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INHIBITION OF HUMAN HEPATIC AND PLACENTAL XENOBIOTIC MONOOXYGENASES BY IMIDAZOLE ANTIMYCOTICS

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Abstract—Three imidazole antimycotic drugs, ketoconazole, clotrimazole and miconazole, were studied to characterize the inhibition of aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin *O*-deethylase (ECDE) and 7-ethoxyresorufin *O*-deethylase (ERDE) activities in human liver and placenta *in vitro* in comparison with liver enzymes from control, phenobarbital (PB) and 3-methylcholanthrene (MC) pretreated rats. All three compounds inhibited rat liver enzymes, although MC pretreatment seemed to lead to a resistance of inhibition relative to PB-treated and control animals. There were large differences in the extent of inhibition of human hepatic and placental activities. Furthermore, while the type of inhibition of the hepatic ERDE was competitive or mixed, that of the placental enzyme cannot be described in ordinary terms of inhibition kinetics. Ketoconazole and clotrimazole were relatively potent inhibitors of maternal cigarette smoking-induced placental ECDE activities (IC_{50} values from 0.5 μM to 5 μM), whereas much less inhibition of the placental AHH activity was obtained with ketoconazole and miconazole (IC_{50} values from 50 μM to 500 μM). In most cases, hepatic enzymes were less sensitive to antimycotics than placental activities. This was in contrast with results from rat enzyme studies, in which MC pretreatment seemed to decrease the inhibitory response.

The mechanism of action of imidazole antimycotics is thought to be the inhibition of conversion of lanosterol to ergosterol in yeast at nanomolar concentrations whereas those concentrations required to inhibit other cytochrome P-450 catalyzed sterol demethylation activites in mammalian cells are at a micromolar range [1–3]. Nevertheless, ketoconazole has been shown to inhibit some cytochrome P-450-dependent steroid oxidations in gonadal, adrenal, placental and hepatic tissues in both experimental animals and man [4–9]. Recently, studies of Sheets and Mason [10] and Meredith *et al.* [11] demonstrated that ketoconazole inhibits xenobiotic metabolism in rat liver microsomes *in vitro*. *In vivo* elimination of aminopyrine and caffeine in rat was also impaired [11]. Furthermore, antimycotics may cause liver damage, the mechanism of which could be metabolic activation [12, 13], implicating the interaction with cytochrome P-450 [14, 15]. Recent studies [16, 17] demonstrated the inhibition of rat liver xenobiotic-metabolizing enzymes by ketoconazole, clotrimazole, miconazole and itraconazole.

Antimycotic drug therapy is long-lasting in practice. Because of potential drug interactions we studied further the inhibitory effect of three imidazole antimycotics, ketoconazole, clotrimazole and miconazole, on aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin *O*-deethylation (ECDE) and 7-ethoxyresorufin *O*-deethylase (ERDE) activities in human liver and placental preparations. Furthermore, for comparative purposes we studied corresponding inhibitions in rat liver microsomes from control, phenobarbital (PB) and 3-methylcholanthrene (MC) pretreated animals.

MATERIALS AND METHODS

Chemicals. Ketoconazole, clotrimazole and miconazole were obtained from Janssen Chemical Company (Beerse, Belgium). All other chemicals were of the highest grade available and were obtained mainly from E. Merck (Darmstadt, F.R.G.).

Pretreatment of experimental animals. Male Wistar rats were used either without any pretreatment (control) or were given PB (0.5 g/l in drinking water during one week) or MC (25 mg/kg body weight in corn oil i.p. three days). The rats were killed 24 hr after the last treatment and liver microsomal fraction was made as follows. Liver samples were homogenized in 0.1 M sodium-phosphate buffer, pH 7.4, with a Potter-Elvehjem glass homogenizer (20 strokes at 600 rpm). The microsomes from the homogenate samples were isolated by a standard ultracentrifugation technique [18]. Protein determinations were carried out according to Lowry *et al.* with bovine serum albumin as a standard [19].

