Antipyrine clearance and metabolite formation in patients with alcoholic cirrhosis

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1 The effect of liver cirrhosis on plasma clearance and metabolite profile of i.v. administered antipyrine was studied in 23 patients with alcoholic liver cirrhosis (age 37–70 years) and 17 healthy subjects (age 28–55 years).

2 Liver volume was also measured and was found to be larger in patients than in controls, mean values being 1.86 and 1.36 l respectively.

3 The elimination half-life of antipyrine in patients with alcoholic liver cirrhosis was significantly longer than in the healthy subjects ($P < 0.001$). Mean values were 39.9 and 10.1 h respectively.

4 Alcoholic liver cirrhosis had no effect on the apparent volume of distribution of antipyrine, but antipyrine plasma clearance was substantially reduced in the patients. Mean clearance values (ranges) were 13.5 (9.3–22.8) ml/min in the patients and 49.3 (31.1–103) ml/min in healthy subjects.

5 Normalization of antipyrine plasma clearance for liver volume resulted in an only slightly increased distinction between patients and healthy subjects, mean values (ranges) being 7.8 (3.3–13.0) ml min⁻¹ l⁻¹ and 36.1 (21.9–35.9) ml min⁻¹ l⁻¹ respectively.

6 The cumulative renal excretion of 4-hydroxyantipyrine (OHA) and norantipyrine (NORA) was significantly lower in patients with alcoholic liver cirrhosis than in healthy subjects, as was the total recovery of antipyrine and major metabolites from urine. Mean values were 15.0, 8.4 and 41.2% of dose in the patients respectively and 24.3, 25.8 and 68.9% of dose in the control subjects. Excreted amounts of total and unconjugated 3-hydroxymethylantipyrine (HMA) and of unchanged antipyrine were the same in the two groups.

7 Clearance for production of all three major metabolites of antipyrine (CL_m) was markedly reduced in patients with alcoholic cirrhosis, while the rate of formation of NORA was significantly more reduced than the rates of formation of OHA and HMA. Mean values for CL_{OHA}, CL_{NORA} and CL_{HMA} were: 2.6, 1.4 and 2.0 ml/min respectively in the patients and 12.9, 13.2 and 7.9 ml/min in the healthy subjects.

8 The results of this study show that antipyrine metabolism is severely impaired in patients with alcoholic liver cirrhosis. However, as the rates of formation of antipyrine metabolites were not reduced to the same extent, different isoenzymes of the cytochrome P-450 system may be differently affected by alcoholic liver cirrhosis.

Keywords antipyrine clearance alcoholic cirrhosis metabolite formation

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Introduction

The disposition of a large number of drugs which are predominantly metabolized in the liver is impaired in patients with alcoholic liver cirrhosis (Wilkinson & Schenker, 1975). As intrinsic drug clearance is a measure of drug-metabolizing enzyme activity, it was suggested that hepatic clearance of a model compound like antipyrine might provide a clinically useful parameter to assess hepatic function in liver disease (Branch *et al.*, 1973; Andreassen *et al.*, 1974; Branch, 1982). In order to reduce the observed wide interindividual variations in antipyrine clearance in healthy and diseased subjects, clearance normalized for liver volume was introduced, because a linear relationship between antipyrine clearance and liver volume in normal subjects was found (Homeida *et al.*, 1979). This also allowed a clearer distinction between subjects with normal and abnormal liver function.

Another major improvement in the value of antipyrine as a model substrate for assessing hepatic drug-metabolizing enzyme activity was made by the estimation of the rates of formation of antipyrine metabolites (Danhof *et al.*, 1979b), because it is likely that different isoenzymes of the mixed-function oxygenase (MFO) system are involved in the formation of the different metabolites of antipyrine in man and rat (Danhof *et al.*, 1979a, 1982a; Toverud *et al.*, 1981; Teunissen *et al.*, 1983a). Hence the activity of various isoenzymes of cytochrome P-450 may be measured with the aid of one compound (Breimer, 1983). Interestingly, an *in vitro* study by Farrell *et al.* (1979) revealed that hepatic aryl hydrocarbon hydroxylase (AHH) activity was significantly lowered in patients with hepatitis or cirrhosis, whereas ethylmor-

phine demethylase activity was almost unchanged, indicating that liver disease may affect the activity of different drug-metabolizing enzymes to a different extent.

