:

$$\text{Acetanilide clearance} = \frac{\text{dose}}{\text{AUC}}$$

Because control values of acetanilide clearance and half-life differed markedly between individuals, the effects of ethanol on these variables have been expressed as percentage deviations from the observations made in the absence of ethanol.

Ethanol elimination rates ($\text{mg l}^{-1} \text{h}^{-1}$) were calculated by least squares regression analysis from the slope of the rectilinear decay of blood ethanol concentrations *v* time.

Since a normal distribution could not be assumed, results are reported as medians and (in parentheses) ranges. Wilcoxon's matched-pairs signed rank test

was used to analyse the significance of differences within groups, and the Mann-Whitney U test to compare the significance of differences between groups. Correlation was assessed by Spearman's rank test (r_s).

Results

(a) Normal subjects

In the absence of ethanol, peak plasma concentrations of acetanilide were observed 1–2 h after dosing. Thereafter plasma concentrations declined mono-exponentially with a median half-life of 1.3 h (1.1–1.7 h). Median acetanilide clearance was 36 l/h (25–65 l/h).

Eight subjects received 25 g ethanol 1.5 h after the administration of acetanilide, and seven subjects received 50 g ethanol. Peak blood ethanol concentrations had occurred by 1.5 h in all subjects: at this time median blood ethanol concentrations were 320 mg/l (80–460 mg/l) after 25 g ethanol, and 740 mg/l (630–910 mg/l) after 50 g ethanol. Thereafter, blood ethanol concentrations declined linearly with time at a median elimination rate of $150 \text{ mg l}^{-1} \text{h}^{-1}$ (110–190 $\text{mg l}^{-1} \text{h}^{-1}$).

After 25 g ethanol (Table 1) the median percentage increase ($P < 0.01$) in half-life was 29%, whilst after 50 g ethanol the median percentage increase ($P < 0.01$) was 45%. The prolongation of acetanilide half-life was significantly longer ($P < 0.05$) after 50 g ethanol than 25 g ethanol.

Median acetanilide clearance (Table 1) after 25 g ethanol decreased by 15% ($P < 0.02$) of control values, and by 34% ($P < 0.02$) after 50 g ethanol. The reduction in acetanilide clearance after 50 g ethanol was significantly ($P < 0.05$) greater than after 25 g ethanol. Furthermore, after combining the results of the 25 g and 50 g ethanol doses there was a highly significant ($r_s = 0.71$, $P < 0.01$) correlation between the blood ethanol concentration, and the percentage reduction in acetanilide clearance (Figure 1).

Table 1 Percentage changes (median and range) in acetanilide half-life ($t_{1/2}$) and clearance (CL) after 25 g and 50 g oral ethanol.

	25 g ethanol		50 g ethanol	
	$t_{1/2}$	CL	$t_{1/2}$	CL
Normals	+ 29%*** (2–49%)	– 15%** (2–36%)	+ 45%*** (19–74%)	– 34%** (17–42%)
Liver disease	+ 7% (–21 – +46%)	– 11%* (1–32%)	+ 50%* (17–98%)	– 13%* (8–40%)

*** $P < 0.01$, ** $P < 0.02$, * $P < 0.05$

Figure 1 90 min blood ethanol concentration *v* acetanilide clearance (% control test value) in 15 control subjects (○ 75 ml vodka test, ● 150 ml vodka test).

(b) Patients with liver disease

In the absence of ethanol, peak plasma acetanilide concentrations were again observed 1–2 h after acetanilide administration. The median elimination half-life of 2.2 h (1.2–4.0 h) in the 10 patients with liver disease was significantly ($P < 0.01$) greater than in the normal subjects. Median acetanilide clearance (16.1/h, range 8–37 1/h) was significantly ($P < 0.01$) reduced in the patients with liver disease as compared to normals.

Peak blood ethanol concentrations were observed by 1.5 h in all liver disease patients, after ethanol administration. After 25 g (six patients), and 50 g (five patients) ethanol median blood ethanol concentrations were 320 mg/l (200–580 mg/l) at this time, and 900 mg/l (880–1250 mg/l) respectively. Median blood ethanol elimination rate was 200 mg l⁻¹ h⁻¹ (160–220 mg l⁻¹ h⁻¹) which was significantly ($P < 0.05$) greater than in normals.

