INDUCTION OF DRUG-METABOLIZING ENZYMES BY TRICYCLIC ANTIDEPRESSANTS IN HUMAN LIVER: CHARACTERIZATION AND PARTIAL RESOLUTION OF CYTOCHROMES P-450

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- 1 Drug-metabolizing enzyme activities were determined in liver microsomes from six kidney-transplant donors, one tricyclic antidepressant-treated and five untreated donors.
- 2 The tricyclic antidepressant treatment modifies neither the overall cytochrome P-450 content of the liver, nor enzymatic activities of 4-nitroanisole demethylase, aniline hydroxylase, epoxide hydrolase and glutathione S-transferase.
- 3 Only benzphetamine and ketotifen demethylation and conjugation of bilirubin with UDP-glucuronic acid are markedly augmented (more than two-fold).
- 4 Separation of the different cytochrome P-450 fractions on a DEAE cellulose column indicates a modification of the elution pattern: the fraction increased by tricyclic antidepressants is responsible for the enhanced monooxygenase activity towards benzopyrene and benzphetamine.

Keywords enzyme induction tricyclic antidepressants cytochrome P-450

Introduction

Hydrophobic molecules are transformed in living organisms by a multistep system which allows their elimination under hydrosoluble forms: molecules are first oxidized and then conjugated with UDP-glucuronic acid, sulphate or glutathione (Lu & West, 1978; Dutton & Burchell, 1977). In the liver of laboratory animals, enzymes catalyzing these reactions have been shown to be present as different isoenzymes exhibiting a broad specificity, and are differently affected by the pretreatment with some inducers (Conney, 1967). These molecules increase the synthesis of specific isoenzymes and have been classified in two main families. The first one includes phenobarbitone and is associated with an increase of cytochrome P-450 species which catalyze preferentially 4-nitroanisole and benzphetamine demethylation, and a rise of bilirubin UDP-glucuronosyltransferase, epoxide hydrolase and glutathione S-transferase activities (Ernster & Orrenius, 1965; Gram *et al.*, 1967; Oesch *et al.*, 1971; Hales & Neims, 1977). In the second family, polycyclic aromatic hydrocarbons, exemplified by 3-methylcholanthrene, increase other cytochrome P-450 species associated with benzopyrene hydroxylase activity and 4-nitrophenol UDP-

glucuronosyltransferase activity (Conney *et al.*, 1956; Conney, 1967; Bock *et al.*, 1973). Some other molecules such as pregnenolone-16-carbonitrile and aroclor 1254 give rise to either different or mixed induction patterns. In man, a relationship between drug administration and pharmacokinetic modifications has been established: barbiturates shorten the half-life of anticoagulants (Robinson & McDonald, 1966), chlorpromazine accelerates the plasma disappearance of antipyrine (Kolakowska *et al.*, 1975), rifampicin is able to inactivate oral contraceptives (Reimers & Jezek, 1971), but only little direct information is available on the modification of drug-metabolizing enzymes during or after therapy (Black *et al.*, 1973; Lecamwasam, 1975; Bolt *et al.*, 1975; Sotaniemi *et al.*, 1978). Several studies described monooxygenase activities in biopsies or in liver samples from renal transplant donors (Beaune *et al.*, 1979; Boobis *et al.*, 1980; Wang *et al.*, 1980; Kremers *et al.*, 1981). During the time-course of these investigations, a piece of liver from a depressive patient treated with tricyclic antidepressants and occasionally with benzodiazepines, was obtained. Drug metabolizing enzyme activities were measured in microsomes

and compared to activities from untreated livers, in order to evaluate modifications due to the drugs. In this way, demethylation of structurally related compounds (chlorcyclizine and ketotifen) has been studied. In addition, cytochromes P-450 were separated on DEAE-cellulose and monooxygenase activities were reconstituted on the obtained fractions.

Methods

Materials

[³H]-benzo(a)pyrene 4,5-oxide was a gift from P.M. Dansette. Benzphetamine chlorhydrate, chlorcyclizine chlorhydrate and ketotifen fumarate were kindly provided by Upjohn Company, Burroughs Wellcome and Sandoz respectively. All other reagents were from commercial sources.

