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DENATURATION OF CYTOCHROME P-450 BY INDOMETHACIN AND OTHER NON-STEROIDAL ANTI-INFLAMMATORY DRUGS: EVIDENCE FOR A SURFACTANT MECHANISM AND A SELECTIVE EFFECT OF A *p*-CHLOROPHENYL MOIETY

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Abstract—Indomethacin added to rat liver microsomes *in vitro* resulted in the denaturation of cytochrome P-450 to cytochrome P-420. This was NADPH independent, appeared to be non-enzyme mediated, did not involve free radicals or lipid peroxidation and was prevented by glycerol, butylated hydroxytoluene or SKF-525A. Indomethacin *in vitro* also caused a loss of cytochrome b_5, NADH-cytochrome b_5 reductase, NADPH-cytochrome *c* reductase and epoxide hydrolase activities, but an activation of UDP-glucuronyltransferase. Amongst a total of 22 non-steroidal anti-inflammatory drugs and derivatives there was a highly significant correlation between the extent of their denaturation of cytochrome P-450 and their surfactant potency. The results suggest that the denaturation of cytochrome P-450 by certain non-steroidal anti-inflammatory drugs was due to a detergent-like, membrane-perturbing action of the drugs and that in most cases the denaturation also involved a specific effect of a *p*-chlorophenyl moiety of the drug.

Indomethacin is a widely used non-steroidal anti-inflammatory drug (NSAID)‡ with a potential for clinical hepatotoxicity [1, 2]. Pharmacological doses in rats (2–5 mg/kg orally) cause a large decrease in the hepatic microsomal cytochrome P-450-dependent monooxygenase enzyme system [3]. Part of this decrease may be due to endotoxins liberated into the blood as a consequence of indomethacin-induced intestinal ulceration, but a direct effect of indomethacin on hepatic cytochrome P-450 is also probably involved [4]. Amongst other NSAID, ketoprofen also depresses rat liver cytochrome P-450 *in vivo* [5], whilst alclofenac destroys mouse liver microsomal cytochrome P-450 *in vitro*, probably through the direct action of an active epoxide metabolite [6]. Aside from the potential effect on drug metabolism, the possibility that NSAID might destroy cytochrome P-450 has increased in importance in view of recent evidence that prostacyclin and thromboxane synthetases are cytochrome P-450 enzymes [7]. In this study we have investigated the mechanism whereby indomethacin and other NSAID cause a loss of liver microsomal cytochrome P-450 *in vitro*, albeit at much higher concentrations than are associated with specific inhibition of prostaglandin synthesis.

MATERIALS AND METHODS

Chemicals. Indomethacin, DMI, DBI, DMBI, L-583,916 [2-(1-*p*-chlorobenzyl-5-methoxy-2-methyl indol-3-yl) propionic acid] and L-593,120 [1-(*p*-chlorobenzylidene-5-methoxy-2-methyl-3-indene) acetic acid] were provided by Dr H. B. Hucker of Merck, Sharp and Dohme (Pennsylvania, U.S.A.). Benoxaprofen, LRCL3861 [2-(2-*m*-methylphenyl-5-benoxazolyl) propionic acid], LRCL3900 [2-(2-*p*-fluorophenyl-5-benzoxazolyl) propionic acid] and LRCL3987B [2-(2-*o*-chlorophenyl-5-benzoxazolyl) propionic acid] were provided by Dr W. Dawson of the Lilly Research Centre (Windlesham, Surrey, U.K.). All other NSAID were provided by their respective manufacturers. Prostaglandin E_2 (PGE_2), 16,16-dimethylprostaglandin E_2 and 16,16-dimethylprostaglandin $F_{2\alpha}$ were gifts from Dr J. E. Pike of the Upjohn Company (Kalamazoo, MI, U.S.A.). SKF-525A was donated by Smith, Kline and French (Welwyn Garden City, Herts., U.K.). Benzo(a)pyrene 4,5-oxide was a generous gift from Dr H. V. Gelboin, National Cancer Institute, Bethesda, MD, U.S.A.

