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Bifonazole, but not the structurally-related clotrimazole, induces both peroxisome proliferation and members of the cytochrome P4504A sub-family in rat liver

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Abstract

Male Wistar rats were treated with a low (150 μmol/kg) and a high (750 μmol/kg) dose of either clotrimazole or bifonazole. Bifonazole, but not clotrimazole, exhibited the characteristics of a peroxisome proliferator including hepatomegaly (increase in liver:body weight ratio), up to a 4-fold induction of lauric acid ω-hydroxylase activity and an 8-fold induction of palmitoyl-CoA oxidation by rat liver peroxisomes. This induction of enzyme activities was paralleled by increased protein levels as determined by immunochemical analysis for both liver microsomal cytochrome P4504A1 and the peroxisomal trifunctional protein of the β-oxidation spiral. In contrast, clotrimazole did not increase protein levels of either cytochrome P4504A or the trifunctional protein. Western blot analyses demonstrated that bifonazole also induced P4502B1/2B2, P4503A and P4501A1, but not P4502E1. Clotrimazole induced a similar spectrum of P450s as determined by Western blotting with the exception that this azole was a marginal P4501A1 inducer under the conditions studied. Taken collectively, our data provides evidence that bifonazole is one of the increasingly recognised, non-carboxylate containing xenobiotics that induce both peroxisome proliferation and the cytochrome P4504A sub-family in rat liver.

Keywords: Bifonazole; Clotrimazole; Cytochrome P4504A; Enzyme induction; Peroxisome proliferators

1. Introduction

Azole anti-fungal drugs, such as clotrimazole, can act as both inhibitors or inducers of the cytochrome P450-dependent mixed function oxidase system (Daujat et al., 1991; Van den Bosche et al., 1991), dependent on the time-course of xenobiotic exposure (Rodrigues et al., 1988). Although clotrimazole will inhibit most cytochrome P450s studied to date, there appears to be a relative specificity for induction of members of the cytochrome P4502B and 3A sub-families in particular (Rodrigues et al., 1988).

Bifonazole is a recently-developed and potent antifungal drug, structurally related to clotrimazole (Fig. 1) but its ability to modulate cytochome

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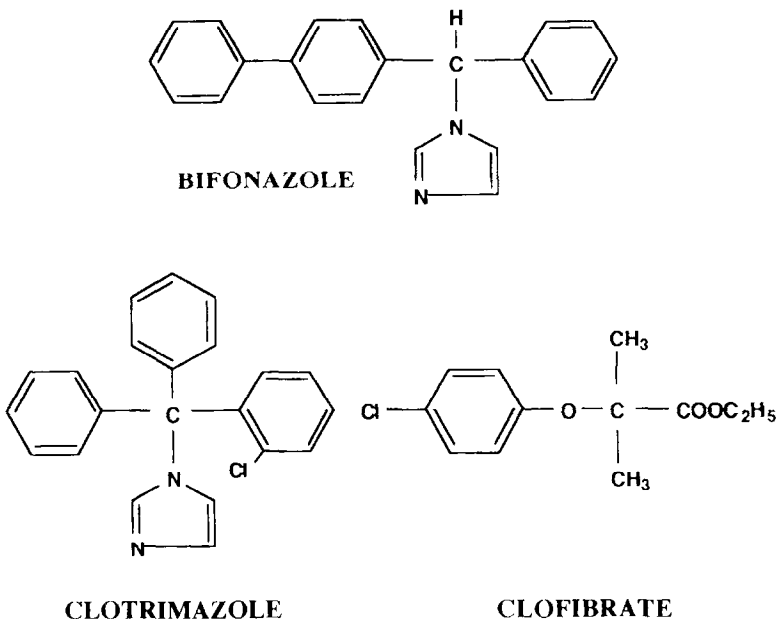

Fig. 1. Chemical structures of bifonazole, clotrimazole and clofibrate.

P450s in the liver of experimental animals has not been described to date. This fact, coupled to the observation that bifonazole is a peroxisome proliferator (Horie et al., 1991) and all peroxisome proliferators studied to date are inducers of the cytochrome P4504A subfamily (Sharma et al., 1988; Hozuka et al., 1991), prompted us to examine the ability of bifonazole to induce the cytochrome P4504A subfamily in rat liver and to compare this to the cytochrome P450 induction profile produced by clofibrate and clotrimazole.

