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Clotrimazole-induced modulation of hepatic cytochrome P450 enzymes in Syrian and Chinese hamsters

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Abstract—Clotrimazole, an imidazole antifungal drug, is known to induce cytochrome P450 isozymes of the P450IIIA and P450IIB subfamilies in rats. This agent modulated hepatic cytochrome P450 enzymes differently in golden Syrian and Chinese hamsters and also in hamsters and rats. Clotrimazole at a daily intraperitoneal dose of 100 mg/kg for three days increased the amount of cytochrome P450 in the livers of the two hamster strains. In Syrian hamsters, clotrimazole significantly induced the activities of 7-pentoxyresorufin *O*-dealkylase, coumarin 7-hydroxylase, benzphetamine *N*-demethylase and testosterone 15α- and 16α-hydroxylases, but reduced those of testosterone 15β-, 7α-, 6β-, 2α- and 2β-hydroxylases. In Chinese hamsters, clotrimazole markedly stimulated the activities of coumarin 7-hydroxylase and testosterone 15α-, 16α- and 2α-hydroxylases as well as the formation of androstenedione. Western blot analysis revealed that clotrimazole treatment induced mainly cytochrome P450 isozymes immunorelated to the P450IIB and P450IIA subfamilies in Syrian hamsters and isozymes immunorelated to the P450IIA subfamily in Chinese hamsters. In contrast, in both hamster strains, clotrimazole did not induce the isozymes corresponding to the P450IIIA subfamily.

N-Substituted imidazole drugs are clinically useful antifungal agents for the treatment of topical and systemic mycoses. The drugs are known to modulate the activities of cytochrome P450 monooxygenases in animals. In rats, imidazole drugs such as clotrimazole, miconazole and ketoconazole increase hepatic cytochrome P450-related activity [1] and clotrimazole has been shown to be one of the most potent inducers of the isozymes of the P450IIIA subfamily [2]. This suggests that administration of these drugs may alter the pharmacological effects of concurrent medication, modify the toxicity of xenobiotics or alter the level of important endogenous substances, since cytochrome P450s are capable of oxidizing these substances.

The mammalian cytochrome P450s have been classified based on their amino acid sequences and it was shown that the mode of induction of cytochrome P450 isozymes belonging to the same subfamily is not similar but depends on species and strains [3]. We have previously isolated a cytochrome P450 isozyme of the P450IIA subfamily from the golden Syrian hamster [4–6], which, unlike the rat isozymes of the P450IIA subfamily, is highly inducible by 3-methylcholanthrene [4, 6].

In the present study, we compared the effects of clotrimazole on hepatic cytochrome P450s in golden Syrian and Chinese hamsters. This study was done to establish whether clotrimazole could induce the P450IIIA subfamily proteins in hamsters as in rats, and whether there are differences between the induction patterns of cytochrome P450 isozymes by clotrimazole in the two strains of hamsters.

Materials and Methods

Chemicals. Clotrimazole, 7-pentoxyresorufin, benzphetamine, erythromycin and testosterone were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). NADPH, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were obtained from Boehringer-Mannheim (Mannheim, Germany). Coumarin and other reagents were purchased from Wako Chemicals (Osaka, Japan). The antibody to P450IIB1 of rat was obtained from OXY gene (Dallas, TX, USA). The antibodies to Syrian hamster P450IIA8, named P450-AFB [5], and to Syrian hamster orthologs of the P450IIA1 and P450IIIA subfamilies were prepared in our laboratory.

Animal treatment. Male golden Syrian hamsters of body weight ranging from 90 to 100 g, obtained from Nippon SLC (Hamamatsu, Japan) and male Chinese hamsters of body weight ranging from 27 to 30 g, inbred in the Animal Experimentation Center of the Institute of Public Health (Tokyo, Japan), were used. Test animals received a daily intraperitoneal injection of clotrimazole in olive oil at a dose of 100 mg/kg for 3 days; control animals received an equal volume of olive oil. The hamsters were killed by decapitation 24 hr after the last administration of clotrimazole. Livers were immediately excised and hepatic microsomes were prepared as described previously [5].

Assays. Microsomal protein concentrations were determined by the method of Lowry *et al.* [7]. Cytochrome P450 contents were determined according to the method of Omura and Sato [8]. The N-demethylation of benzphetamine and erythromycin, 7-pentoxyresorufin O-dealkylation and coumarin 7-hydroxylation were also measured as described by Lu *et al.* [9], Wrighton *et al.* [10], Lubet *et al.* [11] and Kaipanen *et al.* [12], respectively. The testosterone hydroxylase activities were determined as described by Yamazoe *et al.* [13]. The statistical analysis was done using Student's *t*-test.