The objective of the present study was to determine antipyrine clearance (normalized for liver volume) and the rates of formation of its three major metabolites in patients with alcoholic liver cirrhosis in order to investigate whether this disease shows any selective effects on the metabolic pathways of antipyrine.

Methods

Compounds

4-Hydroxyantipyrine (OHA), norantipyrine (NORA) and 3-hydroxymethylantipyrine (HMA) were synthesized according to methods described by Knorr & Pshorr (1896), Koike *et al.* (1954), Yoshimura *et al.* (1971) and Danhof *et al.* (1982b). Ampoules (10 ml) containing 500 mg antipyrine were obtained through the Pharmacy of the University Hospital, Leiden, The Netherlands.

Human experiments

The study was performed at the Department of Gastroenterology and Hepatology of the University Hospital, Leiden, The Netherlands. Twenty-three patients with alcoholic liver cirrhosis, who gave their informed consent participated in this study. Diagnosis was established by clinical and biochemical criteria in all patients and confirmed histologically in eight patients.

Table 1 Clinical and liver function characteristics of normal subjects and patients with alcoholic liver cirrhosis

n (M/F)	Controls 17 (10/7)	Patients 23 (17/6)	Normal laboratory values
Age (years)	37 ± 8 (28–55)	52 ± 10 ^a (37–70)	–
Body weight (kg)	70 ± 10 (47–91)	78 ± 15 (53–113)	–
Plasma albumin (g/l)	ND	34 ± 7 (19–49)	40–50
Prothrombin time (s)	12.8 ± 0.8 (11.2–13.7)	17.2 ± 2.9 ^a (14.0–22.6)	< 15
sGOT (u/l)	8.4 ± 3.3 (5–15)	23 ± 15 ^a (1–68)	2–15
sGPT (u/l)	7.1 ± 3.9 (3–18)	14 ± 8 ^a (6–39)	2–15
Total bilirubin (μmol/l)	ND	124 ± 141 (18–416)	3–17
Alkaline phosphatase (u/l)	33 ± 9 (22–58)	117 ± 70 ^a (46–315)	15–60
γGT (u/l)	15 ± 8 (7–35)	92 ± 96 ^a (13–384)	6–28
Serum creatinine (μmol/l)	ND	100 ± 43 (53–212)	70–133

Mean values ± s.d. and ranges are given

^a: significantly different from controls ($P < 0.01$)

ND: not determined.

Seventeen healthy volunteers were recruited from the hospital staff and served as controls. The mean clinical and liver characteristics are listed in Table 1. The volunteers had neither taken any drugs nor had they been exposed to anaesthetic gases during or at least 4 weeks prior to the study. Most of the patients were treated therapeutically with one or more (up to 5) of the following drugs: spironolactone, cholestyramine, digoxin, cimetidine, ranitidine, oxazepam, chlordiazepoxide, haloperidol, metoclopramide, insulin and vitamins. Liver volume was determined by ultrasound (Carr & Duncan, 1976). Inter-test coefficient of variation of liver volume in 10 persons on eight occasions was 6.3% (Spoelstra *et al.*, 1983).

Antipyrine was administered as a 500 mg i.v. bolus injection at 09.00 h after an overnight fast. Blood samples from patients were collected in heparinized Venoject® tubes at 0, 4, 8, 12, 24, 30, 36, 48, 54 and 60 h and from volunteers at 0, 0.5, 3, 5.5, 8, 24, 28 and 32 h after drug administration. Urine was collected quantitatively after antipyrine administration in 24 h intervals for 2 days in controls and for 4 days in patients. Plasma samples and aliquots of urine samples were stored at -70°C until analysis.

The protocol was approved by the Committee for Medical Ethics of the University Hospital and the Faculty of Medicine, Leiden, The Netherlands.