2. Materials and methods

2.1. Chemicals

Bifonazole and clotrimazole were kindly donated by the Bayer Research Centre (Wuppertal, Germany). Clofibric acid, lauric acid and NADPH were purchased from Sigma (Poole, Dorset, UK). The Western blotting kits for the cytochrome P450s were supplied by Amersham International plc. Antibody to the peroxisomal trifunctional protein was a gift from Dr. Dominick Cinti, University of Connecticut. All other chemicals were obtained from commercial sources and were of the highest purity available.

2.2. Animals and drug treatment

Male Wistar rats (210–230 g initial body weight, University of Surrey Experimental Biology Unit) were dosed by gavage, once daily for 6 consecutive days at the following dose levels (in corn oil): clofibrate, 1 mmol/kg; bifonazole and clotrimazole, each at two dose levels of 150 and 750 μmol/kg. These dose levels were chosen based on previous studies where cytochrome P450 induction was observed for clofibrate (Sharma et al., 1988) and clotrimazole (Rodrigues et al., 1988), with bifonazole being dosed in equimolar amounts to clotrimazole for comparison. In addition, to serve as positive controls for the Western blot experiments, groups of rats (3/group) were treated by i.p. injection, once daily for 3 days, at dose levels of β-naphthoflavone (50 mg/kg), phenobarbital (80 mg/kg), isoniazid (100 mg/kg), dexamethasone (100 mg/kg) and pregnenolone 16α-carbonitrile (100 mg/kg). Control animals were administered the vehicle (corn oil) at 1 ml/kg by gavage. Twenty

four h after the last dose, the animals were killed by cervical dislocation, livers removed, blotted dry, weighed and perfused with ice-cold 0.9% (w/v) saline. The tissues were scissor minced and homogenised in 0.25 M sucrose, adjusted to 25% (w/v) and whole tissue homogenates reserved and stored at −80°C for subsequent determination of peroxisomal enzymes. Microsomes were prepared by ultracentrifugation as previously described (Remmer et al., 1966) and stored in aliquots at −80°C in 50 mM phosphate buffer, pH 7.25 containing 20% (v/v) glycerol, without loss of original activity (cytochrome P450 specific content and laurate hydroxylase activity).

2.3. Enzyme assays

Cytochrome b_5 and total carbon monoxide-discernible cytochrome P450 were determined by the method of Omura and Sato (1964) and NADPH-cytochrome *c* (P450) reductase was determined as previously described (Williams and Kamin, 1962). Protein content was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

The 11-(ω-1) and 12-(ω)-hydroxy metabolites of lauric acid were determined by HPLC (Sharma et al., 1988). Incubation mixtures (final volume 2 ml) contained 1 mg of microsomal protein and 100 μM lauric acid (including 0.875 μM ^{14}C-lauric acid) in a final buffer concentration of 50 mM Tris–HCl (pH 7.4). After a pre-incubation period of 2 min at 37°C, the reaction was initiated by adding 40 μl of NADPH (1 mM) and allowed to incubate for 10 min at 37°C. The mixture was extracted into diethyl ether, dried and analysed by reverse phase HPLC using a Micropak MCH-10 column (30 × 0.4 cm) (Varian Associates Ltd., Walton-on-Thames, UK) using a linear gradient of water/methanol (45:55 containing 0.1% acetic acid) to 100% methanol over a 45 min period and with a flow rate of 1 ml/min. The metabolites were detected by a Berthold radiochemical detector (Sharma et al., 1988).

Whole tissue homogenates were assayed for KCN-insensitive palmitoyl-CoA oxidation activity as previously described (Bronfman et al., 1979). Liver homogenates were diluted 1:1 in 1% (v/v) Triton X-100 in 60 mM Tris–HCl pH 8.3 and solubilised by incubating at 37°C for 2–3 min. The solubilised homogenate was centrifuged at 3000 × *g* for 3 min and 40 μl of the supernatant was added to a cuvette containing reaction mixture (3 ml) composed of 50 μM CoA, 120 μM FAD^+, 370 μM NAD^+ 94 mM nicotinamide, 2.8 mM dithiothreitol, 2 mM potassium cyanide and 150 μg/ml bovine serum albumin (fatty acid free) in 3 ml 60 mM Tris–HCl buffer pH 8.3. The reaction was started by adding 20 μl palmitoyl-CoA (75 μM final concentration).