Human liver samples. Wedge biopsy samples (0.4–1.3 g), which were used for enzyme kinetic studies, were taken in connection with abdominal surgery the cause of which was hepatoma in two cases and exploratory laparatomy in two cases because of suspicion of liver injury. The samples were put immediately in liquid nitrogen and later stored at −70° and thawed just before use. Microsomes were prepared

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¶ Abbreviations used: MC, 3-methylcholanthrene; PB, phenobarbital; AHH, aryl hydrocarbon (benzo(*a*)pyrene) hydroxylase; ECDE, 7-ethoxycoumarin *O*-deethylase; ERDE, 7-ethoxyresorufin *O*-deethylase; IC_{50}, concentration causing a 50 per cent inhibition.

by differential ultracentrifugation as described above.

Needle biopsy homogenates were used for preliminary screening of inhibition of monooxygenases by antimycotics and for the elucidation of approximate IC_{50} values. These samples were surplus tissues from biopsies taken for diagnostic purposes because of suspected liver injury or the involvement of the liver in different chronic diseases. The use of surplus human tissues for investigative purposes has been approved by the Ethical Committee of the Medical Faculty, University of Oulu.

Human placental preparations. Placentas were obtained after normal delivery at term from smoking mothers (smoking status was verified by a plasma cotinine assay [20]). Microsomes were prepared by the standard ultracentrifugation technique [21] and stored at −70° until assayed.

Enzyme assays. The inhibitory effect of various agents on cytochrome P-450 catalyzed enzyme activities were tested with rat liver microsomes and human placental or hepatic microsomes. In every assay control activity has been regarded as the activity measured in the presence of the pure diluent for the inhibitor (H_2O with a few drops of HCl for ketoconazole and clotrimazole; DMSO for miconazole). Inhibitor concentration ranged from 500 to 0.05 μM.

The aryl hydrocarbon hydroxylase (AHH) activity was measured by the fluorometric method of Nebert and Gelboin [22], 7-ethoxycoumarin *O*-deethylase (ECDE) activity according to Greenlee and Poland [23] and the 7-ethoxyresorufin *O*-deethylase (ERDE) activity measurement was carried out according to the end-point fluorometric method of Burke *et al.* [24] as described earlier by Pelkonen *et al.* [20]. The final concentrations of the substrates and lengths of incubation times were 75 μM and 15 min for benzo(*a*)pyrene, 500 μM and 10 min for 7-ethoxycoumarin, and 1 μM and 10 min for 7-ethoxyresorufin. In kinetic studies the range of 7-ethoxyresorufin concentration was between 5 and 0.02 μM, but usually the lowest concentration resulted in values which were too close to blanks to be reliable.

RESULTS

Antimycotic inhibition of rat liver enzymes

All three antimycotic substances inhibited AHH, ECDE and ERDE activities in liver microsomes from variously treated rats (Fig. 1). However, there were some systematic differences in inhibition depending on the pretreatment. The inhibition was most potent in microsomes from control or PB-pretreated rats. IC_{50} values were in most cases between 0.5 μM and 5 μM indicating a powerful inhibitory effect for these antimycotic compounds.

