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EFFECTS OF MICROSOMAL ENZYME INDUCTION ON PARACETAMOL METABOLISM IN MAN

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1 The metabolism of paracetamol after a single oral dose of 20 mg/kg was compared in fifteen patients with microsomal enzyme induction taking anticonvulsants or rifampicin and twelve healthy volunteers.

2 Induction was confirmed by measurement of the plasma antipyrine half-life (mean 6.4 h in the patients compared with 12.8 h in the volunteers).

3 The glucuronide conjugation of paracetamol was enhanced in the induced patients as shown by lower plasma paracetamol concentrations, a shorter paracetamol half-life, higher paracetamol glucuronide concentrations and an increased ratio of the area under the plasma concentration time curves of the glucuronide to the unchanged drug. There were no significant differences in sulphate conjugation.

4 There was a corresponding change in the pattern of urinary metabolite excretion. The induced patients excreted significantly less unchanged drug and sulphate conjugate and more glucuronide conjugate than the healthy volunteers.

5 The urinary excretion of the mercapturic acid and cysteine conjugates of paracetamol was the same in both groups.

6 Conversion of paracetamol to its potentially hepatotoxic metabolite does not seem to be increased in patients induced with anticonvulsants or rifampicin. There would seem to be no contraindication to the use of these drugs in combination.

Introduction

Paracetamol is a widely used and normally very safe analgesic. However, in overdosage it can cause acute hepatic necrosis through the formation of a highly reactive intermediate metabolite by hepatic cytochrome P-450 dependent microsomal enzymes (Mitchell *et al.*, 1973; Mitchell *et al.*, 1974). In most laboratory animal species, the hepatotoxicity of paracetamol is increased by pretreatment with microsomal enzyme inducers such as phenobarbitone, 3-methyl-cholanthrene and ethanol and decreased by inhibitors such as piperonyl butoxide (Mitchell *et al.*, 1973; Mitchell *et al.*, 1974; Jollow *et al.*, 1974; Strubelt, Obermeier & Siegers, 1978; Teschke, Stutz & Strohmeyer, 1979; Streeter & Timbrell, 1979). There have been recent anecdotal reports of liver damage following the alleged therapeutic use of paracetamol in chronic alcoholics (Licht, Seeff & Zimmerman, 1980; Gerber *et al.*, 1980; Goldfinger *et al.*, 1978; McClain *et al.*, 1980) and after overdosage the severity of liver damage appears to be greater in chronic alcoholics and patients who have previously been taking drugs likely to cause induction (Wright & Prescott, 1973).

These observations suggest that microsomal enzyme induction might increase the production of the reactive metabolite of paracetamol and thus enhance its hepatotoxicity. A detailed comparison of paracetamol metabolism was therefore carried out in healthy volunteers and patients with microsomal enzyme induction taking anticonvulsants or rifampicin. Induction in the patients was confirmed by measurement of the antipyrine half-life and the extent of conversion of paracetamol to its potentially toxic metabolite was assessed by the urinary excretion of its cysteine and mercapturic acid conjugates.

Methods

Fifteen fasting patients on long-term therapy with inducing drugs and twelve healthy volunteers received 20 mg/kg of paracetamol in 400 ml of 'Coca Cola' and 18 mg/kg of antipyrine in 200 ml orange juice orally over 2 min on successive mornings. The Coca Cola and orange juice were used to disguise the

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taste of the drugs. All the subjects were ambulant. Fluids and tobacco were withheld for 2 h and food for 4 h after dosing.

All but three of the patients were male and their mean age and body weight were 41 years (range 22–72) and 71 kg (range 53–99) respectively. Thirteen patients were epileptics on long-term therapy with inducing anticonvulsants given singly or in combination – twelve were taking diphenylhydantoin (mean daily dose 315 mg), two phenobarbitone, two carbamazepine and one primidone. The other two patients were taking rifampicin 600 mg daily with isoniazid for tuberculosis. These drugs were continued as usual during the study. The plasma bilirubin, alanine aminotransferase, albumin and creatinine were normal in all the patients, but four had mild elevation of the alkaline phosphatase.