Animals and drug treatments. Adult (220–270 g) male Sprague–Dawley rats, bred in the University of Aberdeen, were kept on Sorbitex mineral bedding (J. Strachan, Perth, U.K.) and fed Oxoid pasteurised breeding pellets and water *ad libitum*. Groups of three rats were induced by treatment with either

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‡ Abbreviations: NSAID, non-steroidal anti-inflammatory drug; DMI, *O*-desmethylindomethacin; DBI, *N*-desbenzoylindomethacin; DMBI, *O*-desmethyl-*N*-desbenzoylindomethacin; CBA, *p*-chlorobenzoic acid; SKF-525A, diethylaminoethyl diphenylpropylacetate; EDTA, ethylenediaminetetracetic acid; BHT, butylated hydroxytoluene; PG, prostaglandin.

phenobarbitone, given as a 0.1% solution in drinking water for six days with a return to normal drinking water 24 hr before death, or with a single dose of 3-methylcholanthrene, 80 mg/kg as a 1% solution in olive oil i.p. three days before death.

Preparation of liver microsomes and incubation with drugs in vitro. Hepatic microsomes were prepared from untreated and induced rats as previously described [3]. Microsomal incubations were carried out in a water bath with continuous shaking at 100 oscillations per min at 37° (except when temperature dependence was studied) in 0.1 M phosphate buffer, pH 7.6, (or in 0.1 M Tris–HCl buffer, pH 7.6, when $MnCl_2$ was included because it precipitated in phosphate buffer), as specified below. In experiments carried out in the presence of an NADPH-generating system, 0.25 mM NADP, 2.5 mM DL-isocitric acid, 0.6 U isocitrate dehydrogenase and 5 mM $MgSO_4$ in phosphate or Tris buffer were used. Microsomal protein was routinely used at a concentration of 1 mg/ml in a total reaction volume of 3, 6 or 18 ml, depending on the number of parameters to be measured. When dependence on protein concentration was studied, 1.5, 3 or 6 mg of microsomal protein was suspended in a 6 ml incubate. In the following details the concentrations in parentheses are the final concentration of a compound in the incubate. Indomethacin (1, 2 or 5 mM) and each of the other NSAID and derivatives (2 mM) were added as 1 M solutions in DMSO, such that the maximum concentration of DMSO in the incubate was 0.6% v/v. The only exception was L-593,120 (1 mM), which had to be dissolved in 0.5% w/v Tween 80 in phosphate buffer, such that the final concentration of Tween 80 in the incubate was 0.48%. SKF-525A (1 mM), EDTA (1 mM), GSH (1 mM), cysteine HCl (1 mM) or UDPGA (1.5 mM) were added to reactions as 2 mM solutions in phosphate buffer. $MnCl_2$ (1 mM) was added as a 2 mM solution in Tris–HCl buffer. PGE_2, 16,16-dimethyl PGE_2 or 16,16-dimethyl $PGF_{2\alpha}$ (1 mM) were added as 160 mM solutions in methanol, such that the maximum concentration of methanol in the incubate was 0.7% v/v. BHT (1 mM) was added as a 0.6 M solution in methanol, such that the concentration of methanol in the incubate was 0.17% v/v. Promethazine HCl (1 mM) was added as a 120 mM solution in ethanol, such that the concentration of ethanol in the incubate was 0.83% v/v. Glycerol (20% v/v) was added as a 40% solution in phosphate buffer.

Measurement of cytochrome P-450, cytochrome b_5 *and various enzyme activities after incubation with indomethacin or other drugs* in vitro. Microsomal incubations were carried out in 0.1 M phosphate buffer pH 7.6 for 30 min at 37° in the presence of 1 mg protein/ml and 1, 2 or 5 mM indomethacin as described above. Following incubation, the reaction was terminated by placing the samples on ice. The microsomes were separated from the indomethacin by centrifugation at 180,000 *g* for 40 min at 4°. Cytochrome P-450 and cytochrome P-420 were measured as described by Omura and Sato [8] and cytochrome b_5 was measured as described by Strittmatter *et al.* [9]. Enzyme assays were carried out as follows: NADH-cytochrome b_5 reductase by the method of Mihara and Sato [10], using an extinction coefficient for ferricyanide of 102 $mM^{-1}\,cm^{-1}$ at 420 nm [11]; NADPH-cytochrome *c* reductase by the method of Phillips and Langdon [12], using an extinction coefficient for reduced cytochrome *c* of 21.1 $mM^{-1}\,cm^{-1}$ at 550 nm [13]; epoxide hydrolase by the method of Dansette *et al.* [14], using benzo(a)pyrene 4,5-oxide as substrate. Microsomal protein was determined by the method of Lowry *et al.* [15], using bovine serum albumin as the standard. Native and Triton X-100-activated UDP-glucuronyltransferase activities were determined by the spectrophotometric method of Mulder and Van Doorn [16], using *p*-nitrophenol as substrate. Lipid peroxidation was measured by the amount of malonaldehyde produced, using the thiobarbituric acid reaction as described by Buege and Aust [17]. At the end of the indomethacin reaction period, a 1 ml aliquot of microsomal incubate (1 mg protein/ml) was mixed with 2.0 ml TCA–TBA–HCl reagent (containing 15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 N hydrochloric acid). The mixture was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 4000 *g* for 10 min. The absorbance of the sample was determined at 535 nm against a blank containing all the reagents minus microsomes. The malondialdehyde concentration was calculated using an extinction coefficient of 156 $mM^{-1}\,cm^{-1}$ [18].