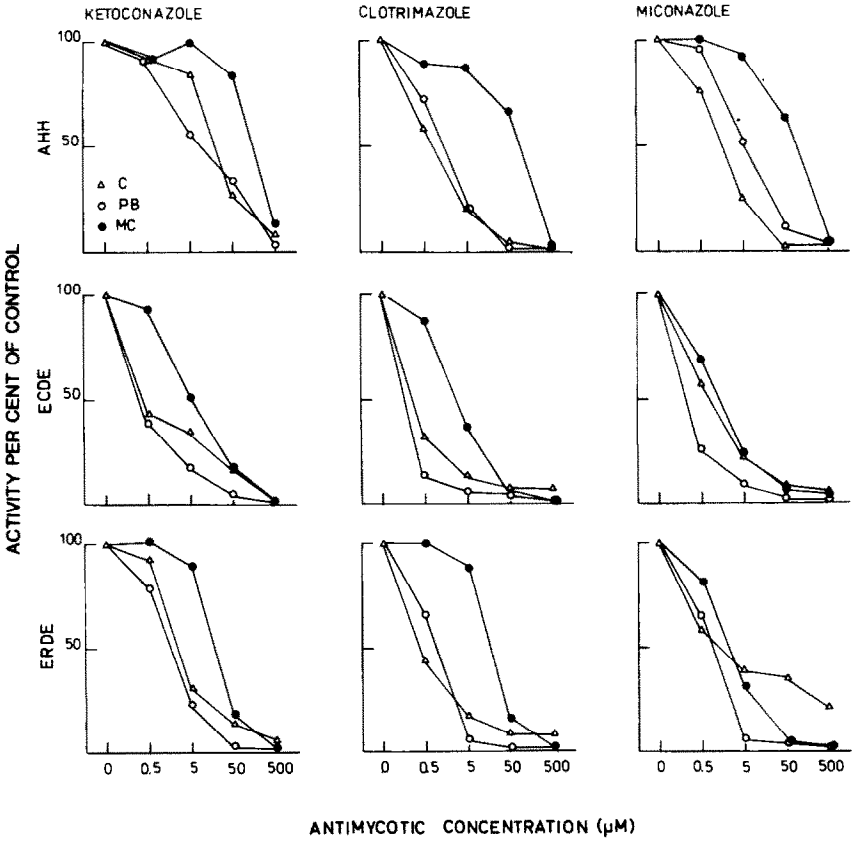

Fig. 1. Inhibition by ketoconazole, clotrimazole and miconazole of aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin *O*-deethylase (ECDE) and 7-ethoxyresorufin *O*-deethylase (ERDE) in liver microsomes from control (C), phenobarbital (PB), and 3-methylcholanthrene (MC) treated rats.

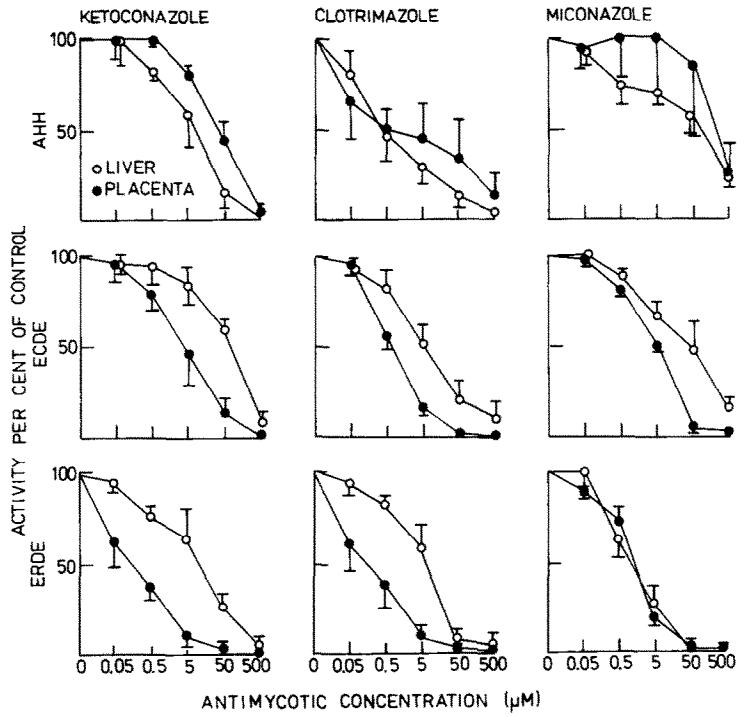

Fig. 2. Inhibition by ketoconazole, clotrimazole and miconazole of AHH, ECDE and ERDE in homogenates from human liver biopsies and in microsomes from placentas from smoking mothers. Inhibition curves are based on experiments with 3–4 liver biopies and 3–4 placental microsomal preparations.