After 25 g ethanol (Table 1) acetanilide half-life was prolonged in only three patients and the median percentage increase of 7% was not significant. After 50 g ethanol, acetanilide half-life increased in all patients by a median of 50%. Median acetanilide clearance fell ($P < 0.05$) by 11% after 25 g ethanol, and by 13% after 50 g ethanol.

The changes in half-life and clearance after the two doses of ethanol were not significantly different from one another, and there was no significant correlation between blood ethanol concentration and changes in acetanilide clearance.

Discussion

Acetanilide was used as a 'model' substrate in this investigation because it is metabolised predominantly (80%) by microsomal oxidation to 4-hydroxyacetanilide (Brodie & McLeod, 1948), and its elimination is independent of hepatic blood flow (McKay, 1979). Moreover, its short half-life (Farah *et al.*, 1977; Cunningham *et al.*, 1974) renders it particularly suitable for examining acute effects such as the influence of single doses of alcohol. In order to prevent interference with the absorption of acetanilide, ethanol was administered 90 min later.

Acetanilide half-lives in our healthy subjects were similar to those previously reported (Cunningham *et al.*, 1974; Farah *et al.*, 1977). In our patients with liver disease, however, the increase in half-life and decrease in clearance suggests considerable impairment of acetanilide elimination. This observation is entirely compatible with the many previous studies showing a reduced rate of microsomal drug oxidation in liver disease (Branch *et al.*, 1973; Sotaniemi *et al.*, 1977). By contrast, we observed a more rapid rate of alcohol elimination in patients with liver disease than in controls. Although the magnitude of the difference is surprising there are at least two possible explanations. First, when expressed as mg l⁻¹ h⁻¹ alcohol elimination rate will be partly determined by its distribution volume which may be altered in liver disease. Secondly, Jenkins & Peters (1978) observed unchanged activities of malate dehydrogenase and aspartate aminotransferase in hepatic mitochondria from patients with liver disease. These enzymes are responsible for the reoxidation of NADPH to NAD, and it is this availability of the latter which is probably rate-limiting for alcohol dehydrogenase. Our findings are compatible with those of Lieberman (1963) who described normal rates of alcohol elimination in patients with acute viral hepatitis and in non-icteric cirrhotics; impaired alcohol elimination was only observed in cirrhotics who were overtly jaundiced.

The present study shows that 25 g and 50 g oral ethanol produces a dose and concentration dependent increase of acetanilide half-life and decrease of acetanilide clearance in normal subjects. These changes, together, indicate that ethanol impairs the microsomal oxidation of acetanilide. However, the magnitude of the observed changes in clearance attributable to ethanol probably underestimate the real change because we calculated clearance from the complete AUC, whereas ethanol

administration was delayed for 90 min after dosing with acetanilide.

Ethanol binds *in vitro* to hepatic microsomal cytochrome P450 from rats, producing a modified type II spectrum (Schenkman *et al.*, 1967). Cohen & Mannering (1972) showed that in rat isolated liver microsomes, ethanol inhibits acetanilide hydroxylation by 50% at a concentration of 1000 mg/l. The magnitude of the interaction between ethanol and acetanilide is thus comparable to that we have observed in man, since we find a median increase in acetanilide half-life of 45% at a median blood ethanol concentration of 740 mg/l.

The effects of ethanol on acetanilide half-life and clearance in patients with chronic liver disease parallel those observed in volunteers. However, the changes were less consistent, particularly with the lower (25 g) ethanol dose; and although the

magnitude of the effect of 50 g ethanol was proportionately similar to that in normal subjects, the actual change was greater since the patients control acetanilide kinetics (in the absence of ethanol) showed evidence of significant impairment of elimination. Patients with chronic liver disease were chosen in preference to patients with alcoholic liver disease, not only on ethical grounds, but in order to obviate difficulties of interpretation in patients who might still be drinking.

We conclude therefore that single doses of ethanol—in quantities that are consumed during 'social' drinking—inhibit the microsomal oxidation of acetanilide both in normal subjects and in patients with chronic liver disease. This may have considerable therapeutic implications for patients taking a wide variety of drugs, who may also consume alcoholic beverages from time to time.