Calculations

The elimination half-life ($t_{1/2}$) of antipyrine was determined for each subject from the log-linear part of the plasma concentration time curve by least squares linear regression. The apparent volume of distribution (V_d) was calculated by dividing the given dose by the extrapolated value of antipyrine concentration at time zero. Total antipyrine plasma clearance (CL) was calculated according to:

$$\text{CL} = \frac{\ln 2 \cdot V_d}{t_{1/2}} \quad (1)$$

Rates of formation of antipyrine metabolites were expressed as clearance for production of a metabolite *m* and were calculated according to:

$$\text{CL}_m = \text{CL} \cdot f_m \quad (2)$$

in which f_m is the fraction of the dose excreted as metabolite *m* upon extrapolation of the 48 h value (for patients: 96 h) to infinity using antipyrine half-life (Danhof *et al.*, 1982c). For statistical analysis Student's *t*-test was used.

Analytical procedures

Antipyrine was measured in plasma; in urine antipyrine, OHA, NORA and HMA were measured by a high-performance liquid chromatographic method (Danhof *et al.*, 1979b; Teunissen *et al.*, 1983b). Total concentrations of free plus conjugated HMA were measured after hydrolysis of the urine samples with β -glucuronidase-sulphatase (Limpet Acetone Powder type I, Sigma Chemical Co., St Louis, U.S.A.), during 3 h at 37°C . Concentrations of free HMA were measured in urine without enzymic hydrolysis.

Results

The mean kinetic data for antipyrine are listed in Table 2, while the values of each individual are shown in Figure 1. Antipyrine half-life was about four times longer in patients with alcoholic cirrhosis than in healthy controls (39.9 vs 10.1 h), while no difference in mean V_d was observed. Plasma clearance was markedly reduced in the patients when compared with the controls: 13.5 vs 49.3 ml/min ($P < 0.001$). As liver volume was increased in the patients (1.86

Table 2 Kinetic parameters for antipyrine and liver volume of patients with alcoholic liver cirrhosis and controls

	Controls (n = 17)	Patients (n = 23)
$t_{1/2}$ (h)	10.1 ± 2.8	39.9 ± 13.8^b
V_d (l)	40.3 ± 7.9	43.6 ± 10.5
V_d (l/kg)	0.57 ± 0.09	0.56 ± 0.09
Liver volume LV (l)	1.36 ± 0.23	1.86 ± 0.63^a
CL (ml/min)	49.3 ± 18.3	13.5 ± 4.0^b
CL/LV (ml min ⁻¹ l)	36.1 ± 10.1	7.8 ± 2.9^b

Mean values $\pm$ s.d. are given

^a: significantly different from controls at $P < 0.01$

^b: significantly different from controls at $P < 0.001$

Figure 1 Individual values of antipyrine half-life, clearance and clearance normalized for liver volume, in 23 patients with alcoholic liver cirrhosis and 17 controls. Mean values and s.d. are indicated by bars.

vs 1.36 l, $P < 0.001$), plasma clearance of antipyrine normalized for liver volume was slightly more reduced (7.8 vs 36.1 ml min⁻¹ l⁻¹).

The cumulative renal excretion of antipyrine and its major metabolites are listed in Table 3, whereas the results in each individual are shown in Figure 2. The excretion of OHA in cirrhotic patients was reduced by 38% ($P < 0.001$) and of NORA by 67% ($P < 0.001$), whereas the excretion of unchanged antipyrine, total HMA and free HMA was not significantly altered. Total recovery of antipyrine and metabolites was reduced by 40%. Interindividual variability among the patients was found to be considerably larger for all metabolites as compared to the control subjects (Figure 2).

Clearance for production of the major metabolites of antipyrine (Table 3, Figure 3) was substantially impaired in the patient group. Mean CL_{OHA}, CL_{NORA} and CL_{HMA} were 20, 10 and 26% of control values, respectively. CL_{NORA} was significantly more affected by alcoholic liver cirrhosis than CL_{HMA} or CL_{OHA} ($P < 0.05$ by analysis of variance).