MC-pretreatment seemed to decrease the inhibition of all three activities by ketoconazole and clotrimazole. The difference in the IC_{50} values between PB-microsomes and MC-microsomes was one or two orders of magnitude. Miconazole displayed the same difference in the IC_{50} values of AHH activity in the three animal groups, but not in the inhibition of ECDE or ERDE.

Inhibition of human enzymes by antimycotics

Antimycotics inhibited to a variable extent also human liver and placental enzymes (Fig. 2). Both ketoconazole and clotrimazole inhibited the placental ECDE and ERDE activities much more than corresponding hepatic activities. The IC_{50} values were from about 0.5 μM to 0.05 μM. Miconazole inhibited these enzymes in both organs roughly to the same extent. The placental AHH activity was inhibited rather weakly by ketoconazole and miconazole, whereas the hepatic enzyme was more strongly inhibited. Clotrimazole was the best inhibitor of the AHH activity in both tissues.

Kinetics and inhibition of hepatic ERDE

To characterize further the kinetics of inhibition by antimycotics, the kinetic constants for ERDE activity and inhibition at selected concentrations were determined for four different liver preparations (Table 1). Michaelis–Menten constants were between 0.15 and 0.27 μM and maximal velocities between 0.20 and 1.56 nmol/mg microsomal protein × min. These values are very similar to those reported by Williams *et al.* [25]. Two liver preparations (3C and 5E) displayed apparently biphasic

Table 1. Kinetic characterization of 7-ethoxyresorufin *O*-deethylation in four human liver wedge biopsy microsomes and its inhibition by ketoconazole, clotrimazole and miconazole

	Kinetic constants		Inhibition (per cent of control)		
Liver sample	K_m (μM)	V_{max} (nmol/mg × min)	ketoconazole (50 μM)	clotrimazole (50 μM)	miconazole (5 μM)
1A	0.27	0.203	(55, 60)*	(47, 57)	NT
4D	0.20	1.560	(43, 46)	(31, 46)	(27, 71)
3C	0.045	0.758	56	47	59
	0.148	1.136	66	62	56
5E	0.047	0.389	69	46	39
	0.183	0.426	61	59	89

* The values for liver 1A and 4D were obtained at two substrate concentrations: 0.05 μM and 0.5 μM. Values for the liver samples 3C and 5E represent inhibitions at the respective low (0.045 to 0.05 μM) and high (0.5 μM) substrate concentrations.

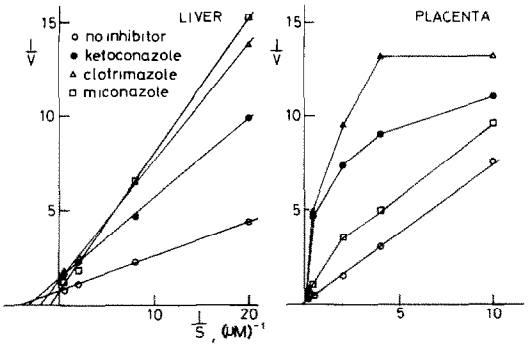

Fig. 3. The graphical analysis of the mode of inhibition of 7-ethoxyresorufin *O*-deethylase activity in human liver microsomes and in human placental microsomes by ketoconazole, clotrimazole and miconazole.

kinetics with an apparent "high-affinity" constant being about 0.045 μM. However, we were not able to study this finding further because of lack of material. Furthermore, because the activity at the lowest substrate concentration used (0.02 μM) was barely above blank, the existence of the "high-affinity" enzyme remained doubtful. Williams *et al.* [25] did not find any high-affinity component of ERDE activity among four liver samples. The enzyme activity was inhibited by all antimycotics at the concentrations selected on the basis of the preliminary screening. We also studied the inhibition at the lower substrate concentration (0.045 to 0.05 μM), but no consistent differences between low and high substrate concentrations were observed.

