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The influence of disulfiram on acetaminophen metabolism in man

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1. Acetaminophen clearance and its partial clearance to its major metabolites has been determined before and after 5 days treatment with the anti-alcohol abuse agent disulfiram (200 mg daily). The study was conducted in 10 subjects, five without liver disease and five with alcoholic cirrhosis of the liver. Acetaminophen was given i.v. at a dose of 500 mg. Plasma samples were obtained up to 8 h after injection and urine collected for 24 h.

2. Across all subjects acetaminophen plasma clearance was reduced from 0·249 ± 0·061 to 0·217 ± 0·066 l/min after disulfiram treatment (mean ± SD, $P < 0·05$). Thus no change in acetaminophen dosage would be required in patients treated with disulfiram.

3. The partial clearance of acetaminophen to its glucuronide, sulphate and glutathione derivatives (i.e. cysteine and *N*-acetyl cysteine) was not significantly changed by disulfiram treatment. Thus it seems unlikely that the previously observed protective effects of disulfiram against acetaminophen-induced hepatotoxicity in animals due to inhibition of metabolism will be seen in man.

Introduction

Disulfiram is used for aversion therapy in alcoholics, a category of patients considered more prone to develop acetaminophen-induced hepatic injury (Maddrey 1987). Acetaminophen overdose is one of the most common causes of acute hepatic injury, although it is a remarkable safe drug at conventional analgesic doses. The hepatotoxicity is considered related to the formation of an arylating metabolite by cytochrome P-450 (Mitchell *et al.* 1984). Following therapeutic doses this species, *N*-acetyl-*p*-benzoquinone imine, is normally detoxified by hepatic glutathione to form 3-(glutathione-*S*-yl)acetaminophen which is readily excreted. Following an overdose glucuronidation and sulphation pathways become saturated, the glutathione detoxification is overwhelmed and the toxic species bind to proteins to form 3-(cysteine-*S*-yl)acetaminophen protein adducts as demonstrated in experimental animals (Roberts *et al.* 1987) and recently also in man (Hinson *et al.* 1990). This is considered to disturb hepatic functions and initiate the subsequent hepatic necrosis.

A variety of inhibitors of cytochrome P-450 reduce the hepatotoxicity of acetaminophen as demonstrated for cimetidine (Mitchell *et al.* 1984, Abernathy *et al.* 1983), alcohol (Critchley *et al.* 1989), metyrapone (Nelson *et al.* 1980, Galinsky *et al.* 1987) and probenecid (Abernathy *et al.* 1985). Disulfiram inhibits various enzyme systems in the liver (O'Reilly 1973, Andersson and Boström 1984, Frank *et al.* 1980), including cytochrome P-450, although disulfiram also increases blood acetone concentrations (De Master and Nagasawa 1977) which actually induces hepatic cytochrome P-450 mixed function oxidase activity (Moldeus and Gergely 1980).

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However, the overall empirically observed effect of disulfiram on acetaminophen-induced hepatic injury in experimental animals is one of protection (Jørgensen *et al.* 1988).

We investigated whether disulfiram at conventional aversion therapy doses affects acetaminophen clearance and metabolism after a standard acetaminophen dose given to individuals with and without cirrhosis of the liver.

Materials and methods

Volunteers and patients

Four male and one female patient with compensated, biopsy-verified, alcoholc cirrhosis and three male and one female patient *without* history and biochemical evidence of liver disease, together with one healthy volunteer from the hospital staff, participated in the study after giving informed consent according to the Helsinki II Declaration. None of the cirrhotic patients had acute exacerbation of their liver disease, although one had ascites. None of the other patients or the volunteer were suffering from *acute* disease. The demographic details and medical condition of the 10 subjects are given in table 1, which also gives an estimate of the liver function by the galactose elimination capacity (Tygstrup 1966). This procedure was performed prior to the experiment, in all but one subject. The subjects continued their regular medication throughout the experiment, the details of which are given in table 1.

Drug administration and sample collection

Acetaminophen was given on two occasions separated by 5 days. After an overnight fast a 500 mg dose of acetaminophen (10 mg/ml adjusted to isotonicity by saline, sterile and pyrogen-tested) was given i.v. over a period of about 1 min through a catheter (Venflon^{R}) placed in the forearm vein. Heparinized venous blood (5 ml) was drawn at regular intervals up to 8 h from an indwelling catheter in the contralateral forearm. Plasma was separated within 2 h and stored at −20°C. Urine was collected immediately after voiding and kept at 5°C until 24 h after injection. All urine was thoroughly mixed, the total volume recorded and aliquots stored at −20°C. After the collection of urine, disulfiram was given as a single morning dose of 200 mg for the next 5 days. At day 5 the acetaminophen injection, blood sampling and urine collection was repeated.

