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A possible cytochrome P-450-mediated *N*-oxidation of diethylcarbamazine

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There was a marked sex difference in the *N*-oxidation of diethylcarbamazine (DEC) in the rat. In 72 h, 41 and 20% of the administered DEC was excreted in urine as the *N*-oxide in male and female rats respectively. Safrole, metyrapone and ethanol inhibited this *N*-oxide formation in male rats by 71, 37 and 44% whereas in female rats the values were 20, 25 and 0% respectively. Phenobarbitone and anthracene enhanced *N*-oxide formation in male rats by 17 and 20% and in female rats by 50 and 90% respectively, but the amount of the *N*-oxide formed was more in the males. The result suggests a possible involvement of a sub-population of cytochrome P-450 isozyme (which is more in male rats) in the *N*-oxidation of DEC.

Cytochrome P-450-dependent monooxygenases are inducible microsomal enzymes that catalyse the oxidation of many structurally diverse substrates. Eight different forms of cytochrome P-450 have been purified to electrophoretic homogeneity and are inducible to varying degrees by different xenobiotics (Ryan et al 1982; Guengerich et al 1982). Kato & Kamataki (1982) have provided evidence for the involvement of multiple forms of cytochrome P-450 in the occurrence of differences in the oxidative metabolism of drugs according to sex.

Recently, Parkinson et al (1982) have shown that metyrapone, a known cytochrome P-450 inhibitor (Netter et al 1967) binds to cytochromes P-450b, P-450e but not to P-450a, P-450c and P-450d (Ryan et al 1982). *N*- and *S*-Oxidations have been shown to be mediated via both cytochrome P-450 and FAD—containing monooxygenases (Ziegler et al 1969). The major metabolites of diethylcarbamazine (DEC) are the *N*-oxide and *N*-de-ethylated product, other, minor, metabolites such as piperazine, *N*-methylpeperazine and diethylcarbamylpiperazine are also formed (Faulkner & Smith 1972).

To evaluate the type of monooxygenase system and possible sub-population of cytochrome P-450 isozyme involved in the *N*-oxidation of DEC, we have examined the influence of sex and some drugs on this metabolic process in-vivo in rats.

Materials and methods

DEC citrate was purchased from Sigma, dipropylacetamide was from our laboratory; metyrapone (Metopirone) was a gift from CIBA-GEIGY Lagos, Nigeria. Phenobarbitone, anthracene, safrole, ethanol and titanous chloride (12·5% w/v $TiCl_3$ in 15% w/v HCl) were from BDH.

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Preparation of DEC-N-oxide. Diethylcarbamazine (1 g, 0·005 mol) and *m*-chloroperoxybenzoic acid (2·0 g, 0·0128 mol) were added to chloroform, 50 ml (previously cooled to 0 °C) and the mixture allowed to warm to room temperature with stirring. The mixture was stirred for a further 20 min and solvent removed under reduced pressure. This was followed by purification of the *N*-oxide on a basic alumina column. The labile *N*-oxide (oil) was stored at −20 °C as such.

Found C, 59·9; H, 10·1; N, 13·6 calculated for $C_{10}H_{21}N_2O_2$: C, 59·7; H, 10·4; N, 13·9%.

Animal treatment. Wistar albino rats (170–200 g) were housed individually in metabolism cages with free access to food and water. Groups of 15 rats were pretreated intraperitoneally for five days with the following: 0·9% NaCl (saline) (0·2 ml), safrole 25% v/v (0·2 ml), ethanol 25% v/v (0·2 ml), metyrapone (50 mg kg^{-1}), phenobarbitone (30 mg kg^{-1}) or anthracene (50 mg kg^{-1}). DEC (20 mg kg^{-1}) was co-administered intraperitoneally with metyrapone and safrole on the fifth day but administered alone to the other groups 24 h after the last drug pretreatment. Urine samples were collected every 24 h for 3 days and frozen at −15 °C until assayed.

Assay. Urine samples (1 ml) were made alkaline with saturated $KHCO_3$ (pH 9) and extracted with n-hexane (1 ml) for 5 min using a Gallenkamp flask shaker. Another sample of urine (1 ml) was treated with $TiCl_3$ (0·02 ml) at pH 3 to reduce the DEC-*N*-oxide to DEC, before being processed.

