Biochemical Pharmacology, Vol. 42, No. 1, pp. 109–115, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

INDUCTION OF THE CYTOCHROME P450 I AND IV FAMILIES AND PEROXISOMAL PROLIFERATION IN THE LIVER OF RATS TREATED WITH BENOXAPROFEN

POSSIBLE IMPLICATIONS IN ITS HEPATOTOXICITY

ANDREW D. AYRTON, COSTAS IOANNIDES* and DENNIS V. PARKE
Molecular Toxicology Research Group, Division of Toxicology, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

(*Received* 11 *January* 1991; *accepted* 25 *February* 1991)

Abstract—Administration of the non-steroidal anti-inflammatory drug benoxaprofen to rats gave rise to significant increases in the hepatic O-dealkylations of ethoxyresorufin and methoxyresorufin and in the 12-hydroxylation of lauric acid but, in contrast, the N-demethylation of dimethylnitrosamine was inhibited. Immunoblot studies employing solubilized microsomes from benoxaprofen-treated rats revealed that benoxaprofen increased the apoprotein levels of P450 IA1 and A2 and of P450 IVA1. The same treatment with benoxaprofen increased the β-oxidation of palmitoyl CoA determined in liver homogenates, and immunoblot analysis showed an increase in the apoprotein levels of the *trans*-2-enoyl CoA hydratase bifunctional protein. It is concluded that benoxaprofen is a peroxisomal proliferator which selectively induces the hepatic cytochrome P450 I and IV families. The possible implications of these findings to the well-known hepatotoxicity of this drug are discussed.

Benoxaprofen [2-(4-chlorophenyl)-5-benzoxazol-2-propionic acid], a non-steroidal anti-inflammatory analgesic, used clinically in the treatment of rheumatoid arthritis and osteoarthritis [1], was associated with a wide variety of adverse effects in man, including cutaneous phototoxicity and onycholysis, soon after its introduction [2]. Following reports of fatal hepatotoxicity resulting from chronic dosage for a month or longer, especially in the elderly, the drug was withdrawn in August 1982 [3].

Consideration of the molecular and electronic structures of benoxaprofen employing computer graphic techniques [4], has revealed that the drug has an area/depth^2 of 2.5 and a $\Delta E(E[\text{LEMO}] - E[\text{HOMO}]$, (where ΔE is the difference in energy between the frontier orbitals, LEMO is the lowest empty molecular orbital and HOMO the highest molecular orbital) of 12.0 which places it in the interface between substrates of the cytochrome P450 I family (cytochromes P448) and other cytochrome P450 families [5, 6]. These observations indicate that benoxaprofen may be a substrate/inducer of the P450 I family, although it is unlikely to show high specificity. Bearing in mind the role of the P450 I family in the production of reactive electrophiles which may manifest toxicity and carcinogenicity [7, 8], it was considered worthwhile to evaluate the ability of the drug to induce the hepatic cytochrome P450 I family in the rat. Moreover, benoxaprofen contains an aryl propionic acid moiety (Fig. 1), which, in the case of compounds such as clofibrate, phthalates and certain herbicides has been associated with the induction of the cytochrome P450 IV family and hepatic peroxisomal proliferation [9, 10] which, at least in some animal species, has been associated with liver tumours [11].

The purpose of the present studies is, therefore, two-fold, namely, to investigate the effect of benoxaprofen administration on the hepatic cytochrome P450 I and IV families and, on the basis of such effects, to consider possible mechanisms for the known hepatotoxicity of the drug.

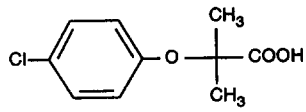
MATERIALS AND METHODS

Methoxy-, ethoxy-, pentoxy-resorufins and resorufin (Molecular Probes, Eugene, OR, U.S.A.), dimethylnitrosamine, lauric acid, palmitoyl CoA and all cofactors (Sigma Chemical Co., Poole, U.K.) were purchased.

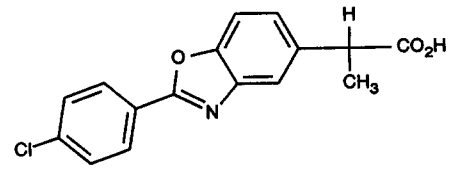
Benoxaprofen, m.p. 189–191° (lit. 189–190°) was synthesized by the method of Dunwell *et al.* [12]. Thin layer chromatography on Merck silica gel plates in methanol/chloroform 95:5 (v/v) gave a single spot with R_f value of 0.5 together with traces of two minor impurities, with R_f values of 0.0 and 0.95. Analysis by HPLC was carried out using a Spherisorb 50DS2 column and a solvent system of water/methanol 15:85, detection being achieved with UV at 280 nm. Benoxaprofen had a retention time of 22.26 min and two minor impurities with retention times of 1.14 min (0.087%) and 4.65 min (0.041%) were also detected. These studies showed that the drug was 99.87% pure. Elemental analysis for C, H and N were within 0.4% of theoretical values, and NMR spectroscopy (200 Mz, DMSO d_6) was consistent with the required structure and showed negligible traces of impurity as singlets at δ 1.22 and 3.5.