Acetaminophen and acetaminophen metabolite assay

Plasma and urine was precipitated with 2 N PCA after the addition of 4-flurophenol as internal standard. Aliquots of the supernatant (25 μl) were injected using a Waters Wisp^{R} autoinjector into an

Table 1. Age, sex, medical condition, medication and galactose elimination (GE) of the volunteers.

Patient identification	Age	Sex	Medical condition	Current medication	GE (μmol/min)
Control					
KUB	46	Male	Chronic bronchitis	t	2·34
JPT	79	Male	Chronic bronchitis	t	1·16
KBC	74	Female	Angina pectoris	m	1·57
HEP	35	Male	Volunteer	None	2·42
LPS	72	Male	Hypertension	f	n.d.
Mean	61				1·87
SD	19				0·61
Cirrhosis					
URH	58	Female	Alcoholic cirrhosis	d, f, s	0·93
BEN	63	Male	Alcoholic cirrhosis†	f, s	1·26
AON	60	Male	Alcoholic cirrhosis	t	1·02
BOC	34	Male	Alcoholic cirrhosis	f, s	1·17
ESA	37	Male	Alcoholic cirrhosis	None	1·49
Mean	50				1·17
SD	14				0·22

† Indicates ascites.

Abbreviations: t: terbutaline; f: furosemide or bumetamide; m: mianserine; s: spironolactone; d: dextropropoxyfene; n.d. = not detected.

h.p.l.c. system consisting of a Waters M600A pump, a Waters C18 10 μ particle Bondpak 30 cm steel column (i.d. 3·9 mm) and a UV M440 model detector recording to 254 nm. Peak areas were calculated by a Waters Integration Module. Acetaminophen plasma concentration was determined from a standard curve from analytical grade acetaminophen. Urinary acetaminophen and acetaminophen metabolite concentrations were determined from an acetaminophen standard curve assuming identical molar extinction coefficients of the metabolites (Moldeus 1978). Since the acetaminophen–cysteine and acetaminophen–mercapturate are formed from acetaminophen–glutathione conjugate the amount of these two metabolites were simply summed and designated glutathione-derived acetaminophen metabolites. Identification of the mercapturate, cysteine, sulphate and glucuronide peaks was done by injection of pure metabolites kindly donated by Sterling Winthrop, Sweden. With this modified assay (Poulsen *et al.* 1985, Knox and Jurand 1977) all metabolites and acetaminophen could be estimated with a day-to-day variation of less than 5%. This day-to-day variation was shown to be consistent from checks made at regular intervals using samples drawn from a single large plasma pool. All samples were analysed in duplicate with a coefficient of variation less than 5%.

Pharmacokinetic and statistical analysis

Acetaminophen plasma clearance was calculated by the trapezoidal rule as dose divided by the area under the acetaminophen concentration–time curve and extrapolation of the area until infinity. In no case did the extrapolated area exceed 10% of the trapezoidal area. Acetaminophen elimination half-life was calculated by the basic ESTRIP program (Brown and Manno 1978) adapted to a Hewlett-Packard 85 desktop computer.

Acetaminophen fractional clearance to its metabolites was calculated by multiplication of the total clearance and fraction of dose found in 24 h urine as a particular metabolite.

Statistical analysis was performed by a double-sided Student's *t*-test using 5% as level of significance, paired and unpaired when appropriate.

Results

The effect of disulfiram on acetaminophen clearance

After disulfiram treatment, acetaminophen plasma clearance decreased across all subjects (normal and liver-impaired) but only slightly. The mean plasma clearance

Table 2. Acetaminophen plasma clearance and partial clearances (l/min) to the major metabolites in individuals with, and controls without, cirrhosis of the liver.