None of the healthy volunteers took drugs or excessive amounts of ethanol regularly. Eleven were male and their mean age and body weight were 31 years (range 21–45) and 67 kg (range 55–82) respectively.

Serial blood samples were taken for 8 and 24 h after ingestion of the paracetamol and antipyrine respectively and urine was collected at 0, 4, 8, 12 and 24 h after the paracetamol. Plasma and urine were stored frozen. Paracetamol and its metabolites in plasma and urine and antipyrine in plasma were estimated by high performance liquid chromatography (Adriaenssens & Prescott, 1978; Prescott *et al.*, 1979). None of the inducing drugs or their metabolites interfered with the assays.

The area under the plasma concentration time curves (AUC) was calculated by the trapezoidal rule, the plasma half-life from the regression of the linear terminal elimination phase and the renal clearances by dividing the amount of drug or metabolites excreted in the urine by the corresponding AUC. The apparent volume of distribution of antipyrine was obtained by dividing the administered dose by the extrapolated plasma concentration at zero time.

The plasma clearance of antipyrine was estimated by multiplying the volume of distribution by 1n2 and dividing by the half-life. Paracetamol clearances were not calculated because its significant and variable first-pass metabolism would invalidate comparisons between the groups (Perucca & Richens, 1979).

Student's *t*-test was used for statistical comparisons of mean data. All results are given as means ± s.d. and concentrations of paracetamol metabolites are expressed as paracetamol equivalents.

Results

Antipyrine half-life

Induction of microsomal enzymes in the patients was confirmed by the mean antipyrine plasma half-life of 6.4 ± 2.1 h compared with 12.8 ± 3.9 h in the healthy volunteers ($P < 0.001$). There was a similar highly significant difference in the mean plasma antipyrine clearances (96 ± 54 ml/min in the induced patients and 41 ± 10 ml/min in the volunteers). The mean apparent volumes of distribution in the two groups were almost identical (0.66 and 0.65 l/kg respectively).

Paracetamol and metabolites in plasma

The mean plasma concentrations of paracetamol and its sulphate and glucuronide conjugates in the induced patients and healthy volunteers are shown in Figure 1. Paracetamol metabolism was enhanced in the patients. The mean plasma paracetamol concentrations were lower than in the volunteers at all time points, the AUC_{0-8h} was significantly less and the plasma half-life was significantly shorter at 1.9 h compared with 2.4 h (Table 1). These differences were due to increased glucuronide conjugation of paracetamol in the patients since the AUC_{0-8h} for the sulphate conjugate was similar in both groups while the initial glucuronide concentrations and AUC_{0-8h} were higher in the patients. The ratio of the AUCs of

Table 1 Plasma paracetamol half-life and areas under the plasma concentration-time curves (AUC) from 0–8 h for paracetamol and its glucuronide and sulphate conjugates in induced patients and healthy volunteers

	Plasma paracetamol half-life (h)	*AUC paracetamol ($\mu g\ ml^{-1} h$)*	*AUC paracetamol glucuronide ($\mu g\ ml^{-1} h$)*	*Ratio of AUC glucuronide/ paracetamol*	*AUC paracetamol sulphate ($\mu g\ ml^{-1} h$)*
Healthy volunteers ($n = 12$)	2.4 ± 0.3	65 ± 9	69 ± 24	1.1 ± 0.5	28 ± 7
Induced patients ($n = 15$)	1.9 ± 0.2*	40 ± 9*	88 ± 28	2.3 ± 0.7*	25 ± 8

*$P = < 0.001$.