Antibodies to purified P450 IA1 protein were raised in sheep as previously described [13, 14]; these

* To whom correspondence should be addressed.



Clofibric acid



Benoxaprofen

Fig. 1. The structures of clofibric acid and benoxaprofen.

antibodies recognized both the AI and A2 proteins. Antibodies to P450 IVA1 and the *trans*-2-enoyl CoA hydratase bifunctional protein (an indicator of peroxisomal proliferation) were generous gifts from Dr G. G. Gibson, University of Surrey, Guildford, and Dr D. L. Cinti, Department of Pharmacology, University of Connecticut Health Center, Farmington, CT, respectively.

Male Wistar albino rats (100–110 g) were purchased from the Experimental Biology Unit, University of Surrey. These were randomly divided into four groups of four animals each, which were given three single daily intraperitoneal doses of benoxaprofen, dissolved in 0.2 M glycine–NaOH, pH 10.5, at dose levels of 25, 100 and 250 mg/kg/day, with the fourth group serving as control, receiving the corresponding volume of vehicle. All animals were killed 24 hr after the last dose of benoxaprofen. To obtain liver microsomal preparations to serve as positive controls in the immunoblot studies, one rat was treated with clofibrate (80 mg/kg/day) and a second rat was treated with 3-methylcholanthrene (25 mg/kg/day), both as single daily intraperitoneal doses for three days, with the animals being killed 24 hr after the last administration.

Livers were immediately excised, homogenates prepared, and microsomal fractions isolated as previously described [15]. The following determinations were carried out on the homogenate: β-oxidation of palmitoyl CoA [16]; and on the microsomal fraction: demethylation of methoxyresorufin [17], deethylation of ethoxyresorufin [18], depentylation of pentoxyresorufin [19], 11- and 12-hydroxylations of lauric acid [20], N-demethylation of dimethylnitrosamine, at a final substrate concentration of 6 mM [21], the NADPH-dependent reduction of cytochrome *c* [22] and total cytochrome P450 [23]. Protein was determined in both microsomes and homogenate using bovine serum albumin as standard [24].

Immunoblotting analysis of electrophoretically-resolved microsomal proteins was carried out using antibodies to P450 IA1 and P450 IVA1 as previously described [25, 26]. Immunological determination of the *trans*-2-enoyl CoA hydratase bifunctional protein was similarly carried out following resolution of the solubilized homogenate proteins and of the standards by electrophoresis. Aliquots of homogenate proteins were solubilized using the same volume of Tris–HCl buffer, pH 8.3 (60 mM) containing Triton X-100 (1% w/v). Solubilized proteins were separated electrophoretically and then transferred onto nitrocellulose paper and immunostained essentially as described by Towbin *et al.* [27]. The immunoblot was carried with rabbit antisera to the bifunctional protein (diluted 1:1000) followed by peroxidase-labelled donkey anti-rabbit IgG (diluted 1:2000).

Statistical analysis was carried out using the Student's *t*-test.

RESULTS

Exposure of rats to benoxaprofen resulted in a significant increase in the hepatic O-deethylation of ethoxyresorufin at the doses of 25 and 100 mg/kg, but at the highest dose employed, 250 mg/kg, the change in activity, although still higher than control, was not statistically significant (Table 1). A similar pattern was obtained in the O-demethylation of methoxyresorufin, but in contrast, treatment with benoxaprofen had no significant effect on the O-depentylation of pentoxyresorufin (Table 1). The hepatic 11-hydroxylation of lauric acid was not altered by treatment of the animals with benoxaprofen, whereas the 12-hydroxylation was enhanced at the higher doses of the drug (Table 1). The N-demethylation of dimethylnitrosamine was significantly inhibited by treatment with benoxaprofen, the effect being more pronounced at the lower doses. Microsomal levels of total cytochrome P450 increased at the two lower doses of the drug. Finally, benoxaprofen had no effect on the NADPH-dependent reduction of cytochrome *c* and microsomal protein (Table 1).

Immunoblot analysis employing polyclonal antibodies against P450 IA1 showed that benoxaprofen, at the two lower doses, increased both A1 and A2 proteins, but to an extent much less than that produced by 3-methylcholanthrene, used as the positive control (Fig. 2A). When polyclonal antibodies against P450 IVA1 were employed, benoxaprofen was seen to cause a marked, concentration-dependent increase in the P450 IVA1 levels, but had no major effect on the other protein, presumably P450 IVA2, recognized by this antibody (Fig. 2B).