Immunoblot analysis. Microsomal proteins were electrophoresed on 10%-acrylamide gels in the presence of sodium dodecyl sulfate according to the method of Laemmli [14] and the resolved proteins were transferred onto nitrocellulose membranes which were developed [15] using antibodies to rat P450IIB1 and to Syrian hamster isozymes corresponding to the P450IIA1, P450IIA8 (P450-AFB) and P450IIIA subfamilies.

Results

The treatment of hamsters with clotrimazole resulted in a significant increase in the microsomal cytochrome P450 content in the liver, particularly in Chinese hamsters (Table 1). The N-demethylation of erythromycin was not elevated in the clotrimazole-treated animals of the two strains. In Syrian hamsters, clotrimazole markedly induced the O-dealkylation of pentoxyresorufin and to a lesser extent the activities of coumarin 7-hydroxylase and benzphetamine *N*-demethylase. In Chinese hamsters, administration of clotrimazole caused a significant increase in the activity of coumarin 7-hydroxylase and to a lesser extent in the activity of benzphetamine *N*-demethylase, whereas the 7-pentoxyresorufin *O*-dealkylase activity was not affected.

As for testosterone hydroxylation (Table 2), the treatment of Syrian hamsters with clotrimazole caused a

Table 1. Effects of clotrimazole on cytochrome P450 enzyme activities in hamster liver

Parameters	Golden Syrian hamster		Chinese hamster	
	Control	Clotrimazole	Control	Clotrimazole
Total cytochrome P450 (nmol/mg protein)	0.97 ± 0.02	1.46 ± 0.01*	0.92 ± 0.03	1.97 ± 0.18*
Erythromycin *N*-demethylase (nmol/min/mg protein)	5.52 ± 0.84	5.03 ± 0.84	3.96 ± 0.66	3.52 ± 1.77
Benzphetamine *N*-demethylase (nmol/min/mg protein)	11.6 ± 0.7	33.9 ± 2.7*	9.1 ± 0.4	15.24 ± 2.4*
Coumarin 7-hydroxylase (pmol/min/mg protein)	23.3 ± 8.2	98.6 ± 21.4*	60.0 ± 19.2	646.8 ± 62.5*
7-Pentoxyresorufin *O*-dealkylase (pmol/min/mg protein)	10.2 ± 2.3	83.0 ± 10.2*	11.5 ± 1.8	11.8 ± 1.5

Values are expressed as means ± SD for four animals.
Significance of the differences vs controls at *$P < 0.01$.

significant increase in testosterone 15α- and 16α-hydroxylation and in 17-oxidation of testosterone to androstenedione, whereas the activities of testosterone 15β-, 7α, 6β-, 2α- and 2β-hydroxylases were reduced. In Chinese hamsters, administration of clotrimazole markedly stimulated the activities of testosterone 15α-, 16α-, 16β- and 2α-hydroxylases and the formation of androstenedione, while the activities of the other testosterone hydroxylases were not affected.

Figure 1 presents representative results of western blot analysis. Clotrimazole treatment induced two isozyme proteins, an intense and a faint band, immunorelated to the P450IIB subfamily in the microsomes from Syrian hamsters, whereas a faint band only was observed in the microsomes from Chinese hamsters (Fig. 1a). Clotrimazole treatment obviously decreased the levels of the proteins corresponding to P450IIIA in Syrian hamsters but the levels were not changed in Chinese hamsters and differences were observed in the intensity of the protein bands between the two strains (Fig. 1b). Clotrimazole markedly induced cytochrome P450 isozyme(s) reacting with the antibodies to the Syrian hamster P450IIA1 in both strains, but in Chinese hamsters, the protein bands were more intense and possibly two closely related isozyme proteins were present (Fig. 1c). An isozyme band immunorelated to P450IIA8 was observed in both of the hamster strains (Fig. 1d).

Discussion

It is well established that, in rats, clotrimazole, miconazole and other N-1 substituted imidazole antifungal drugs are potent inducers of cytochrome P450-mono-oxygenase activities, particularly those associated with the P450IIIA and P450IIB subfamilies, but not the P450IA subfamily [16–18]. In rabbits, imidazole is an inducer of P450IIE1 [19] and oxfendazole is an inducer of the P450IA2 subfamily, but not the P450IIB and P450IIIA subfamilies [20]. In rat liver and in human hepatocyte culture, P450IA1 and IA2 were induced by treatment with albendazole and omeprazole, respectively [21, 22]. These results suggest that imidazole antifungal agents may exhibit different effects on the induction of cytochrome P450 isozymes in rats and other species including humans.