Type of inhibition by antimycotics

Preliminary experiments demonstrated that rat hepatic ECDE and ERDE were competitively or non-competitively (mixed-type) inhibited by all three antimycotics (data not illustrated). Experiments with human liver microsomes were also somewhat variable with respect to the type of inhibition. In some individual samples and with some inhibitors a mixed-type inhibition of ERDE was demonstrated whereas in some other cases a competitive mode of inhibition was observed (Fig. 3). Type of inhibition of ERDE by antimycotics was tested in three different liver microsomal samples and the only consistent finding was the competitive inhibition by miconazole in all samples. Nevertheless, the analysis of the liver experiments was quite straightforward, because straight lines were obtained in the Lineweaver–Burk plots (Fig. 3). On the contrary, attempts to analyze the mode of inhibition in placental microsomes yielded highly curved plots with ketoconazole and clotrimazole in the Lineweaver–Burk model (Fig. 3). With placental microsomes also, miconazole was somewhat different when compared with two other antimycotics. The explanation for this phenomenon has not been developed yet. It should be noted, that the Lineweaver–Burk plot without an inhibitor for the placental ERDE activity yielded a straight line with Michaelis–Mentan constant ranging from 2.5 to 4 μM with three placental preparations. Hence, one explanation may be the existence of multiple forms of P-450 participating in the reaction.

Table 2. IC_{50} values for antimycotic agents in control (C), phenobarbital (PB) and 3-methylacholanthrene (MC) pretreated rat liver and human liver (HL) and placental (HP) microsomal arylhydrocarbon hydroxylase (AHH) 7-ethoxycoumarin *O*-deethylase (ECDE) and 7-ethoxyresorufin *O*-deethylase (ERDE) activities

	IC_{50} μM				
Antimycotic	C	PB	MC	HL	HP
		AHH			
Ketoconazole	<50	5	<500	5	50
Clotrimazole	<5	<5	<500	5	5
Miconazole	<5	5	<500	<500	<500
		ECDE			
Ketoconazole	<0.5	<0.5	5	<500	5
Clotrimazole	<0.5	<0.5	<5	5	<5
Miconazole	<5	5	<500	50	5
		ERDE			
Ketoconazole	<5	<5	<50	<50	<0.5
Clotrimazole	<0.5	<5	<50	<50	<0.5
Miconazole	<5	<5	<5	<5	<5

DISCUSSION

It has been known for some time that imidazole antimycotic therapy can lead to gynecomastia and possibly other hormonal disturbances because of an interaction with cytochrome P-450 isozymes in the sex steroid-synthesizing organs [7, 26]. These side effects have also prompted the attempts to use ketoconazole in the therapy of prostatic cancer at even higher doses [27]. Consequently, therapeutic uses of antimycotics have widened and thus possible interactions with other drugs have become more likely.

In the present study we demonstrate that all three antimycotics, ketoconazole, clotrimazole and miconazole, inhibit cytochrome P-450-linked xenobiotic-oxidizing activites in human liver and placental microsomes and in liver microsomes from variously treated rats. Thus our studies confirm the results of some earlier investigations with respect to ketoconazole [10, 11, 16], clotrimazole [17] and miconazole [16]. However, there are large differences in the inhibitory potency depending on the inhibitor, metabolic pathway and tissue. Although it has been suggested that antimycotics show considerable preference with respect to P-450 isozymes, the present results yield some confusing and contradictory findings as far as rat and human monooxygenases are concerned.

First, the most important finding with rat liver microsomes seemed to be the relative resistance of microsomal activities to inhibition after the MC pretreatment. It is known that the MC pretreatment leads to an increase of specific P-450 isozymes, which are distinctly different from those isozymes catalyzing AHH, ECDE and ERDE activities in control liver microsomes. It seems that the MC-induced isozymes are relatively resistant, by the factor of about 10, to the inhibition by antimycotic drugs. Miconazole was somewhat exceptional, because it displayed only small differences between different pretreatments in ECDE and ERDE activities. Recently, Lavrijsen *et al.* [16] demonstrated that