2.4. Western blotting analyses

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (1970) and subsequent Western blotting was performed as previously described (Burnette, 1981). Western blotting kits (Amersham) used the following primary and secondary antibodies to cytochrome P4501A1 (code number RPN 256) 2B1/2B2 (RPN 257) 2E1 (RPN 259), 3A (RPN 258) or 4A1 (RPN 260). Antibody dilutions and incubation times were according to the manufacturers instructions. Briefly, enhanced chemiluminescence (ECL) detection was used with anti-species IgG biotinylated secondary antibody. The membrane was then incubated with a streptavidin-horseradish peroxidase conjugate in the presence of the substrate, luminol, and the enhanced chemiluminescence captured on Hyperfilm (Amersham). Biotinylated molecular weight marker proteins were used, comprising lysozyme (14.3 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (29 kDa), alcohol dehydrogenase subunit (39.8 kDa), catalase subunit (58.1 kDa) and phosphorylase *b* subunit (97.4 kDa). The cytochrome P450 Western blot kits were specific for each sub-family as described in the manufacturers instruction booklets and references cited therein.

Western blotting for the peroxisomal trifunctional protein was determined as previously described using a horseradish peroxidase/4-chloro-1-naphthol/H_2O_2 colorimetric detection method (Chinje and Gibson, 1991).

3. Results

Bifonazole at both low and high doses (150

Table 1
Influence of bifonazole, clotrimazole and clofibrate on rat liver microsomal mixed-function oxidase components

Treatment group	Total cytochrome P450 (nmol/mg)	Cytochrome b_5 (nmol/mg)	NADPH-cytochrome *c* (P450) reductase (nmol/min/mg)	Liver:body weight Ratio (×100)
Control	0.73 ± 0.03	0.38 ± 0.02	72.3 ± 2.3	4.9 ± 0.2
Bifonazole				
150 μmol/kg	0.76 ± 0.08	0.36 ± 0.02	87.1 ± 1.1**	6.4 ± 0.2*
750 μmol/kg	1.17 ± 0.03*	0.35 ± 0.01	51.9 ± 2.1	7.1 ± 0.3*
Clotrimazole				
150 μmol/kg	1.63 ± 0.2*	0.49 ± 0.02*	89.5 ± 1.5**	5.2 ± 0.1
750 μmol/kg	2.68 ± 0.1**	0.35 ± 0.02	119.0 ± 9.5**	8.1 ± 0.1*
Clofibrate				
1 mmol/kg	0.94 ± 0.08	0.34 ± 0.02	87.3 ± 3.3**	6.4 ± 0.2*

Values are expressed as mean ± S.E.M. from five animals. Statistical analysis was by one way ANOVA and Student's *t*-distribution.
*Significantly different from control at $P < 0.05$.
**Significantly different from control at $P < 0.02$ by Student's *t*-test.

μmol/kg and 750 μmol/kg, respectively) produced significant hepatomegaly, whereas clotrimazole produced this effect at the high dose only (Table 1). At the given dose levels, it would appear that clotrimazole was a better inducer of the mixed-function oxidase components, including the total, carbon monoxide-discernable cytochrome P450, cytochrome b_5 and NADPH-cytochrome P450 reductase (Table 1). However, bifonazole pretreatment resulted in a substantial increase in lauric acid ω-hydroxylase activity in rat liver microsomes (Table 2), an activity associated with induction of the cytochrome P4504A subfamily and P4504A1 in particular (Gibson et al., 1982), whereas clotrimazole at the same molar dose levels was not an inducer of this activity, irrespective of induction of cytochrome b_5 and NADPH-cytochrome P450 reductase. It should be noted that the lower dose of bifonazole appeared to be a better inducer of laurate ω-hydroxylase than the higher dose.