Liver samples were obtained from donors for kidney transplantation. One of them (34-year-old male) was treated for depression with tricyclic antidepressants and benzodiazepines (ATC treatment). The prescription was 100 mg amitriptyline (Laroxyl), 90 mg trimipramine (Surmontil) and 100 mg clomipramine (Anafranil) per day and occasionally 5 mg lorazepam (Temesta) and 10 mg diazepam (Valium). Prior to kidney removal, only traces of tricyclic antidepressants and benzodiazepines were detected in the plasma. Other donors (four males and one female aged from 24 to 34 years) had no therapeutic 'histories'. No information was available about smoking habits. This latter point is probably not of great importance, since it has been reported that smoking is without effect on the demethylation of diazepam and nortriptyline (Jusko, 1978).

Microsomes were prepared as previously described (Kremers *et al.*, 1981). Proteins were measured according to Lowry *et al.* (1951), and cytochrome P-450 determined according to the method of Greim (1970) in liver homogenate or to Omura & Sato (1964) in microsomes.

Enzymatic determinations

Aniline hydroxylation and 4-nitroanisole demethylation (Mazel, 1971), benzo(a)pyrene hydroxylation (Van Cantfort *et al.*, 1977), ethoxycoumarin deethylation (Aitio, 1978) and the demethylation of benzphetamine, chlorcyclizine and ketotifen estimated by the formation of formaldehyde (Nash, 1953) were assayed as previously described (Kremers *et al.*, 1981). Conditions for the demethylation of 1 mM chlorcyclizine and 1 mM ketotifen were verified to be optimal as a function of incubation time, protein and substrate concentrations. 16 α -hydroxylation of

steroids was conducted as previously described (Kremers *et al.*, 1981).

UDP-glucuronosyltransferase was determined in Triton X-100-treated microsomes with bilirubin (Van Roy & Heirwegh, 1968) or 4-nitrophenol (Gorski & Kasper, 1977) as substrates. Microsomal epoxide hydrolase and cytosolic glutathione S-transferase activities were respectively estimated with benzo(a)pyrene 4,5-oxide and chlorodinitrobenzene as substrates (Jerina *et al.*, 1977; Habig *et al.*, 1974). Electrophoresis were performed on 10% polyacrylamide gels (0.75 mm thick) in the presence of sodium dodecylsulphate with 13 μ g microsomal proteins or 5 μ g proteins from separated fractions (Laemmli, 1970).

Cytochrome P-450 fractions were obtained by DEAE-cellulose chromatography of 100 nmol of cytochrome P-450 solubilized from microsomes, in accordance with the procedure of Warner *et al.* (1978) modified as previously described (Le Provost *et al.*, 1981). Cytochrome P-450 was determined on each fraction and detergents were eliminated from concentrated fractions (Amicon PM 30) by calcium phosphate treatment (Coon *et al.*, 1978). Reconstitution experiments were conducted as reported elsewhere (Le Provost *et al.*, 1981).

Results

Drug-metabolizing activities are shown in Table 1. Activities from the five untreated liver samples are given irrespective of the sex, since no differences occurred between males and females with the endogenous and exogenous substrates used in this study; the range of values is in agreement with the previously reported one (Kremers *et al.*, 1981). The cytochrome P-450 content of the liver homogenate or microsomal fraction was not modified by ATC-treatment. No evident modification of SDS-polyacrylamide gel electrophoresis occurred in microsomes from ATC-treated vs untreated liver samples (data not shown). Among monooxygenase activities towards xenobiotics, only benzo(a)pyrene hydroxylation (2.2-fold increased), benzphetamine (2.2-fold) and ketotifen (3.3-fold) demethylations were markedly enhanced, whereas aniline hydroxylation and 4-nitroanisole demethylation remained unchanged. Chlorcyclizine demethylation and ethoxycoumarin deethylation were moderately increased (1.6-fold), but ethoxycoumarin deethylation was not more sensitive to 7,8-benzoflavone or metyrapone inhibition in ATC-treated liver samples than in untreated ones. For ketotifen demethylation, K_m remained nearly constant whereas velocity value was noticeably increased in ATC-treated patients. No change in 16 α -hydroxylation of steroids occurred. Other drug-metabolizing enzymes were differently affected by

Table 1 Some monooxygenase and other drug-metabolizing enzyme activities in human liver samples from untreated and ATC-treated patients.