Patient	Acetaminophen plasma clearance		Gluc.		Sulph.		GSH	
Control								
KUB	0·367	(0·350)	0·191	(0·227)	0·078	(0·067)	0·045	(0·055)
JPT	0·160	(0·120)	0·044	(0·035)	0·026	(0·035)	0·019	(0·012)
KBC	0·240	(0·160)	0·058	(0·063)	0·033	(0·033)	0·031	(0·030)
HEP	0·270	(0·250)	0·157	(0·161)	0·149	(0·138)	0·075	(0·064)
LPS	0·305	(0·272)	0·146	(0·179)	0·110	(0·140)	0·048	(0·106)
Mean	0·268	(0·230)	0·119	(0·133)	0·079	(0·082)	0·044	(0·053)
SD	0·077	(0·092)	0·065	(0·081)	0·052	(0·053)	0·021	(0·036)
Cirrhosis								
URH	0·171	(0·168)	0·042	(0·013)	0·054	(0·037)	0·080	(0·002)
BEN	0·234	(0·224)	0·092	(0·142)	0·034	(0·012)	0·021	(0·001)
AON	0·276	(0·190)	0·138	(0·123)	0·042	(0·025)	0·009	(0·037)
BOC	0·213	(0·190)	0·073	(0·065)	0·053	(0·046)	0·037	(0·021)
ESA	0·256	(0·248)	0·086	(0·072)	0·067	(0·016)	0·087	(0·087)
Mean	0·230	(0·204)	0·086	(0·083)	0·050	(0·027)	0·047	(0·030)
SD	0·041	(0·032)	0·035	(0·051)	0·013	(0·015)	0·035	(0·035)
Mean of all	0·249*	(0·217)	0·103	(0·108)	0·065	(0·055)	0·045	(0·042)
SD of all	0·061	(0·066)	0·052	(0·069)	0·039	(0·047)	0·028	(0·036)

Gluc, Sulph, and GSH represent clearance to acetaminophen–glucuronide, acetaminophen-sulphate and glutathione-derived conjugates; figures in parentheses represent clearance values subsequent to 5 days treatment with disulfiram 200 mg daily.

* $P < 0·05$ for paired test of untreated versus treated situation.