Treatment with benoxaprofen caused a concentration-dependent increase in the β-oxidation of palmitoyl CoA determined in the liver homogenate (Table 1). Immunoblot analysis employing antibodies to the microsomal *trans*-2-enoyl CoA hydratase bifunctional protein showed that benoxaprofen

Table 1. Effect of benoxaprofen on rat hepatic mixed-function oxidases and peroxisomal activities

Parameter	Control	Benoxaprofen (25 mg/kg)	Benoxaprofen (100 mg/kg)	Benoxaprofen§ (250 mg/kg)
Ethoxyresorufin *O*-deethylase (pmol/min/nmol P450)	31 ± 5	58 ± 5*	63 ± 7*	49 ± 5
Methoxyresorufin *O*-demethylase (pmol/min/nmol P450)	23 ± 2	31 ± 1*	41 ± 4*	48 ± 11
Pentoxyresorufin *O*-depentylase (pmol/min/nmol P450)	3.4 ± 0.7	4.1 ± 0.1	4.2 ± 0.4	5.0 ± 0.6
Lauric acid 12-hydroxylase (nmol/min/nmol P450)	3.5 ± 0.4	3.3 ± 0.2	5.8 ± 0.2‡	7.7 ± 0.9†
Lauric acid 11-hydroxylase (nmol/min/nmol P450)	2.1 ± 0.6	2.0 ± 0.4	2.7 ± 0.4	2.9 ± 0.5
Dimethylnitrosamine *N*-demethylase (nmol/min/nmol P450)	7.6 ± 0.6	4.6 ± 0.4†	5.9 ± 0.5*	7.8 ± 1.5
Palmitoyl CoA oxidation (nmol NADH/min/mg protein)	5.4 ± 1.1	8.7 ± 0.7†	9.4 ± 0.9†	11.6 ± 1.4*
Cytochrome P450 (nmol/mg protein)	0.72 ± 0.01	0.99 ± 0.03‡	0.92 ± 0.03‡	0.72 ± 0.08
NADPH-cytochrome *c* reductase (nmol/min/mg protein)	27.8 ± 0.6	27.5 ± 1.1	25.5 ± 1.1	27.0 ± 0.7
Microsomal protein (mg/g liver)	16.3 ± 0.5	18.9 ± 0.4	20.6 ± 1.0	19.8 ± 0.4
Total hepatic protein (mg/g liver)	121 ± 8	118 ± 4	122 ± 9	141 ± 2

Results are presented as means ± SEM for four animals. Rats were treated with single daily intraperitoneal doses of the drug for 3 days, while controls received the corresponding volume of the vehicle. All animals were killed 24 hr after last injection.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$

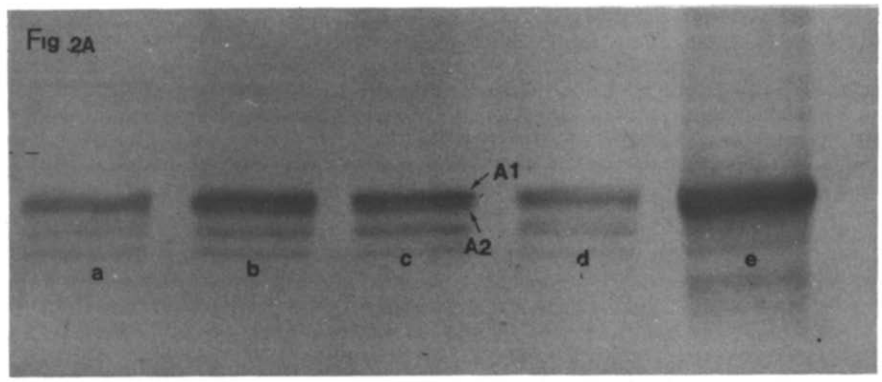
§ At this dose level one of the animals died so that $N = 3$.

administration caused a dose-dependent increase in the levels of this protein, the effect being similar to that of clofibrate, an established peroxisomal proliferator (Fig. 3).