References

- BRANCH, R.A., HERBERT, C.M. & READ, A.E. (1973). Determination of serum antipyrine half-lives in patients with liver disease. *Gut*, **14**, 569–573.
- BRODIE, B.B. & McLEOD, J. (1948). The fate of acetanilide in man. *J. Pharmac. exp. Ther.*, **94**, 29–38.
- BUCHANAN, N., DAVIS, M.D., HENDERSON, D.B., MUCKLOW, J.C. & RAWLINS, M.D. (1980). Acetanilide pharmacokinetics in Kwashiorkor. *Br. J. clin. Pharmac.*, **9**, 525–526.
- COHEN, G.M. & MANNERING, G.J. (1972). Involvement of a hydrophobic site in the inhibition of microsomal *p*-hydroxylation of aniline by alcohols. *Mol. Pharmac.*, **9**, 383–397.
- CUNNINGHAM, J.L., BULLER, B.F. & EVANS, D.A.P. (1974). Pharmacokinetics of acetanilide and diphenylhydantoin sodium. *Eur. J. clin. Pharmac.*, **7**, 461–466.
- FARAH, F., TAYLOR, W., RAWLINS, M.D. & JAMES, O. (1977). Hepatic drug acetylation and oxidation: effects of ageing in man. *Br. med. J.*, **2**, 155–156.
- IBER, G.L. (1977). Drug metabolism in heavy consumers of alcohol. *Clin. Pharmac. Ther.*, **22**, 735–741.
- JENKINS, W.J. & PETERS, T.J. (1978). Mitochondrial enzyme activities in liver biopsies from patients with alcoholic liver disease. *Gut*, **19**, 341–344.
- LIEBER, C.S. (1973). Hepatic and metabolic effects of alcohol (1966–1973). *Gastroenterology*, **65**, 821–846.
- LIEBERMAN, F.C. (1963). The effect of liver disease on the rate of ethanol metabolism in man. *Gastroenterology*, **44**, 261–266.
- McKAY, J. (1979). *The disposition of acetanilide in normals and patients with chronic liver disease and the interaction with ethanol*. Thesis submitted for the degree of Batchelor of Medical Science, University of Newcastle upon Tyne.
- MISRA, P.S., LEFEVRE, A., ISHII, H., ROBIN, E. & LIEBER, C.S. (1971). Increase in ethanol meprobamate and pentobarbital metabolism after chronic ethanol administration in patients. *Am. J. Med.*, **51**, 346–351.
- POWIS, G. (1975). The effect of a single oral dose of methanol, ethanol and propan-2-ol on the hepatic microsomal metabolism of foreign compounds in the rat. *Biochem. J.*, **148**, 269–277.
- POWIS, G. & GRANT, L. (1976). The effect of inhibition of alcohol metabolism upon the changes in the hepatic microsomal metabolism of foreign compounds produced by the acute administration of some alcohols to the rat. *Biochem. Pharmac.*, **25**, 2197–2201.
- RUBIN, E., GANG, H. & LIEBER, C.S. (1970). Interaction of ethanol and pyrazole with hepatic microsomes. *Biochem. Biophys. Res. Comm.*, **42**, 1–8.
- SCHENKMAN, J.B., REMMER, H. & ESTABROOK, R.H. (1967). Spectral studies of drug interactions with hepatic microsomal cytochrome. *Mol. Pharmac.*, **3**, 113–120.
- SEITZ, H.K., GARRO, A.J. & LIEBER, C.S. (1981). Enhanced pulmonary and intestinal activation of procarcinogens and mutagens after chronic ethanol consumption in the rat. *Eur. J. clin. Invest.*, **11**, 33–38.
- SOTANIEMI, E.A., AHLQVIST, J., PELKONEN, R.O., PIIRTTIAHO, H. & CUOMA, P.U. (1977). Histological changes in the liver and indices of drug metabolism in alcoholics. *Eur. J. clin. Pharmac.*, **11**, 295–303.

(Received January 26, 1982,
accepted June 21, 1982)