The present study has also shown that clotrimazole modulated the cytochrome P450 enzymes differently in the rat and hamster, and in the two hamster strains. Clotrimazole may be unable to induce the P450IIIA subfamily proteins in hamsters. In Syrian hamsters, this is shown by the reduced levels of proteins corresponding to the P450IIIA subfamily (Fig. 1b) and by the reduced activities of testoserone 6β-hydroxylation (Table 2) which is considered to be specific for the rat P450IIIA1 [23]. In Chinese hamsters, the activity of testosterone 6β-hydroxylase and the levels of the proteins immunorelated to the Syrian hamster P450IIIA subfamily were substantially unchanged by clotrimazole. Although the possibility exists that the immunoreactivity of the isozyme proteins of Chinese hamsters is not equal to that of Syrian hamsters, as shown in the present study (Fig. 1b), these results support the idea that clotrimazole is not capable of inducing the P450IIIA subfamily in hamsters.

Table 2. Effects of clotrimazole on testosterone hydroxylase activity in hamster liver

Testosterone metabolites	Golden Syrian hamster		Chinese hamster	
	Control	Clotrimazole	Control	Clotrimazole
6α	98 ± 38	92 ± 1	97 ± 6	81 ± 26
15β	1800 ± 200	1000 ± 36†	310 ± 73	230 ± 68
15α	200 ± 80	440 ± 14†	88 ± 17	290 ± 13†
7α	2900 ± 1000	1600 ± 71†	580 ± 36	450 ± 140
6β	6400 ± 500	3700 ± 120†	2500 ± 560	2000 ± 680
16α	20 ± 8	44 ± 3*	28 ± 3	100 ± 34†
16β	290 ± 23	270 ± 20	94 ± 4	420 ± 130†
2α	66 ± 4	57 ± 2*	12 ± 1	56 ± 2†
2β	2800 ± 470	1700 ± 66†	280 ± 62	280 ± 96
Androstenedione	850 ± 190	1200 ± 76*	1200 ± 360	4400 ± 490†

Values are expressed as the means ± SD for four animals; pmol/min/mg protein.
Significance of differences vs controls at *$P < 0.05$ and †$P < 0.01$.