Measurement of the surfactant potency of drugs. Surfactant potency was measured as the activity for lowering surface tension, using a White torsion balance (Gallenkamp Ltd., East Kilbride, U.K.) with a 1-cm platinum ring immersed in 6 ml of the drug solution. The surface tensions of distilled water and ethanol at 18° read 73.5 (lit. 73.05) and 23.5 (lit. 24.5) dynes/cm, respectively. All drugs were dissolved in DMSO then diluted with 0.1 M phosphate buffer, pH 7.6, giving a solution containing 0.1–2.0 mM drug and 0.4% v/v DMSO. The balance tension was increased until the ring broke clear of the solution surface, at which point the reading was taken as the surface tension of the solution. Measurements were made at 18° at 5, 15 and 30 min after preparing the solution and repeated for three identically prepared solutions: surface tension replicates varied by not more than ±1 dyne/cm. For each drug there was a linear relationship between its log concentration (0.1–2.0 mM) and surface tension.

Statistics. Statistical analysis was carried out using ANOVA followed by Dunnett's test [19], taking $P < 0.05$ as significant, and Spearman's rank correlation test.

RESULTS

Effects of indomethacin on drug metabolising enzymes

Incubation of hepatic microsomes with 1–5 mM indomethacin for 30 min at 37° resulted in a drug concentration-dependent loss of cytochrome P-450, cytochrome b_5, NADH-cytochrome b_5 reductase, NADPH-cytochrome *c* reductase and epoxide hydrolase (Fig. 1). In contrast, UDP-glucuronyltransferase native activity was increased approximately 10-fold by 1 or 2 mM indomethacin, although it was not affected by 5 mM indomethacin (Table 1).

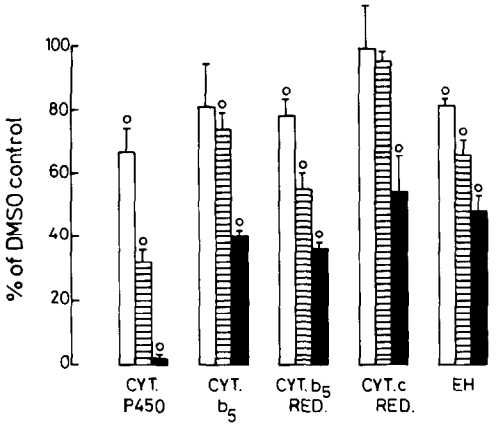

Fig. 1. The destruction of microsomal cytochrome P-450, cytochrome b_5, NADH-cytochrome b_5 reductase, NADPH-cytochrome *c* reductase and epoxide hydrolase (EH) after incubation with indomethacin *in vitro* for 30 min at 37°. The assays were carried out as described in the Methods in the presence of 1 mM (□), 2 mM (▤) or 5 mM indomethacin (■) in 0.4% (v/v) DMSO. Results are the percentage activity remaining after incubation compared to an appropriate control incubate containing 0.4% DMSO (control activity = 100%). Values are means ± SD using four different microsome preparations, each from a different untreated rat. ○—activity significantly lower ($P < 0.05$) than the DMSO control incubate. Control (100%) activities are (means ± SD, N = 4): cytochrome P-450 = 0.80 ± 0.12 nmol/mg microsomal protein; cytochrome b_5 = 0.57 ± 0.07 nmol/mg; NADH-cytochrome b_5 reductase = 2.55 ± 0.32 μmol/min/mg; NADPH-cytochrome *c* reductase = 190.1 ± 19.9 nmol/min/mg; epoxide hydrolase = 7.5 ± 0.51 nmol/min/mg.

UDP-glucuronyltransferase detergent-activated activity, however, was not affected by 1 or 2 mM indomethacin, but was decreased approximately 8-fold by 5 mM indomethacin. The maximum concentration of DMSO present in the incubates as a result of adding the indomethacin solution (0.6% v/v) had no effect alone on cytochrome P-450.