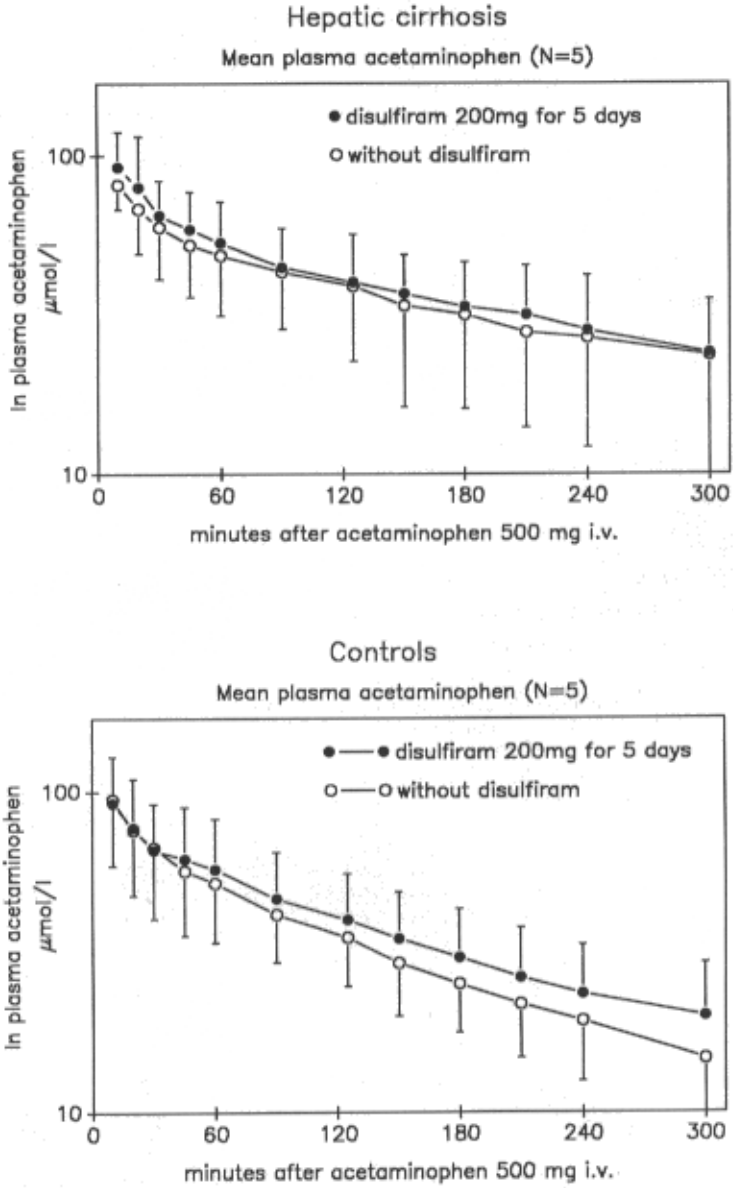

Figure 1. *Upper panel*: mean plasma acetaminophen concentrations in five patients with alcoholic cirrhosis of the liver before and after disulfiram treatment 200 mg per day for 5 days. Bars indicate SD. *Lower panel*: plasma acetaminophen concentrations in five individuals without disease of the liver before and after disulfiram treatment 200 mg per day for 5 days. Bars indicate SD.

before and after disulfiram treatment was 0·249 and 0·217 l/min respectively. The average reduction following disulfiram treatment was 32 ± 7 ml/min ($P < 0{\cdot}05$) corresponding to a 10% decrease. The individual plasma clearances, their means and SD values are given in table 2. The reduction in plasma clearance could not be related to any of the individual metabolic pathways (data shown in table 2). Acetaminophen clearance to glutathione-derived metabolites, i.e. clearance to acetaminophen–cysteine and acetaminophen–mercapturate (table 2) was apparently not changed. However, the minimum statistically significant decrease that could have been detected, given the number of individuals used in this study and the variation observed (assuming a power requirement of 80% and a significance level of 5%), was calculated to be 22%.

The total urinary recovery of acetaminophen and the measured metabolites was consistent with earlier reports, $93 \pm 25\%$ (mean $\pm$ SD).

The effect of hepatic cirrhosis on acetaminophen clearance

This study also provided the opportunity to examine the effect of liver cirrhosis on acetaminophen clearance (see figure 1).

The plasma clearance of acetaminophen was reduced slightly from 0·268 l/min in the group without hepatic cirrhosis to 0·230 l/min in patients with liver cirrhosis. However, this difference did not reach statistical significance, despite galactose elimination being significantly ($P < 0{\cdot}05$) reduced in patients with hepatic cirrhosis ($1{\cdot}17$ mmol/min $\pm$ 0·31 (mean $\pm$ SEM) vs. $1{\cdot}75 \pm 0{\cdot}42$, in the control).

Discussion

We have shown that disulfiram reduces acetaminophen plasma clearance, although only to a small degree. At the conventional aversion therapy dose of 200 mg, corresponding to 3–4 mg per kg body weight daily, the reduction was about 10% and could not be related to any of the major metabolic pathways, i.e. sulphation or glucuronidation. The formation of glutathione-derived metabolites was reduced, but did not reach statistical significance. Therefore it can be inferred that the conventional aversion dose of disulfiram to man is too small to inhibit cytochrome P-450 isozyme activity forming the toxic metabolite of acetaminophen to any significant degree. Thus disulfiram is unlikely to have any modulating effect on an overdose of acetaminophen. The clearance values for the quantitatively dominant metabolic pathways of acetaminophen, i.e. sulphation and glucuronidation, were consistent with earlier reports (Forrest *et al.* 1979, Villeneuve *et al.* 1983), as too were the values for the clearance of the drug in cirrhotics and controls (Andreasen and Hutters 1979).

In rats we have earlier demonstrated that disulfiram, given at 100 mg per kg body weight, protected against the hepatotoxicity of acetaminophen and that excretion of glutathione-derived acetaminophen metabolites (Jorgensen *et al.* 1988) was reduced. This suggested a reduced formation of the reactive metabolite of acetaminophen. This contention was further supported by the major reduction in P-450 activity in rat liver microsomes (80%) seen after disulfiram and by the dose-dependent inhibitory effect (Melandri *et al.* 1980). In man we have recently reported a considerable reduction in the clearance of antipyrine after the same disulfiram dose as in the present study (Loft *et al.* 1986) also demonstrating inhibition of P-450. The dose of disulfiram in the present study, 3–4 mg per kg, was considerably lower than

that used in earlier animal studies and did not apparently significantly reduce the clearance of acetaminophen to its glutathione-conjugated metabolite.

The results of the present study have not revealed any major effect of liver cirrhosis on acetaminophen clearance. This is consistent with earlier data in which cirrhosis patients were not considered to be more susceptible to acetaminophen overdose than others without such disease (Villeneuve *et al.* 1983).

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