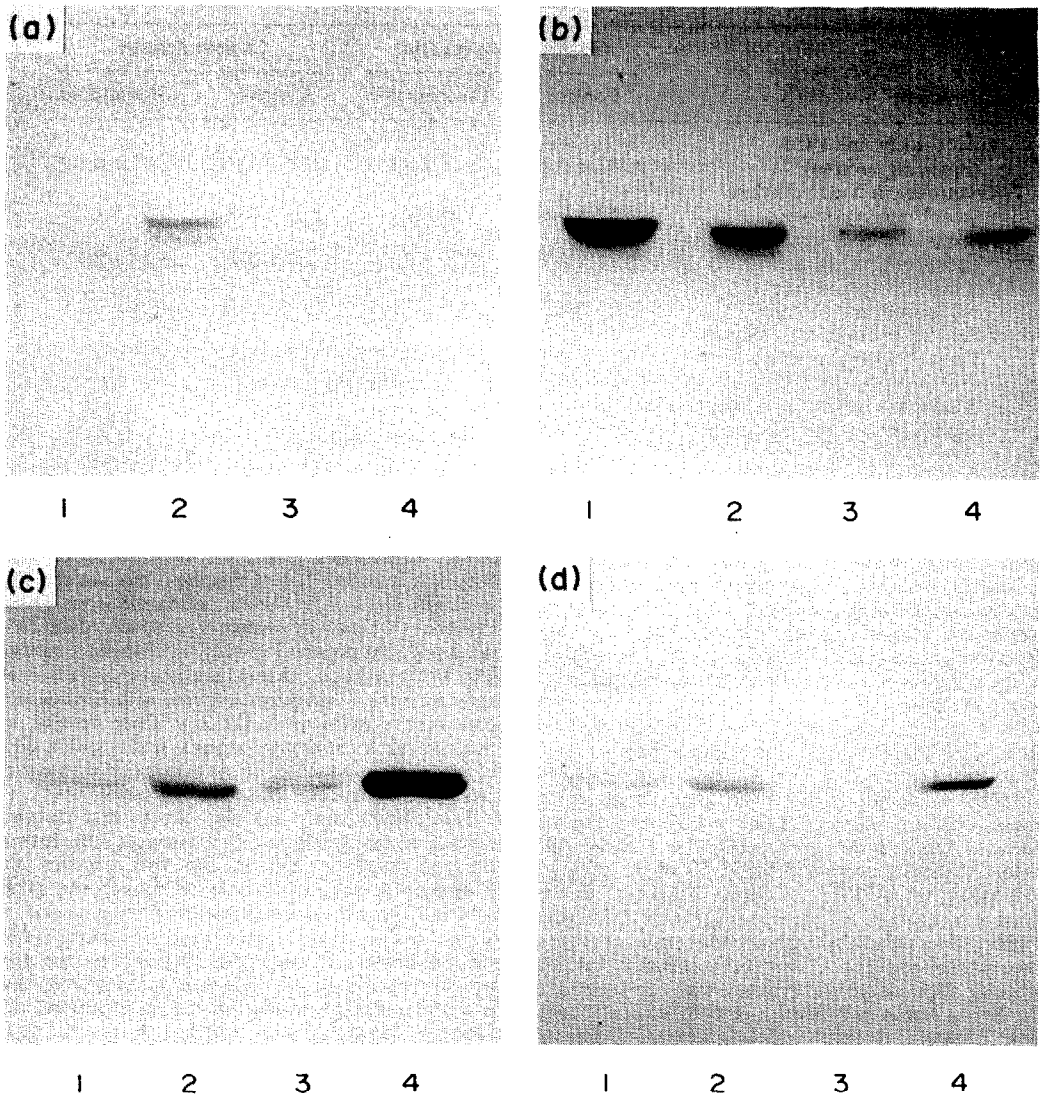

Fig. 1. Western blot analysis of the microsomal proteins from untreated and clotrimazole-treated hamsters. Hepatic microsomes containing 4 μg of proteins were analysed using the antibodies to rat P450IIB1 (a) and Syrian hamster P450IIIA (b), IIA1 (c) and IIA8 (d). Lanes 1, 2, 3 and 4 represent the hepatic microsomes from untreated Syrian, clotrimazole-treated Syrian, untreated Chinese and clotrimazole-treated Chinese hamsters, respectively.

Marked strain differences were observed between the two hamster strains: clotrimazole induced the isozyme proteins immunorelated to the P450IIB subfamily in the Syrian but not the Chinese hamsters. The two bands detected by anti-P450IIB1 probably correspond to the Syrian hamster homologs of P450IIB1 and P450IIB2. In accordance with this, a significant increase was obtained in Syrian hamsters but not in Chinese hamsters in the activity of pentoxyresorufin *O*-dealkylase, the enzyme reaction catalysed primarily by the rat P450IIB1 [11]. However, the testosterone 16β-hydroxylase activity which is also preferentially catalysed by the rat P450IIB1 [24] was not induced in Syrian hamsters. On the other hand, in Chinese hamsters, a marked increase in 16α- and 16β-hydroxylation of testosterone as well as androstenedione formation was observed. This indicates that the activities of 16α- and 16β-hydroxylases and the formation of androstenedione may not be associated with the cytochrome P450IIB subfamily proteins in Chinese hamsters, or that the proteins of the cytochrome P450IIB subfamily in Chinese hamsters have a low immuno-cross-reactivity with those of rat P450IIB1. The results suggest, however, that clotrimazole may induce the isozymes corresponding to the P450IIB subfamily in Syrian hamsters as in rats [17, 18].

Differences between the two strains were also observed in the induction rate of the enzymes that are associated with the P450IIA subfamily of the rat and mouse, namely, testosterone 7α-, or 15α-hydroxylation and coumarin 7-hydroxylation [25–28]. Although the induction of testosterone 15α-hydroxylase activity was observed equally in both the hamster strains, coumarin 7-hydroxylase activity was far more elevated in Chinese than in Syrian hamsters. A marked induction in coumarin 7-hydroxylase activity in clotrimazole-treated Chinese hamsters might be mediated by the isozyme that was observed in large amounts in Western blot analysis using Syrian hamster anti-P450IIA1

(Fig. 1c). The enhanced activity of testosterone 15α-hydroxylase in Syrian hamsters might be mediated by P450IIA8 as it has the activity of testosterone 15α-hydroxylase (unpublished data). The other enzyme activities induced in both of the hamster strains might be mediated by an isozyme corresponding to either P450IIA1 or P450IIA8, as both of these isoenzyme proteins were shown to be induced by clotrimazole (Fig. 1c, d). These results indicate that clotrimazole induces isozymes of the cytochrome P450IIA subfamily with the activity of coumarin 7-hydroxylase and testosterone 15α-hydroxylase and not those with tesosterone 7α-hydroxylase activity in both of the hamster strains. Since the induction of these enzymes by clotrimazole was not reported in rats, species differences might be present in this respect between hamsters and rats.

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