DISCUSSION

Treatment with benoxaprofen modestly enhanced the O-deethylation of ethoxyresorufin, an activity exclusively catalysed by the P450 I family, and particularly the A1 isoenzyme [28, 29]. Similar changes were observed in the O-demethylation of methoxyresorufin, an activity also associated with the P450 I family, and particularly the A2 isoenzyme [30] and these findings are supported by the immunoblot studies. The P450 I family has been implicated in the metabolic activation and toxicity of many chemicals including drugs [7, 8], and the induction of the P450 I family by benoxaprofen raises the possibility that these enzymes may metabolically convert benoxaprofen to a "reactive electrophile(s)", which by virtue of its interaction with cellular nucleophiles may mediate the known hepatotoxicity of the drug. The fact that no induction has been observed at the highest benoxaprofen dose probably reflects the toxicity of the drug as one of the animals died. It would be reasonable to assume that reactive metabolites of benoxaprofen formed by P450 I metabolism would subsequently react preferentially with this enzyme leading to its ultimate destruction. However, in contrast to this hypothesis, it has previously been concluded, from studies in rat hepatocytes, that benoxaprofen hepatotoxicity was due to the parent compound and that cytochrome P450-dependent metabolic oxidation was not involved, as the toxicity was unaffected by P450 inducers and inhibitors [31].

Nevertheless, benoxaprofen interacts *in vitro* with hepatic microsomes to elicit a type I spectral change [31], indicative of binding of the drug to the substrate binding site of cytochromes P450. Moreover, various other benzoxazole derivatives have been shown in studies *in vitro* to modulate mixed-function oxidase activities [32]. Furthermore, although metabolic studies in animals and man revealed that the only metabolite of benoxaprofen is an ester glucuronide, with no oxidation metabolites identified [33], minor metabolites, of a polar character, were also reported, and although not identified, might represent oxidation metabolites formed by the cytochrome P450 system. It should also be noted that benoxaprofen metabolism was studied following single administration of the drug to animals not pretreated with inducing agents [33]; in such circumstances the concentrations of hepatic P450 I are low, comprising less than 5% of total hepatic cytochrome P450 [34] so that oxidation of benoxaprofen would be minimal. However, repeated administration of benoxaprofen would result in induction of P450 I so that oxidative metabolism to hepatotoxic intermediates might assume greater importance, thus explaining why long term administration of the drug was necessary for hepatotoxicity to occur. Continuous high production of reactive



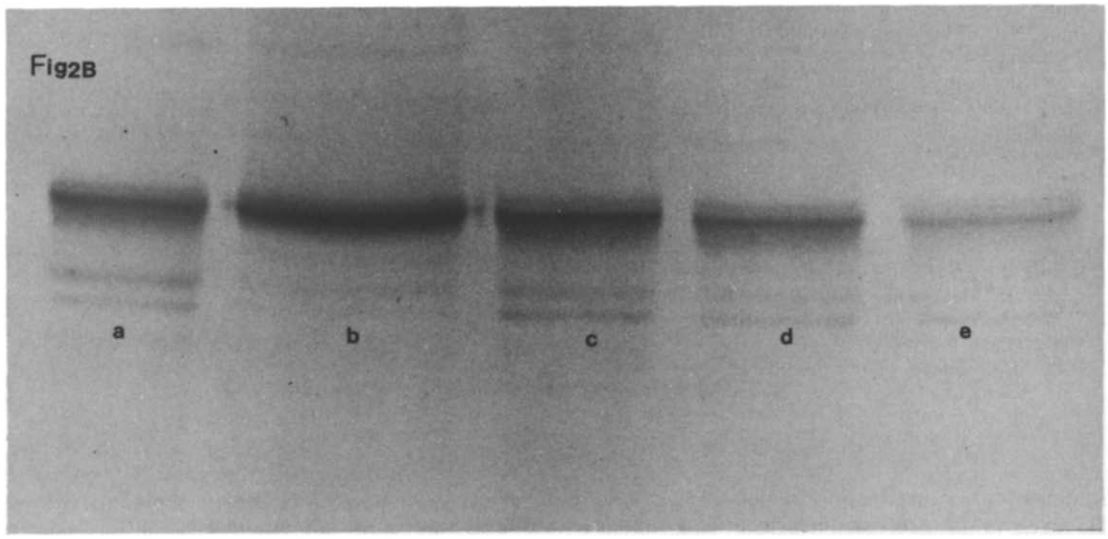


Fig. 2. Immunoblot analysis of microsomes from benoxaprofen-treated rats using anti-cytochrome P450 IA1 and anti-cytochrome P450 IVA1 polyclonal antibodies. Microsomal proteins (20 μg) were resolved by electrophoresis in a 10% (w/v) SDS–polyacrylamide gel and transferred electrophoretically to nitrocellulose. In the case of P450 IA1 (A) the immunoblot was carried out with sheep anti-cytochrome P450 IA1 (diluted 1:12,000) followed by peroxidase-labelled donkey anti-sheep IgG (diluted 1:1000). a: Control, b: benoxaprofen 25 mg/kg, c: benoxaprofen 100 mg/kg, d: benoxaprofen 250 mg/kg and e: 3-methylcholanthrene 25 mg/kg (10 μg loaded). In the case of P450 IVA1 (B) the immunoblot was carried out with sheep anti-cytochrome P450 IVA1 (diluted 1:1600) followed by peroxidase-linked donkey anti-sheep IgG (diluted 1:2000). a: benoxaprofen 250 mg/kg, b: clofibrate (10 μg loaded), 80 mg/kg, c: benoxaprofen 100 mg/kg, d: benoxaprofen 25 mg/kg and e: control.

intermediates would consequently deplete protective intracellular glutathione, that could further exacerbate benoxaprofen toxicity. This illustrates the need, in drug safety evaluation studies, to examine the metabolism of the drug after repeat dosage.