Effect of indomethacin on cytochrome P-450

The effects of 2 mM indomethacin on cytochrome P-450 were studied in more detail. The extent of the loss of cytochrome P-450 was independent of the microsomal protein concentration (0.25–1.0 mg/ml), independent of the incubation temperature (18–37°) and occurred rapidly, reaching its maximum extent (i.e. the losses shown in Fig. 1) within 5 min at either 1, 2 or 5 mM indomethacin. There was, however, no significant loss of cytochrome P-450 (2 ± 0.2%) during 30 min incubation at 4°. After 30 min incubation at 37° the loss of cytochrome P-450 was similar in liver microsomes of either control rats (62 ± 5%), phenobarbitone-induced rats (60 ± 4%) or 3-methylcholanthrene-induced rats (67 ± 5%) and was not affected, in either control or induced microsomes, by the presence of an NADPH-generating system.

In order to understand the mechanism of the indomethacin action on cytochrome P-450 (2 mM indomethacin incubated with liver microsomes for 30 min at 37°), the effects of compounds known to inhibit various different types of chemically mediated destructions of cytochrome P-450 were studied. The results, summarised below, were not dependent on the presence of an NADPH-generating system during the incubation. The indomethacin-mediated loss of cytochrome P-450 was not affected by the presence of 1 mM concentrations of reduced glutathione, cysteine, EDTA, $MnCl_2$ or promethazine. Indomethacin caused only a very small (1.4-fold) increase in microsomal NADPH-dependent lipid peroxidation (0.19 ± 0.04 and 0.27 ± 0.03 nmol malondialdehyde/30 min/mg protein in the absence and presence of indomethacin, respectively), compared with a 10-fold increase caused by incubation with NADPH and 2.5 mM carbon tetrachloride, a compound known to enhance lipid peroxidation [20]. Although prostaglandins protect against the loss of hepatic cytochrome P-450 caused by indomethacin *in vivo* [4], the loss of cytochrome P-450 *in vitro* was not affected by the presence of PGE_2, 16,16-dimethyl PGE_2 or 16,16-dimethyl $PGF_{2\alpha}$ at 1 mM. Three compounds did, however, individually protect cytochrome P-450

Table 1. Effect of indomethacin on UDP-glucuronyltransferase

Indomethacin concentration	Glucuronyltransferase activity (nmol/min/mg microsomal protein) Native*	Activated†
Control	4.3 ± 0.3‡§	44.9 ± 3.5
DMSO	4.1 ± 0.8§	42.0 ± 6.4
1 mM	42.9 ± 2.8	43.1 ± 1.6
2 mM	40.8 ± 2.0	39.9 ± 4.1
5 mM	5.0 ± 0.1§	5.4 ± 0.3§

* Liver microsomes were incubated with Tris buffer (75 mM, pH 7.3) for 10 min at room temperature, then for a further 30 min at 37° in the buffer alone (=control) or plus 0.4% DMSO or plus indomethacin in 0.4% DMSO. Transferase was measured at the end of this period using *p*-nitrophenol as substrate as described in the Methods.

† Liver microsomes were incubated as above except that the initial incubation was in Tris buffer containing 0.25% (w/v) Triton X-100.

‡ Values are means ± SD using four different microsome preparations, each from a different untreated rat.

§ Significantly different from the activated control value ($P < 0.05$).

against indomethacin. The loss of cytochrome P-450 was significantly reduced from 62 ± 5% (with 2 mM indomethacin alone) to 42 ± 9% in the presence of 1 mM butylated hydroxytoluene, 38 ± 4% in the presence of 20% (v/v) glycerol and 30 ± 2% in the presence of 1 mM SKF-525A.

In all the experiments the indomethacin-mediated loss of cytochrome P-450 was accompanied by the approximately stoichiometric formation of its denatured form, cytochrome P-420. Each of the chemicals that protected cytochrome P-450 against indomethacin (BHT, glycerol and SKF-525A) resulted in a corresponding reduction in the amount of cytochrome P-420 formed.