The aqueous layer was removed with a Pasteur pipette and the n-hexane fraction (0·5 µl) was injected into a Varian 3740 gas chromatograph with thermionic specific detector. The glass column (2 m × 2 mm, i.d.) was packed with 10% DEGS on chromosorb W, HP 80/100 mesh. Injection port temperature was 200 °C, column 180 °C and detector 240 °C. Nitrogen flow rate was 30 ml min^{-1}, hydrogen 4·2 ml min^{-1} and air 175 ml min^{-1}.

The internal standard, dipropylacetamide was added to samples before extraction at a concentration of 20 µg ml^{-1}. DEC in the urine sample was measured by the ratio of its peak height or area to that of the internal standard using a Varian CDS III integrator. DEC-*N*-oxide was quantified as the difference in the amounts of DEC in urine before and after treatment with $TiCl_3$.

Table 1. Effect of some chemicals on the *N*-oxidation of DEC in male rats.

	% dose excreted in 72 h as		
	DEC	DEC-*N*-oxide	% Effect
Control	34 ± 2	41 ± 2	
Safrole	43 ± 4	12 ± 4	71−
Metyrapone	35 ± 4	26 ± 4	37−
Ethanol	37 ± 3	23 ± 3	44−
Phenobarbitone	14 ± 4	48 ± 4	17+
Anthracene	24 ± 3	52 ± 3	27+

− = Inhibition. + = Enhancement.

Results

The result shows a marked sex difference in the *N*-oxidation of DEC (see Tables 1, 2). About 41 and 20% of the administered DEC were excreted as the *N*-oxide in male and female rats respectively.

Safrole, metyrapone and ethanol inhibited this *N*-oxide formation in male rats by about 71, 37 and 44%, whereas in female rats the values were 20, 25 and 0% respectively. Phenobarbitone and anthracene enhanced the *N*-oxide formation in male rats by about 17 and 20% and in female rats by 50 and 90% respectively.

Table 2. Effect of some chemicals on the *N*-oxidation of DEC in female rats.

	% dose excreted in 72 h as		
	DEC	DEC-*N*-oxide	% Effect
Control	48 ± 3	20 ± 3	
Safrole	44 ± 3	16 ± 3	20−
Metyrapone	41 ± 2	15 ± 2	25−
Ethanol	45 ± 2	20 ± 2	0
Phenobarbitone	25 ± 1	30 ± 1	50+
Anthracene	34 ± 2	38 ± 2	90+

− = Inhibition. + = Enhancement.

Discussion

The enhanced *N*-oxidation of DEC by both phenobarbitone and anthracene pretreatment and the inhibition by both safrole and metyrapone suggest that at least a part involvement of cytochrome P-450 in this oxidation pathway. There is a possibility that the FAD-containing monoxygenase also contributes to the *N*-oxidation of DEC. Gorrod & Damani (1979) did report that heteroaromatic *N*-oxidation is not mediated via the microsomal flavin monooxygenase, which oxidizes tertiary aliphatic, alicyclic and aromatic amines, but is catalysed by a phenobarbitone-inducible cytochrome P-450 system. It has been shown in this laboratory that safrole and metyrapone inhibit the 7-hydroxylation of chlorpromazine in-vivo, a reaction mediated by cytochrome P-450, but do not inhibit the *S*-oxidation which might be mediated via an FAD-containing monooxygenase (Oforah & Dixon 1983).

The inhibitory effect of ethanol on the *N*-oxidation of DEC in the male rats only, is of interest. Ethanol is known to induce a particular form of cytochrome P-450 (Ingelman-Sundberg & Hagbjork 1982), a process which could lead to a suppression of other cytochrome P-450(s) (Ryan et al 1982). Ethanol is metabolized via a cytochrome P-450-dependent hydroxyl radical-mediated oxygenation mechanism (Ingelman-Sundberg & Hagbjork 1982), a process which could be competing with the *N*-oxidation of DEC. But this does not explain the observed sex difference, in its inhibitory effect.

Usually there is about 20% difference in the amount of cytochrome P-450 between male and female microsomes (more in male) but Schenkman et al (1967) and Kato & Onoda (1970) have demonstrated that microsomal cytochrome P-450 from male rats showed different affinities from that of female rats for binding with certain drug substances. For example, ethoxyresorufin *O*-de-ethylation activity is higher in female microsomes than in male microsomes (Vodicnik et al 1981). The administration of morphine is known to decrease hexobarbital hydroxylation only in male rats (Kato 1974). Kato & Kamataki (1982) have provided evidence for the involvement of multiple forms of cytochrome P-450 in the occurrence of the sex difference. One of such sub-population of cytochrome P-450 could be involved in the *N*-oxidation of DEC.

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