Table 2
Influence of bifonazole, clotrimazole and clofibrate pretreatment on fatty acid hydroxylation and β-oxidation in rat liver

Treatment group	Lauric acid hydroxylase activity (nmol/min/mg)		Palmitoyl-CoA oxidation (nmol/min/mg)
	ω-1-hydroxylase	ω-hyroxylase	
Control	3.0 ± 0.2	3.9 ± 0.3	5.0 ± 0.5
Bifonazole			
150 μmol/kg	3.7 ± 0.3*	13.2 ± 1.2**	39.3 ± 3.1**
750 μmol/kg	2.6 ± 0.1	8.1 ± 0.6**	27.6 ± 4.4**
Clotrimazole			
150 μmol/kg	2.2 ± 0.2	2.2 ± 0.3	8.5 ± 1.3
750 μmol/kg	0.9 ± 0.05*	1.7 ± 0.2	2.5 ± 0.1
Clofibrate			
1 mmol/kg	2.7 ± 0.3	13.1 ± 0.9**	58.2 ± 5.7**

Values are expressed as mean ± S.E.M. from five animals.
Statistical analysis was by one-way ANOVA and Student's *t*-distribution.
*Significantly different from control at $P < 0.05$.
**Significantly different from control at $P < 0.01$ by Student's *t*-test.

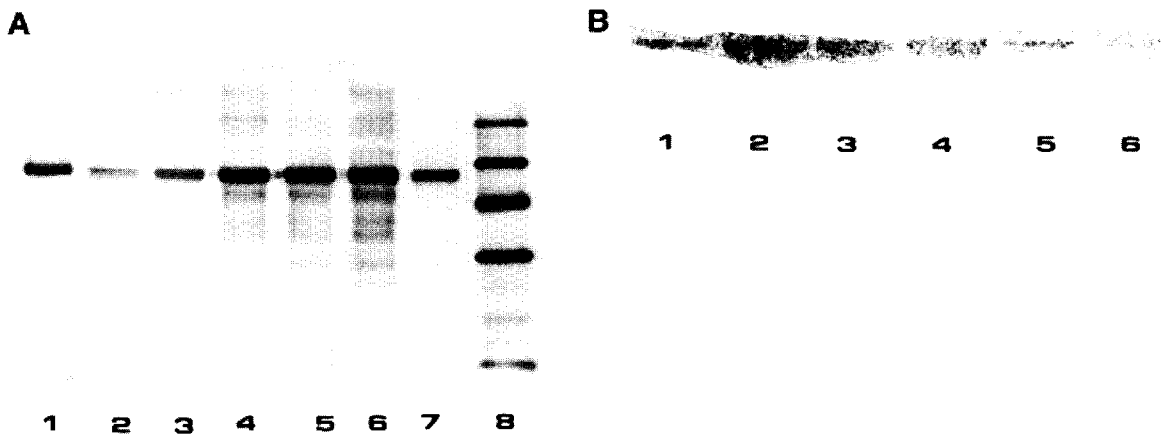

Fig. 2. Western blot analysis for microsomal P4504A1 and peroxisomal trifunctional protein. Rats were pretreated with either clofibrate, bifonazole or clotrimazole and liver microsomal fractions prepared and analysed by Western blotting as described in Materials and methods (Section 2.4). A 20-μg aliquot of microsomal protein was loaded in each track of Fig. 2A and 20 μg of total homogenate protein in each track of Fig. 2B. (A) Tracks were loaded as follows: authentic P4504A1 (0.5 pmole, track 1), high dose clotrimazole group (track 2), low dose clotrimazole group (track 3), high dose bifonazole group (track 4), low dose bifonazole group (track 5), clofibrate group (track 6), control group (track 7) and standard molecular-weight markers (track 8). (B) Tracks were loaded with rat liver homogenate protein derived from, 1, control; 2, clofibrate; 3, bifonazole low dose; 4, bifonazole high dose; 5, clotrimazole low dose and 6, clotrimazole high dose. The blot shown is representative of the five animals in each treatment group.

Whether this is a result of general hepatotoxicity or complexation of residual bifonazole with hepatic cytochrome P450 at the higher dose remains to be determined. In addition, bifonazole pretreatment is consistent with hepatic peroxisome proliferation, as assessed by palmitoyl-CoA oxidation in fatty acid β-oxidation, another characteristic feature of P4504A family inducers (Sharma et al., 1988; Hozuka et al., 1991), whereas clotrimazole was without effect (Table 2).