Treatment of rats with benoxaprofen gave rise also to a concentration-dependent increase in the 12-hydroxylation of lauric acid; this effect was specific in that the 11-hydroxylation of the fatty acid was unaffected. The hydroxylation of lauric acid at the 12-position is specifically catalysed by the P450 IV family of enzymes, and benoxaprofen caused a concentration-dependent increase in the apoprotein levels of this family, determined immunologically. Similarly, the cyanide-insensitive β-oxidation of palmitoyl CoA, a marker enzyme for peroxisomes, was increased in a dose-dependent manner. Furthermore, the same treatment increased the hepatic apoprotein levels of a protein present in the peroxisomes, namely, *trans*-2-enoyl CoA hydratase, which is characterized by bifunctional activity catalysing the hydration and dehydrogenation in the β-oxidation of fatty acids [35, 36] and is induced by a variety of peroxisomal proliferators [37]. Recent studies have revealed that the bifunctional protein is in fact a trifunctional enzyme, also possessing Δ^3, Δ^2-enoyl CoA isomerase activity [38]. A number of chemicals such as the drug clofibrate and its analogues, phthalate esters, and chlorinated phenoxy acids, have been shown to induce both P450 IVA1 activity and peroxisomal proliferation, and an interrelationship appears to exist between these two phenomena [39, 40]. It has been argued that such peroxisome proliferators function as epigenetic

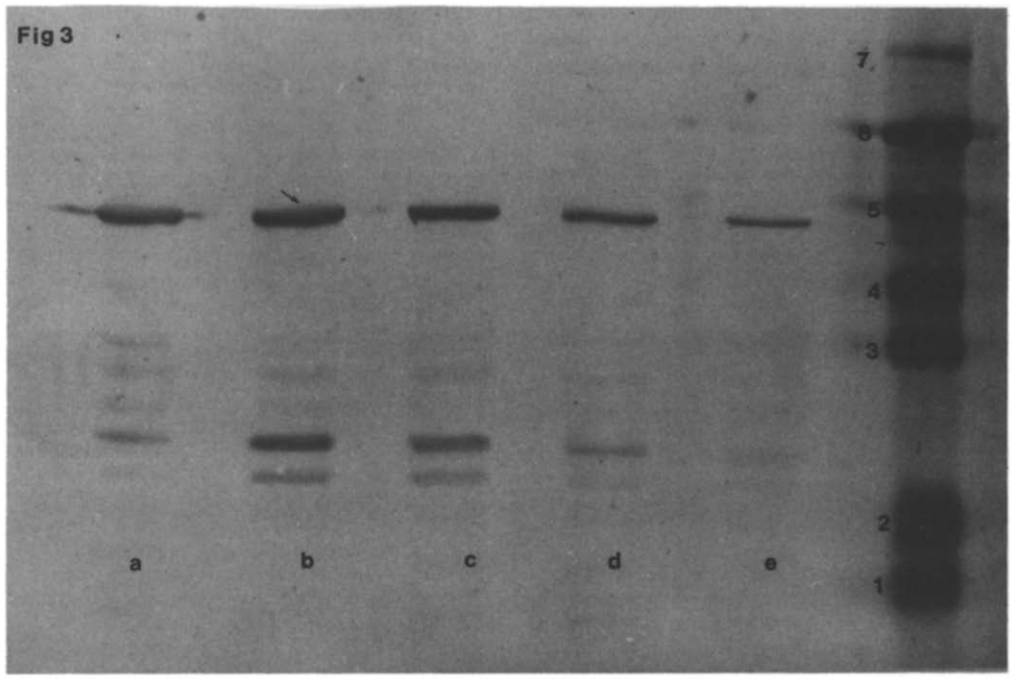


Fig. 3. Immunoblot analysis of microsomes from benoxaprofen-treated rats using antibodies to the peroxisomal bifunctional protein. Homogenate proteins (30 μg) were resolved by electrophoresis in a 3% (w/v) SDS–polyacrylamide gel and transferred electrophoretically to nitrocellulose. The immunoblot was carried out with rabbit antisera to the peroxisomal bifunctional protein (diluted 1:1000) followed by peroxidase-linked anti-rabbit IgG (diluted 1:2000). a: clofibrate 80 mg/kg, b: benoxaprofen 250 mg/kg, c: benoxaprofen 100 mg/kg, d: benoxaprofen 25 mg/kg and e: control. The molecular weight markers were: 1: triosephosphate isomerase (26,600), 2: lactate dehydrogenase (36,500), 3: fumarase (48,500), 4: pyruvate kinase (58,000), 5: fructose 6-phosphate kinase (84,000), 6: β-galactosidase (116,000) and 7: α_2-macroglobulin (180,000).