	Untreated samples (n = 5)	ATC-treated sample
Cytochrome P-450 ^a _b	10.2 (8.9–12.8) 0.32 (0.22–0.41)	10.3 (101%) 0.38 (119%)
Aniline hydroxylase ^c	0.21 (0.11–0.29)	0.29 (137%)
4-nitroanisole demethylase ^c	1.44 (0.74–2.00)	1.71 (119%)
Benzphetamine demethylase ^c	1.70 (1.04–2.33)	3.70 (218%)
Ketotifen demethylase ^c 1 mM <i>K_M</i>	0.48 (0.36–0.75) 110 μ M	1.71 (356%) 90 μ M
Chlorcyclizine demethylase ^c	0.08 (0.06–0.11)	0.13 (165%)
Benzopyrene hydroxylase ^c	0.16 (0.09–0.24)	0.36 (220%)
Ethoxycoumarin deethylase ^c	0.17 (0.14–0.26)	0.28 (166%)
inhibition 10 ⁻⁵ M metyrapone	5%	9%
inhibition 10 ⁻⁵ M ANF	30%	33%
Progesterone 16 α -hydroxylase ^d	10.5 (9.0–13.0)	11.0 (105%)
Dehydroepiandrosterone 16 α -hydroxylase ^d	42 (27–83)	46 (112%)
Epoxide hydrolase ^c	6.61 (6.04–7.16)	7.83 (118%)
Glutathione S-transferase ^c	0.56 (0.41–0.68)	0.67 (120%)
UDP-glucuronosyltransferase ^c bilirubin	0.34 (0.12–0.62)	0.76 (223%)
4-nitrophenol	15.1 (6.5–24.5)	24.2 (160%)

Results are mean for determinations of five different human livers with extreme values between brackets and expressed as:

^a nmol g⁻¹ liver

^b nmol mg⁻¹ microsomal protein

^c nmol min⁻¹ mg⁻¹ microsomal protein

^d pmol min⁻¹ mg⁻¹ microsomal protein

^e μ mol min⁻¹ mg⁻¹ protein from 100,000 supernatants

ATC treatment: benzo(a)pyrene, 4,5-oxide hydroxylase, chlorodinitrobenzene glutathione S-transferase and 4-nitrophenol UDP-glucuronosyltransferase activities were only slightly augmented, whereas bilirubin UDP-glucuronosyltransferase activity was increased by ATC treatment (2.2-fold).

An attempt to correlate enhanced monooxygenase activities and induction of a specific form of cytochrome P-450 was made: the relative percentages of different cytochrome P-450 fractions recovered after DEAE-cellulose chromatography are shown in Table 2. The pattern of cytochrome P-450 fractions and the reconstituted monooxygenase activities in the present work were in agreement with those previously reported in human liver (Cresteil *et al.*, 1982). Results clearly show that fraction Ba from ATC-treated liver was notably increased at the expense of fraction A when compared to untreated liver, since specific activities of cytochrome P-450 in microsomes were roughly similar (0.38 to 0.30 nmol mg⁻¹ protein). This allows one to calculate specific activities for each fraction of cytochrome P-450: 0.22 and 0.06 nmol mg⁻¹ protein for fractions A and Ba from untreated samples vs 0.06 and 0.28 nmol mg⁻¹

protein in ATC-treated samples. Rechromatographed on DEAE-cellulose, the fraction Ba from ATC-treated sample was resolved into a single peak, which excluded an artefact in chromatographic behaviour. Reconstitution experiments confirmed results obtained in microsomes. Aniline hydroxylase was mainly catalyzed by fraction A (Beaune *et al.*, 1980) and was unchanged by ATC-treatment. On the other hand, a marked increase of the benzphetamine demethylation rate was observed in fraction Ba from ATC-treated liver. A similar pattern was obtained for benzo(a)pyrene hydroxylation. So, the increase of benzo(a)pyrene hydroxylation and benzphetamine demethylation observed in microsomes, is due to both a considerable change in the pattern of cytochrome P-450 fractions and an increase of catalytic activity towards these substrates.