The above conclusions on bifonazole induction of the P4504A subfamily were further substantiated by the Western blot data shown in Fig. 2. Fig. 2A demonstrates that bifonazole induces a protein of similar molecular weight to authentic P4504A1 in a similar manner to clofibrate, whereas this immunochemical analysis demonstrates no induction with clotrimazole pretreatment. This pattern of bifonazole-dependent induction was also mirrored by immunochemical analysis for the peroxisomal trifunctional protein (Fig. 2B).

The ability of bifonazole and clotrimazole to induce other cytochrome P450 families are shown in Fig. 3, where clotrimazole induced P4502B1/2B2 and P4503A4 and bifonazole induced P4501A1, P4502B1/2B2 and P4503A4. Therefore, clotrimazole and bifonazole are mixed cytochrome P450 inducers, in contrast to clofibrate which appears to

Table 3
Summary of cytochrome P450 isoenzymes induced by clotrimazole, bifonazole or clofibrate

P450 isoenzyme induced[a]	Inducer		
	Clotrimazole	Bifonazole	Clofibrate
P4501A1	–	+	–
P4502B1/2B2	+	+	minor
P4502E1	–	–	–
P4503A4	+	+	minor
P4504A1	ND	+	+

[a]Induction was assessed from the Western blot data in Figs. 2 and 3.
–, no detectable induction.
+, detectable induction.
Minor, minimal induction.
ND, not determined.