inhibition by ketoconazole and miconazole of rat hepatic activities, the *N*-demethylation of *N*,*N*-dimethylaniline, the *O*-demethylation of *p*-nitroanisole and the hydroxylation of aniline, was more pronounced in microsomes from PB- or MC-pretreated animals. The reason for this difference between the present results and those of Lavrijsen *et al.* [16] probably resides in the the use of different substrates, which are markers for different isozymes. Studies on the mode of inhibition of AHH and ECDE activities by ketoconazole and clotrimazole demonstrated the competitive or mixed type of inhibition, as shown earlier by Sheets *et al.* [10] and Meredith *et al.* [11]. On the other hand, in reconstituted systems all three antimycotics have been shown to be inhibitors of both P-450b and P-450c and that they were more potent inhibitors for P-450b catalyzed reactions [17].

The IC_{50} values with control and PB treated microsomes were usually between about 1 and 5 μM, which are in a good agreement with the values ranging from 1 to 10 μM for the inhibition of different *N*-demethylations and ERDE activity by ketoconazole [8]. Clotrimazole seemed to be the most potent inhibitor. Sheets *et al.* [9] also demonstrated that clotrimazole was the most potent inhibitor of androstendione hydroxylations in rat liver microsomes. Lavrijsen *et al.* [16] published IC_{50} values ranging from 2.6 μM for miconazole and *N*,*N*-dimethylaniline *N*-demethylation in PB-induced microsomes, to about 25–42 μM for ketoconazole and aniline hydroxylase in control liver microsomes. They demonstrated that miconazole was more potent than ketoconazole.

It was striking to find out that the human liver and placental enzymes behaved quite differently than anticipated on the basis of rat studies. Hepatic enzyme activities were inhibited by all three antimycotics, but the concentrations needed for 50% inhibitions were usually between 5 and 50 μM and only exceptionally less than 5 μM. On the other hand, placental activities were usually inhibited much more efficiently. Especially ECDE and ERDE activities were inhibited more than 50% by ketoconazole and clotrimazole at concentrations between 0.5 and 0.05 μM. Miconazole was again an exception in this respect and also ketoconazole was a rather weak inhibitor of both hepatic and placental AHH activity. These findings were unexpected, because all the earlier studies have shown that the placental cigarette smoke-induced xenobiotic-metabolizing monooxygenase system resembles the one induced by polycyclic aromatic hydrocarbons in rat liver microsomes [20, 28]. This correspondence has been shown with the aid of differential substrates and inhibitors and with monoclonal antibodies to MC and PB-induced rat liver P-450 isozymes [20, 29, 30].

It has been demonstrated that all three antimycotics interact with cytochrome P-450 in both human liver and placental microsomes giving rise to a type II spectral interaction [8, 9]. However, the isozymic specificity of this interaction has not been elucidated. Clotrimazole and ketoconazole are relatively potent inhibitors of placental mitochondrial cholesterol side chain cleavage activity, whereas miconazole is relatively weak [31]. All three antimycotics are potent inhibitors of placental aromatase activity with IC_{50} values between 0.6 and 60 μM [5]. However, although these steroid-synthetizing P-450 isozymes are regarded separate from those isozymes catalyzing xenobiotic oxidations [32, 33], there is some evidence that at least P-450 associated with constitutive ECDE activity and aromatase P-450 may be identical [34]. Because human placenta contains only one P-450 protein catalyzing aromatase reaction the Lineweaver–Burk plots for inhibition of atromatase by antimycotics are linear [7]. Hence, the discrepancy of the inhibitory effect of antimycotics on xenobiotic metabolism between MC-rat liver and human placenta may partly be due to species specific differences in the ratio between induced and constitutive P-450 isozymes. It is concluded that isozyme specificity of antimycotic inhibition of human monooxygenases cannot be predicted on the basis of rat data.

Studies on the type of inhibition complicated the interpretation of results in humans tissues even more. Although competitive and non-competitive mixed-type inhibition curves were obtained with human liver microsomes, quite in line with results obtained with rat liver microsomes and human placental aromatase, highly unusual curved plots were obtained with placental microsomes. The reason for this unusual behaviour of antimycotics with placental enzymes is unknown. Studies on mice have shown the existence of two P-450 isozymes catalyzing ERDE activity, which are, however, equally sensitive to the inhibition by ketoconazole [35]. With respect to placenta, the curved lines may suggest the presence of multiple enzymes or active sites of catalyzing ERDE activity, each with differing affinities for the inhibitors. However, evidence to support the existence of multiple ERDE isozymes in human placenta is lacking.