Discussion

In man, benzodiazepines produce only slight modifications of drug metabolism: (i) the long-term dosage

Table 2 Recovery of cytochrome P-450 and reconstitution of monooxygenase activities in fraction from tricyclic antidepressant-treated and untreated human liver samples

	Untreated liver sample	ATC-treated liver sample
<i>Percentage of cytochrome P-450</i>		
in fraction A	73	16
Ba	21	73
Bb	6	5
Bc	—	6
<i>Aniline hydroxylase</i>		
in microsomes	1.13	1.15
in fraction A	0.48	0.41
Ba	0.29	0.19
Bb	nd	0.16
<i>Benzphetamine demethylase</i>		
in microsomes	4.6	9.7
in fraction A	0.16	<0.050
Ba	1.11	3.97
Bb	nd	0.72
<i>Benzopyrene hydroxylase</i>		
in microsomes	0.51	0.93
in fraction A	0.024	<0.010
Ba	0.091	0.169
Bb	nd	<0.010

Percentage of cytochrome P-450 are calculated using total cytochrome P-450 recovered after chromatography as 100 per cent.

Enzymatic activities expressed as nmol products formed in 1 min by 1 nmol cytochrome P-450, are the results of typical experiments performed in duplicate.
nd: not determined.

of diazepam slows its own elimination in healthy subjects, as well as in patients with liver diseases (Klotz *et al.*, 1976, 1977), (ii) administration of lorazepam does not modify plasma half-life of antipyrine (Greenblatt *et al.*, 1979) and (iii) no significant effect of the simultaneous administration of four benzodiazepines was found on amitriptyline and nortriptyline plasma level (Silverman & Braithwaite, 1972, 1973; Dugal *et al.*, 1975). Thus, in view of these pharmacokinetic data, no apparent inductive effect of benzodiazepines seems to occur in man.

The administration of tricyclic antidepressants leads to conflicting results in man: for O'Malley *et al.* (1973), chronic treatment with tricyclic antidepressants resulted in a reduction of antipyrine half-life, suggesting an induction of drug-metabolizing enzymes. Nortriptyline was also shown to impair the metabolism of dicoumarol and antipyrine (Vesell *et al.*, 1970), whereas Vesell *et al.* (1975) and Pond *et al.* (1975) failed to find any alteration of drug metabolism. On the other hand, no induction of cytochrome P-450 and related monooxygenase activities was noted after treatment of rats with nortriptyline (Vesell *et al.*, 1975). In the present study, human monooxygenase activities were shown to be differently affected by pretreatment with a cocktail of tricyclic antidepress-

sants and benzodiazepines: neither the overall cytochrome P-450 content, nor activities towards aniline, 4-nitroanisole and steroids in position 16 α were markedly augmented. A second group of substrates, including chlorcyclizine and ethoxycoumarin, exhibited a moderately enhanced metabolism. Lastly, benzo(a)pyrene hydroxylation, benzphetamine and ketotifen demethylations were more doubled, as well as bilirubin UDP-glucuronosyltransferase activity.

Two points can be taken into consideration to comment on the inducing effects of ATC treatment: (i) tricyclic antidepressants were prescribed in appreciable amounts for a long time, whereas the patient received benzodiazepines only occasionally, (ii) no inductive effect of benzodiazepines on drug metabolism can be deduced from pharmacokinetic data (Dugal *et al.*, 1975). Therefore, the observed changes in drug-metabolizing enzyme activities might be mainly, if not fully, attributed to tricyclic antidepressants. It might also be postulated that tricyclic antidepressants induce enzyme forms specifically involved in their own metabolism. In this view, the important increase of ketotifen demethylation is of interest, since ketotifen and amitriptyline exhibit structural similarities (Figure 1). The demethylase activity towards another structurally related com-

Figure 1 Structure of tricyclic antidepressants and related compounds used in this study.

pound, chlorcyclizine, is also increased, but to a smaller extent. However, ATC also enhances drug-metabolizing activities which are not related to tricyclic antidepressant metabolism, e.g. benzphetamine demethylase, benzo(a)pyrene hydroxylase and ethoxycoumarin deethylase.

These data represent the first direct demonstration of a modification of the pattern of cytochrome P-450 species in response to drug administration in man.

Further studies are now in progress in order to obtain a better characterization of the inducing effect of tricyclic antidepressants upon drug-metabolizing enzymes in man.

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