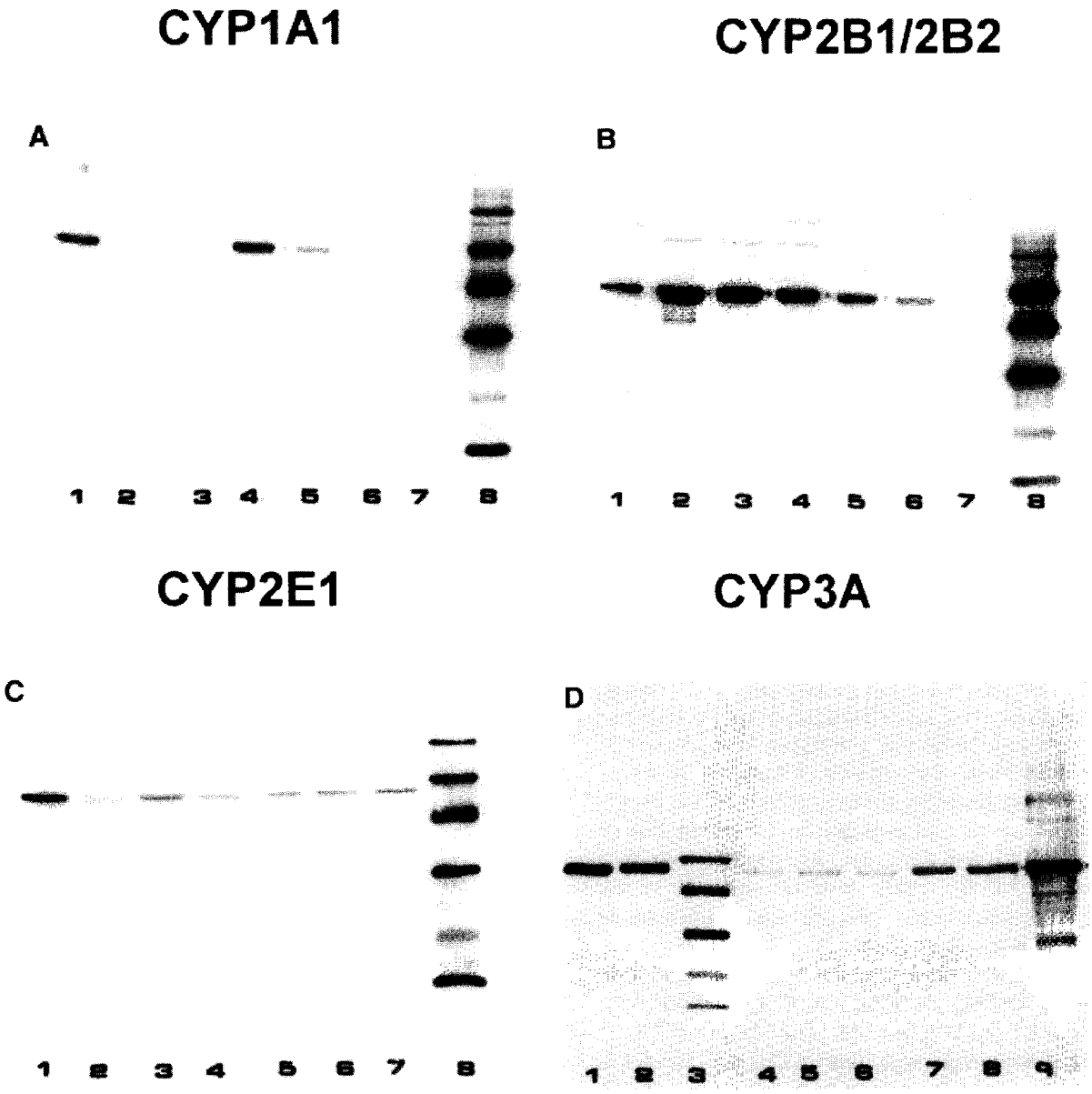

Fig. 3. Western blot analysis for cytochromes P4501A1, 2B1/2B2, 2E1 and 3A in clofibrate, clotrimazole and bifonazole treated animals. Rats were treated with either clofibrate, clotrimazole or bifonazole and ECL Western blot analysis determined for CYP1A1 (A), CYP2B1/2B2 (B), CYP2E1 (C) and CYP3A (D) as described in Materials and methods (Section 2.4). Microsomal fractions from control or drug-treated animals (5/group) were pooled and 5 μg microsomal protein subjected to Western blot analysis. For blots A–C, track loading was: 1, positive control i.e. β-naphthoflavone (A), phenobarbital (B) or isoniazid (C); 2, high dose clotrimazole; 3, low dose clotrimazole; 4, high dose bifonazole; 5, low dose bifonazole; 6, clofibrate; 7, control; 8, standard molecular weight markers. For blot D (CYP3A antibody), track loading was: 1, dexamethasone; 2, pregnenolone 16-α-carbonitrile; 3, standard molecular weight markers; 4, control; 5, clofibrate; 6, low dose bifonazole; 7, high dose bifonazole; 8, low dose clotrimazole and 9, high dose clotrimazole. The blot shown is representative of the five animals used in each treatment group.

be more specific for the P4504A sub-family (Figs. 2, 3), as summarised in Table 3.

4. Discussion

Based on their relative abilities to induce lauric acid ω-hydroxylase activity and immunochemical reactivity with an antibody raised against P4504-A1, our results indicate that bifonazole, but not the structurally-related clotrimazole, is an inducer of the cytochrome P4504A subfamily in rat liver microsomes. There are at least three closely related enzymes in the P4504A subfamily in rat liver, namely P4504A1, P4504A2 and P4504A3 (Kimura et al., 1989; Nebert et al., 1991; Sundseth and Waxman, 1992). Which of these enzymes are induced by bifonazole is not clear at present as they all display lauric acid ω-hydroxylase activities and the antibody to P4504A1 used in our immunoblot studies (Fig. 2A) also recognises additional proteins that are probably P4504A2 and/or P4504A3 (Sharma et al., 1989).

The above induction of the P4504A sub-family and the fact that bifonazole induces peroxisomal palmitoyl-CoA oxidation (Table 2) and hepatomegaly (Table 1) would classify bifonazole as a typical peroxisome proliferator (Reddy and Lalwani, 1983), in agreement with the data of Horie et al. (1991). Until recently, it was considered that most peroxisome proliferators contained a carboxyl group in their structure or could be metabolised to one (Reddy and Lalwani, 1983; Lock et al., 1989). However, emerging evidence suggests that this need not always be the case and non-carboxylate peroxisome proliferators have been described, including dehydroepiandrosterone (Wu et al., 1989) and certain leukotriene antagonists such as the tetrazole alkoxyacetophenones (Eacho et al., 1986). Thus, it would appear that bifonazole is a member of this latter group of non-carboxylate peroxisome proliferators. Alternatively, the ability of bifonazole to act as a P4504A1 inducer, whereas the closely related clotrimazole does not, may be related to possible differences in the production of active metabolites directly acting as inducers. Although the full metabolic profiles for bifonazole and clotrimazole have not been determined, it is instructive to note that the imidazole-containing drug, midaglizole, is metabolised by imidazole ring-opening with the subsequent production of carboxylic acid metabolites (Nakaoka and Hakusi, 1987). Thus, it is possible that bifonazole, but not clotrimazole, produces carboxylic acid metabolites, due to the steric hindrance to initial imidazole oxidation in the latter bulky molecule, a hypothesis that requires further evaluation.