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REFERENCES

1. Restrepo A, Stevens DS and Utz JP. First International Symposium on ketokonazole. *Rev Infect Dis* **2**: 519–562, 1980.
2. Van den Bossche H, Bellens D, Cools W, Gorrens J, Marichal P, Verhoeven H, Willemsens G, De Coster R, Beerens D, Haelterman C, Coene M-C, Lauwers W and Le Jeune L. Cytochrome P-450: target for itraconazole. *Drug Dev. Res* **8**: 287–298, 1986.
3. Feldman D, Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. *Endocrine Rev* **7**: 409–420, 1986.
4. Kowal J, The effect of ketokonazole on steroidogenesis in adrenal cortex tumor cells. *Endocrinology* **112**: 1541–1543, 1983.
5. Loose DS, Kan PB, Hirst MA, Marcus RA and Feldman D Ketokonazole blocks adrenal steroidogenesis by inhibiting cytochrome P-450-dependent enzymes. *J. Clin Invest* **71**: 1495–1499, 1983.

6. Santen RJ, Van den Bossche H, Symoens J, Brugmans J and De Coster R, Site of action of low dose ketokonazole on androgen biosynthesis in men. *J Clin Endocrinol Metab* **57**: 732–736, 1983.
7. Mason JI, Murry BA, Olcott M and Sheets JJ, Imidazole antimycotics: inhibitors of steroid aromatase. *Biochem Pharmacol* **34**: 1087–1092, 1985.
8. Schuster I, The interaction of representative members from two classes of antimycotics—the azoles and the allylamines—with cytochromes P-450 in steroidogenic tissues and liver. *Xenobiotica* **15**: 529–546, 1985
9. Sheets JJ, Mason JI, Wise CA and Estabrook RW, Inhibition of rat liver microsomal cytochrome P-450 steroid hydroxylase reactions by imidazole antimycotic agents. *Biochem Pharmacol* **35**: 487–491, 1986.
10. Sheets JJ and Mason JI, Ketokonazole: a potent inhibitor of cytochrome P-450-dependent drug metabolism in rat liver. *Drug Metab Disp* **12**: 603–606, 1984.
11. Meredith CG, Maldonado AL and Speeg KV, The effect of ketoconazole on hepatic oxidative drug metabolism in the rat *in vivo* and *in vitro*. *Drug Metab Disp* **13**: 156–162, 1985.
12. Ritter JK and Franklin MR, Induction and inhibition of rat hepatic drug metabolism by N-substituted imidazole drugs. *Drug Metab Dispos* **15**: 335–343, 1987.
13. Lavrijsen K, Van Houdt J, Thijs D, Meuldermans W and Heykants J, Induction potential of antifungals containing an imidazole or triazole moiety. Miconazole and ketoconazole, but not itraconazole are able to induce hepatic drug metabolizing enzymes of male rats at high doses. *Biochem Pharmacol* **35**: 1867–1878, 1986.
14. Buchi KN, Gray PD and Tolman KG, Ketoconazole hepatotoxicity: an *in vitro* model. *Biochem Pharmacol* **35**: 2845–2847, 1986.
15. Lake-Bakaar G, Scheuer PJ and Sherlock S, Hepatic reactions associated with ketoconazole in the United Kingdom. *Brit Med J* **294**: 419–421, 1987.
16. Lavrijsen K, Van Houdt J, Thijs D, Meuldermans W and Heykants J, Interaction of miconazole, ketoconazole and itraconazole with rat-liver microsomes. *Xenobiotica* **17**: 45–57, 1987.
17. Rodrigues AD, Gibson GG, Ioannides C and Parke DV, Interactions of imidazole antifungal agents with purified cytochrome P-450 proteins. *Biochem Pharmacol* **36**: 4277–4281, 1987.