Discussion

From the results obtained in the present study it is evident that hepatic drug-metabolizing enzyme activity is substantially impaired in alcoholic liver cirrhosis. Antipyrine half-life had increased four-fold, while clearance as well as clearance normalized for liver volume had decreased considerably (Figure 1). These results are in agreement with those reported by others (Branch *et al.*, 1973; Andreasen *et al.*, 1974; Homeida *et al.*, 1979). Moreover, no overlap was observed for any of these parameters between cirrhosis patients and controls. Therefore it was not deemed necessary to normalize antipyrine clearances for liver volume to allow a clear distinction between patients with alcoholic cirrhosis and control subjects. For other types of liver disease however, normalization has proved to be of considerable value (Homeida *et al.*, 1979; Spoelstra *et al.*, in preparation). It is well recognized that among healthy subjects there is a wide variability in the clearance of drugs that are predominantly metabolized by

Table 3 Excretion and clearance for production of antipyrine metabolites in normal subjects and in patients with alcoholic liver cirrhosis

	Controls (n = 17)	Patients (n = 23)
<i>Excretion of antipyrine and metabolites (% of dose)</i>		
OHA	24.3 ± 5.3	15.0 ± 10.1 ^a
NORA	25.8 ± 4.8	8.4 ± 5.3 ^a
HMA	15.1 ± 3.4	12.3 ± 10.5
Antipyrine	3.7 ± 1.6	5.4 ± 5.4
Total	68.9 ± 9.9	41.2 ± 15.8 ^a
% glucuronidation of HMA	50.5 ± 8.5	45.4 ± 24.1
<i>Clearance for production of antipyrine metabolites (ml/min)</i>		
CL _{OHA}	12.9 ± 6.8	2.6 ± 2.3 ^a
CL _{NORA}	13.2 ± 4.9	1.4 ± 1.1 ^a
CL _{HMA}	7.9 ± 3.5	2.0 ± 1.7 ^a
<i>Normalized clearance for production of antipyrine metabolites (ml min⁻¹ l⁻¹)</i>		
CL _{OHA} /LV	9.2 ± 3.4	1.2 ± 1.0 ^a
CL _{NORA} /LV	9.7 ± 2.7	0.8 ± 0.7 ^a
CL _{HMA} /LV	5.7 ± 2.0	1.2 ± 1.1 ^a

Mean values ± s.d. are given

^a: significantly different from controls $P < 0.001$.

Figure 2 Individual values of excreted amounts of 4-hydroxyantipyrine (OHA), norantipyrine (NORA) and 3-hydroxymethylantipyrine (HMA) as percent of dose in 23 patients with alcoholic liver cirrhosis and 17 controls. Mean values and s.d. are indicated by bars. Five individual patients are indicated by special symbols.

Figure 3 Individual values of the clearance for production of OHA, NORA and HMA in 23 patients with alcoholic liver cirrhosis and 17 controls. Mean values $\pm$ s.d. are indicated by bars. Abbreviations as in Figure 2.

the liver (Vesell, 1977; Breimer, 1983). This is also reflected by the 3½-fold range in antipyrine clearance (31–103 ml/min) in the control group of the present study. The normalization of antipyrine clearance for liver volume (Roberts *et al.*, 1976; Homeida *et al.*, 1979) resulted in a reduction of interindividual variability to a 2½-fold range (22–55 ml min⁻¹ l⁻¹).

The observed differences between patients and control subjects may be somewhat exaggerated, because these two groups were not entirely comparable with respect to age, gender and concomitant drug intake. However, antipyrine clearance has been shown to decrease only slightly with age (Vestal *et al.*, 1975) and no difference was found in total clearance of

antipyrine between males and females (Teunissen *et al.*, 1982). Hence these influences were regarded to contribute to the differences observed between patients and controls for only a small percentage. The enhancing effect of smoking has been well established in healthy subjects (Vestal *et al.*, 1975; Jusko, 1979), but in the present study no influence of smoking was found in the patient group (unpublished results). Smokers were equally represented in the two groups and therefore differences between them may have been more pronounced because of the differential effect of smoking. Concomitant drug intake in the patient group was inevitable and some of these have been shown to affect the metabolism of other drugs.

Cimetidine for instance inhibits oxidative metabolism in healthy subjects (Puurunen *et al.*, 1980) as well as in patients with liver cirrhosis (Röllinghof & Paumgartner, 1982). Spironolactone has been demonstrated to be a weak inducer of drug-metabolizing enzyme activity (Huffman *et al.*, 1973; Miguët *et al.*, 1980). The use of these drugs may thus have affected antipyrine metabolism in some patients. Nonetheless, the pronounced decrease in antipyrine clearance in the patients was probably mainly caused by impaired hepatic function.