Regarding the molecular mechanisms of P4504A induction and peroxisome proliferation, it would appear that the recently described peroxisome proliferator activated receptor (PPAR), (Issemann and Green, 1990; Green, 1992) may have a role to play in the induction process (Tugwood et al., 1992) and there appears to be significant sequence identity in the 5′ flanking regions containing the PPAR recognition/regulating elements in both the peroxisomal acyl-CoA oxidase gene and cytochrome P4504A6 gene (Muerhoff et al., 1992). Whether bifonazole induction of the P4504A sub-family acts via this PPAR mechanism and whether the ligand-binding site in the PPAR can readily discriminate between bifonazole and clotrimazole remains to be determined.

References

Bronfman, M., Inestrosa, N.G. and Leighton, F. (1979). Fatty acid oxidation by human liver. Biochem. Biophys. Res. Commun. 88, 1030–1036.

Burnette, W.N. (1981) Western blotting; electrophoretic transfer of proteins from sodium dodecyl sulphate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio-iodinated protein A. Anal. Biochem. 112, 195–203.

Chinje, E. and Gibson, G.G. (1991) Stereochemical selectivity in the induction of cytochrome P450IVA (P452)-dependent fatty acid hydroxylation and peroxisome proliferation. Biochem. Pharmacol. 41, 769–774.

Daujat, M., Pichard, L., Fabre, I., Pineau, T., Fabre, G., Bonfils, C. and Maurel, P. (1991) Induction protocols for cytochromes P450IIIA in vivo and in primary cultures of animal and human hepatocytes. In: M.R. Waterman and E.F. Johnson (Eds), Methods in Enzymology: Cytochrome P450, Vol. 206, Academic Press, New York, pp. 345–353.

Eacho, P.I., Foxworthy, P.S., Johnson, W.D., Hoover, D.M. and White, S.L. (1986) Hepatic peroxisomal changes induced by a tetrazole-substituted alkoxy acetophenones in rats and comparison with other species. Toxicol. App. Pharmacol. 83, 430–437.

Gibson, G.G., Orton, T.C. and Tamburini, P.P. (1982) Cytochrome P452 induction by clofibrate: purification and

properties of a hepatic cytochrome P450 relatively specific for the 12- and 11-hydroxylation of dodecanoic acid (lauric acid). Biochem. J. 203, 161–168.

Green, S. (1992) Peroxisome proliferators: A model for receptor- mediated carcinogenesis. In: Growth Regulation by Nuclear Hormone Receptors, Cancer Surveys, Vol. 14, ICRF, London, pp. 221–232.

Horie, S., Fukumori, N. and Suga, T. (1991) Induction of hepatic peroxisomes by a new, non-carboxylate-containing drug, bifonazole. Toxicol. Lett. 55, 249–254.

Hozuka, H., Watanabe, T., Hepie, S., Yamada, J., Suga, T. and Ikeda, T. (1991) Characteristics of peroxisome proliferation: co-induction of peroxisomal fatty acid oxidation related enzymes with microsomal laurate hydroxylase. Chem. Pharm. Bull. 39, 1267–1271.

Issemann, I. and Green, S. (1990) Activation of a member of the steroid hormone receptor super-family by peroxisome proliferators, Nature 347, 645–650.

Kimura, S., Hardwick, J.P., Kozak, C.A. and Gonzalez, F.J. (1989) The rat clofibrate inducible 4A subfamily II. cDNA sequence of IVA3, mapping of the cyp4A locus to mouse chromosome 4 and coordinate tissue-specific regulation of the cyp4A genes. DNA 8, 517–525.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T. Nature 227, 680–685.