18. Pelkonen O, Kaltiala EH, Larmi TKI and Kärki NT, Cytochrome P-450-linked monooxygenase system and drug-induced spectral interactions in human liver microsomes. *Chem-Biol Interact* **9**: 205–216, 1974.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin Phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Pelkonen O, Pasanen M, Kuha H, Gachalyi B, Kairaluoma M, Sotaniemi EA, Park SS, Friedman FK and Gelboin HV, The effect of cigarette smoking on 7-ethoxyresorufin *O*-deethylase and other monooxygenase activities in human liver: analyses with monoclonal antibodies. *Br J Clin Pharmacol* **22**: 125–134, 1986.
21. Pelkonen O and Pasanen M, Effect of heparin on the subcellular distribution of human placental 7-ethocoumarin *O*-deethylase activity. *Biochem Pharmacol* **30**: 3254–3256, 1981.
22. Nebert DW and Gelboin HV, Substrate inducible microsomal aryl hydrocarbon hydroxylase in mammalian cell culture. I. Assay and properties of the induced enzyme. *J Biol Chem* **243**: 6242–6249, 1968.
23. Greenlee WF and Poland A, An improved assay of 7-ethoxycoumarin *O*-deethylase activity: Induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J Pharmacol Exp Ther* **205**: 596–605, 1978.
24. Burke MD and Mayer RT, Ethoxyresorufin: direct fluorometric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab Disp* **6**: 583–588, 1974.
25. Williams FM, Mutch E, Woodhoue KW, Lambert D and Rawlins MD, Ethoxyresorufin *O*-deethylation by human liver microsomes. *Br J Clin Pharmacol* **22**: 263–268, 1986.
26. Berg D, Buchel K-H, Plempel M and Regel E, Antimycotic sterol biosynthesis inhibitors. *Trends Pharmacol Sci* June: 233–238, 1986.
27. Amery WK, De Coster R and Caers I, Ketoconazole: From an antimycotic to a drug for prostate cancer. *Drug Development Res* **8**: 299–307, 1986.
28. Pelkonen O, Differential inhibition of aryl hydrocarbon hydroxylase in human foetal liver, adrenal gland and placenta. *Acta Pharmacol Toxicol* **41**: 306–316, 1977.
29. Fujino T, Park SS, West D and Gelboin HV, Phenotyping of cytochromes P-450 in human tissues with monoclonal antibodies. *Proc Natl Acad Sci* **79**: 3682–3686, 1982.
30. Pelkonen O, Vähäkangas K, Kärki NT and Sotaniemi EA, Genetic and environmental regulation of aryl hydrocarbon hydroxylase in man: studies with liver, lung placenta and lymphocytes. *Toxicol Pathol* **12**: 256–260, 1984.
31. Nagai K, Miyamori I, Ikeda M, Koshida H, Takeda R, Suhara K and Katagiri M, Effect of ketoconazole (an imidazole antimycotic agent) and other inhibitors of steroidogenesis on cytochrome P-450-catalyzed reactions. *J Steroid Biochem* **24**: 321–332, 1986.
32. Juchau MR, Drug biotransformation in the placenta. *Pharmacol Ther* **8**: 501–524, 1980.
33. Pelkonen O and Pasanen M, Enzymology and regulation of xenobiotic and steroid metabolism in placenta. *Biochem Soc Trans* **12**: 42–44, 1984.
34. Meigs RA, The constitutive 7-ethoxycoumarin O-deethylase activity of human placental microsomes: relationship to aromatase. *Biochem Biophys Res Commun* **145**: 1012–1018, 1987.
35. Ballard SA, Tarbit MH, Burnet FR and Lodola A, Selective interaction of two N-substituted imidazole antifungal compounds with cytochromes P-450 catalyzing ethoxyresorufin O-de-ethylation. *Biochem Soc Transact* **15**: 1116–1117, 1987.