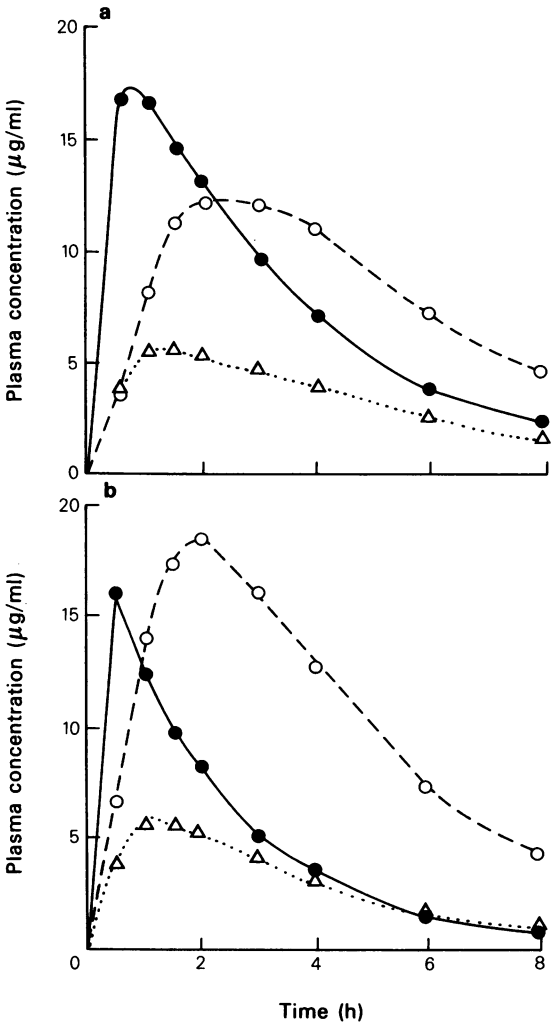

Figure 1 Mean plasma concentrations of paracetamol (●) and its glucuronide (○) and sulphate (△) conjugates in a) twelve healthy volunteers and b) fifteen induced patients after a single oral dose of 20 mg/kg of paracetamol.

paracetamol glucuronide to the parent drug in the patients was more than double that in the volunteers (Table 1).

There was no statistically significant correlation between the plasma half-lives of antipyrine and paracetamol.

Urinary excretion of paracetamol and metabolites

In the healthy volunteers about 5% of the total amount recovered in 24 h was excreted unchanged, with 57% as glucuronide, 30% as sulphate, 4.5% as mercapturic acid and 3.7% as cysteine conjugates. However, in the patients, significantly less paracetamol was recovered unchanged and as sulphate while significantly more was excreted as glucuronide. The excretion of the mercapturic acid and cysteine conjugates was no greater in the induced patients than in the volunteers (Table 2). There was no evidence of initial more rapid production of mercapturic acid and cysteine conjugates in the patients. The pattern of urinary excretion of paracetamol and metabolites from 0–4 h and 0–24 h was similar and if anything the fraction excreted as mercapturic acid and cysteine conjugates from 0–4 h was less in the patients (3.6 and 2.5%) than in the controls (3.8 and 2.8%).

The total urinary recovery of drug was slightly but not significantly lower in the patients than in the volunteers (Table 2) while the mean 24 h urine volumes were virtually identical (1783 and 1798 ml respectively).

The mean renal clearances of paracetamol and its glucuronide and sulphate conjugates were 11.9, 131 and 166 ml/min respectively in the healthy volunteers. The clearances of all three compounds were somewhat lower in the induced patients but the differences were not statistically significant (Table 3).

Discussion

Induction of drug metabolising enzymes was confirmed in the patients taking anticonvulsants and

Table 2 Urinary excretion of paracetamol and its glucuronide, sulphate, mercapturic acid and cysteine conjugates in healthy volunteers and induced patients

	Percentage excreted in 24 h as:					*% of dose recovered in 24 h*
	Paracetamol	*Glucuronide*	*Sulphate*	*Mercapturic acid*	*Cysteine*	
Healthy volunteers ($n = 12$)	4.8 ± 1.6	57 ± 11	30 ± 10	4.5 ± 1.2	3.7 ± 1.1	92 ± 10
Induced patients ($n = 15$)	2.4 ± 0.9***	68 ± 6**	22 ± 4*	4.2 ± 2.1	3.7 ± 1.3	83 ± 19

*$P = < 0.05$, **$P = < 0.01$, ***$P = < 0.001$.