Lock, E.A., Mitchell, A.M. and Elcombe, C.R. (1989) Biochemical mechanisms of induction of hepatic peroxisome proliferation. Annu. Rev. Pharmacol. Toxicol. 29, 145–163.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, P.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 365–275.

Muerhoff, A.S., Griffin, K.J. and Johnson, E.F. (1992) Characterization of a rabbit gene encoding a clofibrate-inducible fatty acid ω-hydroxylase: CYP4A6. Arch. Biochem. Biophys. 296, 66–72.

Nakaoka, M. and Hakusui, H. (1987) Identification of a new hypoglycaemic agent, midaglizole, in dogs. Xenobiotica 17, 1329–1339.

Nebert, D.W., Nelson, D.R., Coon, M.J., Estabrook, R.W., Feyereisen, R., Fujji-Kuriyama, Y., Gonzalez, F.J., Guengerich, F.P., Gunsalus, I.C., Johnson, E.F., Loper, J.C., Sato, R., Waterman, M.R. and Waxman, D.J. (1991) The P450 superfamily: update on new sequences, gene mapping and recommended nomenclature. DNA Cell Biol. 10, 1–14.

Omura, T. and Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification and properties. J. Biol. Chem. 239, 2379–2385.

Reddy, J.K. and Lalwani, N.D. (1983) Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidaemic drugs and industrial plasticisers to man. CRC Crit. Rev. Toxicol. 12, 1–58.

Remmer, H., Schenkman, J.B., Estabrook, R.W., Sasame, H., Gillette, J.R., Narasimhulu, S., Cooper, D.Y. and Rosenthal, O. (1966) Drug interaction with hepatic microsomal cytochrome. Mol. Pharmacol. 2, 187–190.

Rodrigues, A.D., Waddell, P.R., Ah-Sing, E., Morris, B.A., Wolf, C.R. and Ioannides, C. (1988). Induction of the rat hepatic microsomal mixed-function oxidase by three imidazole-containing antifungal agents: selectivity of the cytochrome P450IIB and P450III families of cytochrome P450. Toxicology 50, 283–301.

Sharma, R., Lake, B.G., Foster, J. and Gibson, G.G. (1988). Microsomal cytochrome P452 induction and peroxisome proliferation by hypolipidaemic drugs in rat liver: a mechanistic inter-relationship. Biochem. Pharmacol. 37, 1913–1201.

Sharma, R., Lake, B.G., Makowski, R., Bradshaw, T., Earnshaw, D., Dale, J.W. and Gibson, G.G. (1989). Differential induction of fatty acid oxidation enzymes by peroxisome proliferators in rat liver and kidney. Characteristics of a novel cytochrome P450 and implications for peroxisome proliferation, Eur. J. Biochem. 184, 69–78.

Sundseth, S.S. and Waxman, D.J. (1992) Sex-dependent expression and clofibrate-inducibility of cytochrome P4504A fatty ω-hydroxylases. J. Biol. Chem. 267, 3915.

Tugwood, J., Issemann, I., Anderson, R., Bundell, K., McPheat, W. and Green, S. (1992). The mouse peroxisome proliferator activated receptor recognises a response element in the 5′ flanking sequence of the rat acyl-CoA oxidase gene. EMBO 11, 433–439.

Van den Bosche, H., Marichal, P., Willensens, G. and Janesen, P.A.J. (1991) Effects of inhibitors in the P450-dependent metabolism of endogenous compounds in fungi, protozoa and vertebrates: In: E. Arinc, J.B. Schenkman and E. Hodgson (Eds), Molecular Aspects of Monoxygenases and Bioactivation of Toxic Compounds, NATO ASI Series A, Life Sciences, Vol. 202, Plenum Press, New York, pp. 345–363.

Williams, C.H. and Kamin, H. (1962). Microsomal triphosphopyridine nucleotide cytochrome *c* reductase of liver. J. Biol. Chem. 237, 587–595.

Wu, H.Q., Masset-Brown, J., Tweedie, D.J., Milewich, L., Frenkl, R.A., Martin-Wixtrom, C., Estabrook, R.W. and Prough, R.A. (1989) Induction of microsomal NADPH cytochrome P450 reductase and cytochrome P450IVA1 ($P450LA_{\omega}$) by dehydroepiandrosterone in rats: a possible peroxisome proliferator. Cancer Res. 49, 2337–2343.