Effect of various NSAID and derivatives on cytochrome P-450

Each compound was dissolved in DMSO and incubated at 2 mM with hepatic microsomes for 30 min at 37° in the absence of an NADPH-generating system, as in Fig. 1, unless otherwise stated. The maximum concentration of DMSO present in the incubates as a result of adding the drug solutions (0.4% v/v) had no effect alone on cytochrome P-450. In Table 2 the NSAID and their derivatives are ranked in order of their effectiveness in causing a loss of cytochrome P-450. Since L-593,120 had to be dissolved in Tween 80 [0.48% (w/v) in the incubate] instead of DMSO, in this case the loss of cytochrome P-450 was measured relative to microsomes incubated with 0.48% Tween 80 alone (which caused no significant loss of cytochrome P-450 relative to microsomes incubated in phosphate buffer).

L-593,120 and L-583,916 are derivatives of indomethacin, while DMI, DBI, DMBI and CBA are its major non-conjugated metabolites. Chlorobenza-

Table 2. Abilities of non-steroidal anti-inflammatory drugs and derivatives to decrease the cytochrome P-450 concentration of microsomes and the surface tension of aqueous buffer

Compound	% Decrease in cytochrome P-450 concentration*	P-420 formed† / P-450 lost	% Decrease in surface tension‡
L-593,120	85 ± 3 (7)	1.07	39
L-583,916	85 ± 2 (5)	0.81	26
Benoxaprofen	63 ± 4 (6)	0.87	18
Indomethacin	62 ± 5 (7)	0.84	21
DMI	46 ± 3 (4)	0.83	24
Flurbiprofen	23 ± 3 (3)	0.82	10
Zomepirac	22 ± 3 (3)	0.84	10
DBI	16 ± 2 (4)	0.85	3
LRCL3861	15 ± 2 (3)	0.83	23
Sulphinpyrazone	14 ± 4 (3)	1.0	ND
Naproxen	12 ± 2 (3)	0.44	4
LRCL3987B	12 ± 3 (3)	0.67	20
Piroxicam	12 ± 3 (3)	0.89	ND
Ibuprofen	11 ± 1 (3)	NF	8
Alclofenac§	10 ± 1 (3)	0.71	2
Paracetamol	8 ± 2 (3)	0.50	ND
Ketoprofen	5 ± 2 (3)	0.50	5
DMBI	4 ± 1 (4)	NF	0
LRCL3900	3 ± 2 (3)	NF	16
CBA	2 ± 1 (4)	NF	1
p-Chlorobenzamide	1 ± 1 (4)	NF	0
p-Chlorobenzoylglycine	1 ± 1 (4)	NF	0

* % Decrease in P-450 concentration (nmol/mg protein) resulting from incubation of hepatic microsomes with 2 mM compound (in 0.4% DMSO or 0.48% Tween 80 in phosphate buffer, pH 7.6, as described in the text), for 30 min at 37°, expressed relative to P-450 concentration in microsomes incubated with buffer and vehicle alone. P-450 concentration of fresh microsomes = 0.68 ± 0.05 nmol/mg protein, which was not significantly affected by incubation with 0.4% DMSO or 0.48% Tween 80 alone. Values are means ± SEM, with N (number of individual microsome samples, each from a different rat) in parentheses. The eight results above the dotted line show significant decreases in P-450 ($P < 0.05$), whereas the 14 results below the line show no significant decreases in P-450 ($P > 0.05$).

† Mean ratios between the amounts (nmol per incubation) of cytochrome P-420 formed and of P-450 lost in the incubations described above. No P-420 was formed in incubations with DMSO or Tween 80 alone. NF = no P-420 formed.

‡ % Decrease in surface tension of 0.4% DMSO in phosphate buffer, pH 7.6, due to the presence of 1 mM compound at 18°, expressed relative to the surface tension of buffered 0.4% DMSO alone (72.4 dynes/cm), which was not significantly different from the surface tension of distilled water (=73.5 dynes/cm). Results are means for triplicate determinations and replicates varied by not more than ±1 dyne/cm. ND = not determined.

§ The values for all compounds are for incubations in the absence of NADPH. Alclofenac plus an NADPH-generating system caused a 20 ± 2% loss of P-450 ($P < 0.05$, N = 3) with no formation of P-420.