carcinogens, i.e. acting through a non-genotoxic mechanism [41]. The increased production of H_2O_2 through peroxisomal proliferation, may yield active oxygen species which may give rise to lipid peroxidation and possibly DNA damage. The possibility, therefore, exists that benoxaprofen may induce its hepatotoxicity as a consequence of this proliferative effect on peroxisomes.

However, the relevance of peroxisomal proliferation to chemical toxicity/carcinogenicity has been questioned and may apply to small animals only, such as rat and mouse [42]. In recent studies the diphenyl ether herbicide, fomesafen, was shown to induce peroxisomal proliferation in isolated hepatocytes from rats and mice but not from guinea pig, marmoset and man [43]. Moreover, because of their high rates of metabolic oxidation and oxygen uptake, the mouse and rat may more readily generate reactive oxygen radicals, following peroxisomal proliferation, which can function as tumour promoters and initiators [44]. Indeed, some larger animals, like the marmoset, are refractive to the toxicity and hepatocarcinogenicity of known peroxisomal proliferators [11]. However, potent hypolipidaemic drugs like ciprofibrate have been reported to induce peroxisomal proliferation in cats and in rhesus and cynomolgus monkeys [45], but it is not clear if this is associated with toxicity in these species. It should be noted that drugs like clofibrate, a potent inducer of peroxisome proliferation and of P450 IV activity, have been used extensively to treat hyperlipidaemia with no increase in human liver tumours [46].

The O-dealkylation of pentoxyresorufin, a diagnostic substrate for the phenobarbital-inducible forms of cytochrome P450 [19] was unaffected by the treatment with benoxaprofen showing that the inductive effect of the drug is selective for the P450 I and IV families. Moreover, the N-demethylation of dimethylnitrosamine which, at the substrate concentration employed (6 mM), serves as a probe for the P450 IIE subfamily [47], was inhibited by benoxaprofen treatment indicating that the induction of the P450 I and IV families may have occurred at the expense of the P450 IIE subfamily, and other constitutive forms of the cytochrome that participate in dimethylnitrosamine demethylation.

In the present study, induction of P450 I was observed at a daily dose of 25 mg/kg, given intraperitoneally to rats for 3 days, whereas clinically, the drug was administered to patients at an oral dose of 10 mg/kg/day; orally administered benoxaprofen is readily absorbed from the gastrointestinal tract [33]. The extent of enzyme induction is directly related to the elimination $t_{\frac{1}{2}}$ of the inducing agent [15]. Benoxaprofen is characterized by a long

biological $t_{\frac{1}{2}}$ of nearly 30 hr [48], similar to that seen in the rat [33], but in elderly patients, in whom benoxaprofen hepatotoxicity was manifested, the $t_{\frac{1}{2}}$ was over 100 hr, with consequent drug accumulation [49, 50]. After 1 month of repeated oral dosage of benoxaprofen, drug accumulation would result in tissue concentrations of the drug 10 times greater than after a single dosage, so at the clinical doses employed benoxaprofen may potentially be an inducer of the cytochrome P450 I and IV families.

In summary, the present studies demonstrate that benoxaprofen, at doses similar to those used clinically, induces the cytochrome P450 I and IV families in the rat. On the basis of these observations mechanisms are proposed that could account for the known hepatotoxicity of benoxaprofen in elderly patients.

