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Effect of ethoxyquin on the carbon tetrachloride-induced changes in rat hepatic microsomal enzymes

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Recent studies on the hepatotoxic effects of carbon tetrachloride (CCl_4) have related the toxic effects of CCl_4 to the activity of the hepatic cytochrome P-450 dependant-mixed function oxidases.[1,2] It is now believed that the toxic effects are mediated by an active metabolite of CCl_4.[3] In accordance with this view, pre-treatment of rats with phenobarbitone or DDT produces an increase in the rate of metabolism of CCl_4;[2–4] an increased destruction of cytochrome P-450 and drug metabolizing enzyme activity;[5–7] more extensive liver necrosis[8] and a reduction in the LD_{50} of the toxicant.[1–3] Further support for the central role of the drug metabolizing system in development of CCl_4 hepatotoxicity is provided by the findings that treatments that reduce the activity of the enzyme system (such as low-protein diets,[1,2] small doses of carbon disulphide[9] or inhibitors of drug metabolism such as SKF 525A[10] and disulphiram[11]) decrease CCl_4 hepatotoxicity.

In an earlier publication,[12] it was shown that 6-ethoxy-2,2,4-trimethyl-1-2-dihydroquinoline (ethoxyquin) given to rats 48 hr before CCl_4 protected them from a normally lethal dose of CCl_4. Ethoxyquin also prevented the CCl_4-induced necrosis and hepatic fat accumulation. Whilst ethoxyquin is a powerful antioxidant *in vitro*, the inability to detect a significant concentration of ethoxyquin in the liver at the time of dosage with CCl_4 suggested that the theory of antioxidant protection could not be invoked to explain the action of ethoxyquin. In fact, it was shown that ethoxyquin was an inducer of hepatic microsomal drug metabolizing enzymes and it was suggested that this could be the basis of its protective action against CCl_4-induced hepatotoxicity.[12] However, in contrast to the action of phenobarbitone, ethoxyquin presumably induces a metabolic pathway that leads to a reduction in the concentration of the postulated toxic metabolite.

Among the early effects of CCl_4 toxicity is the loss of drug metabolizing enzymes and glucose-6-phosphatase, perhaps due to the close proximity of these enzymes to the site of production of the postulated toxic metabolite. It therefore seemed useful to study the effects of ethoxyquin on the CCl_4-induced changes in these enzymes. In these studies, the rats were killed by cervical dislocation 24 hr after CCl_4 treatment, and their livers removed. Aminopyrine demethylase[13] and glucose-6-phosphatase activities[14] were measured as described previously. Hexobarbitone oxidase and aniline hydroxylase activities were determined by the methods of Gilbert and Golberg.[15] The amount of cytochrome P-450 was determined spectrophotometrically as described by Omura and Sato.[16] Protein concentrations were measured by the method of Lowry *et al.*[17] using bovine serum albumin as standard.

Our results (Table 1) show that, in control rats, CCl_4 produces a loss of cytochrome P-450 and a loss of activity of drug metabolizing enzymes and glucose-6-phosphatase. These findings are in agreement with the results of other workers.[6,18,19] Pretreatment of rats with ethoxyquin decreased the effect of CCl_4 on all the enzyme activities although it was without effect on the CCl_4-induced destruction of cytochrome P-450.

TABLE 1. EFFECT OF CCl_4 AND ETHOXYQUIN ON HEPATIC MICROSOMAL ENZYME ACTIVITY AND CYTOCHROME P-450

	Glucose-6-phosphatase (μmoles/phosphate liberated/mg protein per hr)	Aminopyrine demethylase (μmoles formaldehyde formed/g liver per hr)	Hexobarbitone oxidase (μ moles hexobarbitone metabolized/g liver per hr)	Aniline hydroxylase (μmoles 4-amino phenol formed/g liver per hr)	Cytochrome P-450 (nmoles/mg protein)
Control	4·48 ± 0·89 (3)	3·54 ± 0·48 (8)	7·19 ± 0·83 (3)	0·62 ± 0·04 (4)	0·31 ± 0·02 (4)
CCl_4	1·72 ± 0·46 (3)	0·94 ± 0·16 (8)*	4·88 ± 1·18 (3)	0·15 ± 0·02 (4)*	0·12 ± 0·04 (4)†
Control (%)	38·4	26·7	58·2	24·8	40·6
Ethoxyquin	3·16 ± 0·45 (3)	3·16 ± 0·49 (8)	6·26 ± 1·63 (3)	0·58 ± 0·06 (4)	0·36 ± 0·10 (4)
Ethoxyquin + CCl_4	2·94 ± 0·07 (3)	1·83 ± 0·24 (8)‡	5.55 ± 1.25 (3)	0·27 ± 0·03 (4)†	0·13 ± 0·01 (4)‡
Control (%)	93·1	58·0	88·7	46·8	35·4

Male Wistar CFHB rats (170–200 g) were fed the diet FFG *ad lib*. Ethoxyquin (500 mg/kg), diluted with methyl oleate (1:1), was administered orally 72 hr before the animals were killed. CCl_4 was administered orally 24 hr before the rats were killed for the enzyme assay. Results are given as mean ± S.E. No. of animals is given in parentheses.

* Significantly lower than similar animals not given CCl_4 ($P < 0·001$).

† Significantly lower than similar animals not given CCl_4 ($P < 0·01$).

‡ Significantly lower than similar animals not given CCl_4 ($P < 0·05$).

Our findings on the effect of ethoxyquin are similar to those observed by Stripp *et al.*[20] in their study on the protective effect of 3-methylcholanthrene against CCl_4-induced loss of hepatic drug metabolizing enzyme activities. These authors also suggested that CCl_4 can be metabolized through alternative toxic and non-toxic pathways and an inducer such as 3-methylcholanthrene might affect these processes.

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