As to antipyrine metabolite formation in patients with alcoholic cirrhosis the present results were quite interesting. Interindividual variability in excretion of antipyrine metabolites as percent of dose was extremely large, ranging from practically zero to higher than normal values, whereas overall excretion was reduced. The highest values for HMA (patients indicated with * and ▼, Figure 2) were associated with the lowest values of OHA and NORA. The reverse (high OHA and NORA with low HMA) was also observed in one patient (▲), indicating that formation of OHA and NORA may be related, whereas HMA formation may be inversely related to the other two, as was also found in rats after induction with 3-methylcholanthrene (Danhof *et al.*, 1979a; Teunissen *et al.*, 1983a). However, the association between the metabolites is not absolute as was shown in patient ●, who excreted very little NORA but exhibited normal excretions of OHA and HMA. In general, excretion of NORA was reduced to a greater extent than that of OHA, while the mean HMA excretion was not significantly different from that in control subjects.

In several patients (e.g. patient ■, Figure 2) excretion of all three metabolites was markedly reduced. In the same patient ■, glucuronidation of HMA was also much impaired (< 10% of total HMA, normal range 33–63%), which was observed in only three out of 23 patients. Interestingly, the percentage of dose excreted in 96 h by these three patients was very low (11–30%), while renal function was not impaired. Serum creatinine was within normal range and concentrations of the metabolites of antipyrine and their conjugates in plasma were too low to be detected, i.e. < 25 ng/ml (Teunissen *et al.*, 1983b). The percentage of HMA that was glucuronidated was determined by analysis of urine samples with and without enzymic hydrolysis (Danhof *et al.*, 1979b). Without hydrolysis OHA and NORA could not be detected in urine. However, if glucuronidation of OHA and NORA in the above three patients had been impaired it might have resulted in (1)

accumulation of the relatively lipophilic unconjugated compounds in the cirrhotic liver, (2) further metabolism and (3) decomposition, as these phase I metabolites are relatively unstable at physiological pH (Teunissen *et al.*, 1983b). Impaired glucuronidation would then account for low metabolite recoveries, but it would also result in underestimation of clearance for production of these metabolites, since for its calculation it had to be assumed that the excreted amounts of the metabolites are equal to the amounts formed. However, only in three patients glucuronidation of HMA was impaired, but this was not significant for the entire patient group (Table 3). Though ester-glucuronidation of zomepirac was found to be decreased in liver disease (Witassek *et al.*, 1983), conjugation reactions are often found not to be impaired: e.g. conjugation of oxazepam was found to be unaltered (Sellers *et al.*, 1979). Clearance of phenobarbitone was even increased in patients with alcoholic cirrhosis, but this may have been due to decreased plasma protein binding (Kitteringham *et al.*, 1983).

Clearance for production of the three major metabolites of antipyrine was significantly reduced in cirrhotic patients, though not to the same extent and again large interindividual differences were observed (Table 3, Figure 3). The reduction of the clearances for production of NORA (CL_{NORA}) was significantly more pronounced than the reduction of CL_{HMA} and CL_{OHA} ($P < 0.05$). These findings support the supposition that liver disease may affect different oxidative drug-metabolizing enzymes to a different extent, as was previously suggested by Farrell *et al.* (1979).

In conclusion, the marked reduction of antipyrine clearance in patients with alcoholic liver cirrhosis as observed in this study was not the result of an equal impairment of the three major metabolic pathways of antipyrine. In different patients with the same diagnosis, different metabolic pathways of antipyrine were found to be affected differentially (in some cases they were even absent), resulting in very large interindividual variability in the excretion of antipyrine metabolites. In the present patient group the rate of formation of all three metabolites was significantly impaired in the following rank order: NORA > OHA > HMA. Obviously different isoenzymes of the cytochrome P-450 system are differentially affected by alcoholic liver cirrhosis. As to discrimination between patients and controls, normalization for liver volume of plasma clearance as well as of clearance for production of the metabolites of antipyrine did not result in further improvements.

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