REFERENCES

1. Dawson W, Boot JR, Harvey J and Walker JR, The pharmacology of benoxaprofen with particular reference to effects on lipoxygenase product formation. *Eur J Rheum Inflamm* **5**: 61–68, 1982.
2. Rainsford K, Side effects of anti-inflammatory/analgesic drugs: epidemiology and gastrointestinal tract. *Trends Pharmacol Sci* **5**: 205–208, 1984.
3. Taggart HM and Adlerdice JM, Fatal cholestatic jaundice in elderly patients taking benoxaprofen. *Br Med J Clin Res* **284**: 1372, 1982.
4. Lewis DFV, Ioannides C and Parke DV, Structural requirements for substrates of cytochromes P450 and P448. *Chem Biol Interact* **64**: 39–60, 1987.
5. Lewis DFV, Ioannides C and Parke DV, Prediction of chemical carcinogenicity from molecular and electronic structure: a comparison of MINDO/3 and CNDO/2 molecular orbital methods. *Toxicol Lett* **45**: 1–13, 1989.
6. Lewis DFV, Ioannides C and Parke DV, A retrospective study of the molecular toxicology of benoxaprofen. *Toxicology* **65**: 33–47, 1990.
7. Ioannides C and Parke DV, The cytochrome P450 I gene family of microsomal hemoproteins and their role in the metabolic activation of chemicals. *Drug Met Rev* **22**: 1–85, 1990.
8. Ioannides C and Parke DV, The cytochromes P448—A unique family of enzymes involved in chemical toxicity and chemical carcinogenesis. *Biochem Pharmacol* **36**: 4197–4207, 1987.
9. Moody DE and Reddy JK, The hepatic effects of hypolipidemic drugs (clofibrate, nafenopin, fibric acid and Wy-14,643) on hepatic peroxisomes and peroxisome-associated enzymes. *Am J Path* **90**: 435–445, 1978.
10. Schmidt U and Machemer L, Difference between species in response to a 3,5-dichloropydyloxyphenoxy compound: Induction of cytochrome P450 and/or peroxisome proliferation. *Fd Addit Cont 6, Suppl* **1**: S41–S55, 1989.
11. Gibson GG, Comparative aspects of the mammalian cytochrome P450 IV gene family. *Xenobiotica* **19**: 1123–1148, 1989.
12. Dunwell DW, Evans D, Hicks TA, Cashin EA and Kitchen A, 2-Aryl-5-benzoxazole-alkanoic acid derivatives with notable anti-inflammatory activity. *J Med Chem* **18**: 53–58, 1975.
13. Rodrigues AD, Lewis DFV, Ioannides C and Parke DV, Spectral and kinetic studies of the interaction of imidazole antifungal agents with microsomal cytochromes P450. *Xenobiotica* **17**: 1315–1327, 1987.
14. Rodrigues AD, Gibson GG, Ioannides C and Parke DV, Interactions of imidazole antifungal agents with purified cytochrome P450 proteins. *Biochem Pharmacol* **36**: 4277–4281, 1987.
15. Ioannides C and Parke DV, Mechanism of induction of hepatic microsomal drug metabolising enzymes by a series of barbiturates. *J Pharm Pharmacol* **27**: 739–746, 1975.
16. Bronfman M, Inestrosa NC and Leighton F, Fatty acid oxidation by human liver peroxisomes. *Biochem Biophys Res Commun* **88**: 1030–1036, 1979.
17. Burke MD and Mayer RT, Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P450 binding of phenoxazone and a homologous series of its n-alkyl ethers (alkoxy resorufins). *Chem Biol Interact* **45**: 243–248, 1983.
18. Burke MD and Mayer RT, Ethoxyresorufin: direct fluorometric assay of microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab Dispos* **2**: 583–588, 1974.
19. Lubet RA, Mayer RT, Cameron JW, Nims RW, Burke MD, Wolf T and Guengerich FP, Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238**: 43–48, 1985.
20. Parker G and Orton T, Induction by oxyisobutyrates of hepatic and kidney microsomal cytochrome P-450 with specificity towards hydroxylation of fatty acids. In: *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. Gustafsson JA, Mode A and Rafter J), pp. 373–377. Elsevier/North Holland Biomedical Press, Amsterdam, 1980.
21. Holtzman JL, Gram TE, Gigon PL and Gillette JR, The distribution of the components of the mixed-function oxidase between the rough and smooth endoplasmic reticulum of liver cells. *Biochem J* **110**: 401–406, 1968.
22. Williams Jr CH and Kamin H, Microsomal triphosphosphopyridine cytochrome c reductase of liver. *J Biol Chem* **237**: 587–595, 1962.
23. Omura T and Sato R, The carbon monoxide pigment of liver microsomes. I. Evidence for its haemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
24. Lowry OH, Rosebrough NJ, Farr AL and Randall AJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
25. Ayrton AD, McFarlane M, Walker R, Neville S and Ioannides C, The induction of P450 I proteins by aromatic amines may be related to their carcinogenic potential. *Carcinogenesis* **11**: 803–809, 1990.
26. Barnett CR, Gibson GG, Wolf CR, Flatt PR and Ioannides C, Induction of cytochrome P450 III and P450 IV family proteins in streptozotocin-induced diabetes. *Biochem J* **268**: 765–769, 1990.
27. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4355, 1983.
28. Guengerich FP, Dannan GW, Wright ST, Martin MV and Kaminsky LS, Purification and characterization of cytochrome P-450s. *Xenobiotica* **12**: 701–716, 1982.
29. Phillipson CE, Godden PMM, Lum PY, Ioannides C and Parke DV, Determination of cytochrome P-448 activity in biological tissues. *Biochem J* **221**: 81–88, 1984.
30. Namkung MJ, Yang HL, Hulla JE and Juchau MR, On the substrate specificity of cytochrome P450 IIIA1. *Mol Pharmacol* **34**: 628–637, 1988.
31. Knights KM, Cassidy MR and Drew R, Benoxaprofen induced toxicity in isolated rat hepatocytes. *Toxicology* **40**: 327–339, 1986.