Table 3 Renal clearances (0–8 h) of paracetamol and its glucuronide and sulphate conjugates in healthy volunteers and induced patients

	Renal clearance (ml/min)		
	Paracetamol	*Glucuronide*	*Sulphate*
Healthy volunteers ($n = 12$)	11.9 ± 4.9	131 ± 22	166 ± 29
Induced patients ($n = 15$)	9.0 ± 5.5	116 ± 30	138 ± 46

rifampicin by the greatly shortened antipyrine half-life and increased clearance without any change in the apparent volume of distribution. The glucuronide conjugation of paracetamol was also enhanced in the patients as compared to the healthy volunteers. The plasma concentrations of paracetamol were lower and the half-life was shorter while plasma concentrations of the glucuronide conjugate and the ratio of the AUC of the glucuronide to the unchanged drug were increased. There were corresponding changes in the urinary excretion of paracetamol and its metabolites. The patients excreted less unchanged drug and sulphate conjugate but more glucuronide conjugate than the volunteers. Although the renal clearances of paracetamol and its conjugates were slightly lower in the patients, this alone could not account for the observed differences in the disposition of paracetamol. Unlike glucuronyl transferase, sulphotransferase is not an inducible microsomal enzyme, and as expected sulphate conjugation was not increased in the induced patients.

Little is known of the effects of microsomal enzyme induction on paracetamol metabolism in man and previous studies have given conflicting results. Perucca & Richens (1979) demonstrated reduced oral bioavailability of paracetamol which they attributed to increased first-pass metabolism in six epileptics compared with six normal subjects. The mean total body clearance and elimination rate were higher in the former group but the differences were not significant. No data was provided on the individual metabolites of paracetamol and the mercapturic acid and cysteine conjugates were not measured. On the other hand, Mitchell *et al.* (1974) found that pretreatment with 3 mg/kg of phenobarbitone for 5 days had no effect on the plasma half-life of paracetamol in seven healthy volunteers although formation of the mercapturic acid conjugate was increased.

An important but unexpected finding in the present study was that the urinary excretion of the mercapturic acid and cysteine conjugates of paracetamol was not increased in the induced patients. However, in view of the enhanced glucuronide conjugation in these patients, significant reduction in the recovery of the mercapturic acid and cysteine conjugates would have been expected as occurred with the unchanged drug and the sulphate conjugate. Some induction of the oxidative metabolism of paracetamol cannot therefore be excluded but would seem to be of no clinical significance at recommended therapeutic doses. It cannot be assumed that this would necessarily be the case after overdosage since paracetamol metabolism is dose-dependent (Prescott, 1980).

The mercapturic acid and cysteine conjugates reflect the conversion of paracetamol to the potentially hepatotoxic metabolite. In animals the fraction of a dose converted to the mercapturic acid conjugate is highest in species most susceptible to the hepatotoxicity of paracetamol and lowest in those most resistant. Similarly, inducing agents which potentiate paracetamol liver toxicity also increase mercapturic acid conjugate production while its formation is decreased by treatments which protect (Jollow *et al.*, 1974; Sato, Matsuda & Lieber, 1979). The situation is far from simple however, and extrapolations must be made with great caution. Thus phenobarbitone actually protects against paracetamol liver injury and inhibits mercapturic acid formation in hamsters (Jollow *et al.*, 1974) and potentiation of hepatotoxicity by established inducers is not necessarily related to increased hepatic cytochrome P-450 (Streeter & Timbrell, 1979; Peterson, Holloway, McClain & Holtzman, 1979). Similarly, acute administration of ethanol prevents paracetamol hepatotoxicity in rats (Sato & Lieber, 1980). Other unknown factors must be responsible. Some discrepancies could be explained by the selective activation of one or more of the multiple forms of cytochrome P-450 which, depending on the particular inducer and species, may or may not be responsible for the oxidative metabolism of paracetamol. Whatever the explanation, patients with enzyme induction due to treatment with anticonvulsants and rifampicin do not seem to be at greater risk of paracetamol hepatotoxicity because of increased production of the toxic metabolite.

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