32. Little PJ and Ryan AJ, Inhibitors of hepatic mixed-function oxidases—V. Inhibition of aminopyrine N-demethylation and enhancement of aniline hydroxylation by benzoxazole derivatives. *Biochem Pharmacol* **31**: 1795–1798, 1982.
33. Chatfield DH and Green JN, Disposition and metabolism of benoxaprofen in laboratory animals and man. *Xenobiotica* **8**: 133–144, 1978.
34. Luster MI, Lawson LD, Linko P and Goldstein JA, Immunochemical evidence for two 3-methylcholanthrene-inducible forms of cytochrome P-448 in rat liver microsomes using double antibody radioimmunoassay. *Mol Pharmacol* **23**: 252–257, 1982.
35. Lazarow PB, Rat liver peroxisomes catalyze the β-oxidation of fatty acids. *J Biol Chem* **253**: 1522–1528, 1978.
36. Reddy MK, Qureshi SA, Hollenberg PF and Reddy JK, Immunochemical identity of peroxisomal enoyl-CoA hydratase with the peroxisome proliferation associated 80,000 mol. wt. polypeptide in rat liver. *J Cell Biol* **89**: 406–417, 1981.
37. Reddy MK, Hollenberg PF and Reddy JK, Partial purification and immunoreactivity of an 80,000 molecular weight peptide associated with peroxisome proliferation in rat liver. *Biochem J* **188**: 731–740, 1980.
38. Palosaari PM and Hiltunen JK, Peroxisomal bifunctional protein from rat liver is a trifunctional enzyme processing 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and Δ^3,Δ^2-enoyl-CoA isomerase activities. *J Biol Chem* **265**: 2446–2449, 1990.
39. Sharma R, Lake BG, Foster J and Gibson GG, Microsomal cytochrome P-452 induction and peroxisomal proliferation by hypolipidaemic agents in rat liver. A mechanistic inter-relationship. *Biochem Pharmacol* **37**: 1193–1201, 1988.
40. Bacher M and Gibson GG, Chlorophenoxy acid herbicides induce microsomal cytochrome P450 IVA1 (P-452) in rat liver. *Chem Biol Interact* **65**: 145–156, 1988.
41. Reddy JK and Lalwani ND, Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit Rev Toxicol* **12**: 1–58, 1983.
42. Lock EA, Mitchell AM and Elcombe CR, Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annu Rev Pharmacol Toxicol* **29**: 145–163, 1989.
43. Smith LL and Elcombe CR, Mechanistic studies: their role in toxicological evaluation. *Fd Addit Cont 6, Suppl* **1**: S57–S65, 1989.
44. Parke DV and Ioannides C, Role of cytochromes P-450 in mouse liver tumor production. In: *Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons* (Eds. Stevenson DE, McClain RM, Popp JA, Slaga TJ, Ward JM and Pitot HC), pp. 215–230. Wiley-Liss, New York, 1990.
45. Reddy JK, Qureshi SA, Lalwani ND, Reddy MK and Moehle CM, Induction by ciprofibrate of hepatic peroxisome proliferation in rats, pigeons, chickens, cats and Rhesus monkeys, *Fed Proc* **41**: 1741, 1982.
46. IARC, *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 24: Some Pharmaceutical Drugs*. pp. 39–58. IARC, Lyon, 1980.
47. Thomas PE, Bandiera S, Maines SL, Ryan DE and Levin W, Regulation of cytochrome P450 j, a high affinity N-nitrosodimethylamine demethylase in rat hepatic microsomes. *Biochemistry* **26**: 2280–2289, 1987.
48. Brogard JM, Comte F, Madon M and Spack MO, Pharmacokinetic profile of benoxaprofen in subjects with normal and impaired renal function. Prediction of multiple-dose kinetics. *Eur J Rheumatol Inflamm* **5**: 113–123, 1982.
49. Hamdy RC, Murnane B, Perera N, Woodcock K and Koch IM, The pharmacokinetics of benoxaprofen in elderly subjects. *Eur J Rheumatol Inflamm* **5**: 69–75, 1982.
50. Kamal A and Koch IM, Pharmacokinetic studies of benoxaprofen in geriatric patients. *Eur J Rheumatol Inflamm* **5**: 76–81, 1982.