mide and chlorobenzoylglycine are analogues of CBA. LRCL3861, LRCL3987B and LRCL3900 are derivatives of benoxaprofen. Significant loss of cytochrome P-450 was caused by L-593,120, L-583,916, benoxaprofen, indomethacin, flurbiprofen, zomepirac, DMI and DBI (shown above the dotted line in Table 2). No significant loss of cytochrome P-450 was caused by the other compounds (below the dotted line). The effects of benoxaprofen were similar to those of indomethacin in being rapid, drug concentration-dependent, not occurring at 4°, prevented by 20% glycerol (although not by BHT) and not prevented by EDTA. In all cases except alclofenac the losses in cytochrome P-450 were the same whether an NADPH-generating system was present or not and were, at least for those compounds causing a significant loss of cytochrome P-450, accompanied by the approximately stoichiometric formation of cytochrome P-420 (the ratio between nmol cytochrome P-420 formed and nmol cytochrome P-450 lost ranged from 0.81 to 1.07). With some of the compounds that caused non-significant losses in cytochrome P-450 the ratios of cytochrome P-420 formed/cytochrome P-450 lost were much less than unity: this was probably due to inaccuracy incurred in measuring the very small amounts of cytochrome P-420 formed. In contrast, alclofenac caused a small loss of cytochrome P-450, which was NADPH dependent and not accompanied by cytochrome P-420 formation.

Surfactant potencies of various NSAID and derivatives

The results of the study on the indomethacin-mediated loss of cytochrome P-450 suggested that the mechanism might involve a detergent-like effect of the drug. Accordingly, the surface activities of a number of NSAID and derivatives were investigated and the possibility explored of a correlation with their effects on cytochrome P-450. Table 2 shows the relative surfactant potencies of the NSAID and their derivatives, measured as the percentage decrease in surface tension due to the presence of the compound in solution relative to the surface tension of 0.4% DMSO (the solution in which the compounds were dissolved). The results for surface tension effects are shown for compounds used at 1 mM concentration (instead of 2 mM as used when studying cytochrome P-450 loss). This was because 1 mM was the highest concentration of certain of the compounds that could be dissolved in 0.4% DMSO (the concentration used in the studies on cytochrome P-450 loss) without recourse to either Tween 80 (not suitable because it is itself a detergent) or sonication of the solution (not suitable because it gave rise to unreproducible results, presumably due to the compounds gradually coming out of solution; the high reproducibility of the results for cytochrome P-450 loss with 2 mM compounds was probably because these were retained in solution by the microsomes). There was a wide variation in surface activity between the different NSAID and derivatives. There was a highly significant correlation among the NSAID between their relative surface activities (as defined above) and the extents of their destruction/denaturation of cytochrome P-450: the Spearman rank correlation coefficient, $r_s = 0.720$ ($P < 0.01$) for a comparison of all the compounds in Table 2, whilst $r_s = 0.899$ ($P < 0.01$) for a comparison of just the eight compounds that caused a significant loss of cytochrome P-450.

DISCUSSION

This study has shown that indomethacin and a number of other NSAID and derivatives cause an NADPH-independent denaturation of hepatic microsomal cytochrome P-450 to cytochrome P-420 *in vitro*. Can this be explained by any of the known mechanisms whereby drugs and other chemicals cause the loss of cytochrome P-450 *in vitro*?

NADPH-dependent lipid peroxidation destroys cytochrome P-450 (without the formation of cytochrome P-420) [21] and carbon tetrachloride destroys cytochrome P-450 at least partly by stimulating lipid peroxidation via a free-radical metabolite [20, 22]. However, this mechanism is unlikely for indomethacin, since the loss of cytochrome P-450 was accompanied by the formation of cytochrome P-420, was not NADPH dependent, was not prevented by lipid peroxidation inhibitors (EDTA or $MnCl_2$ [21]) or free radical scavengers (reduced glutathione or promethazine [20]), and was not accompanied by an increase in microsomal lipid peroxidation. The mechanism of the loss of cytochrome P-450 due to indomethacin appeared not to be related to the mechanisms whereby cytochrome P-450 is destroyed by thiono-sulphur compounds (e.g. parathion [23]) or allyl compounds (e.g. secobarbital [24]), since these compounds, in contrast to indomethacin, cause loss of cytochrome P-450 haem without the formation of cytochrome P-420 and have little effect on cytochrome b_5.

The denaturation of cytochrome P-450 to cytochrome P-420 by indomethacin and its partial prevention by glycerol bears a close similarity to the glycerol-preventable denaturation of cytochrome P-450 by cholate [25] and non-ionic detergents. The idea of a detergent-like action of indomethacin is supported by its activation of UDP-glucuronyltransferase, which is a well-documented effect of non-ionic detergents [26]. Cytochrome P-450 can also be denatured to cytochrome P-420 by compounds reacting with its sulphydryl groups, e.g. by 4-hydroxycyclophosphamide, but this is prevented by reduced glutathione or cysteine [25, 27] and therefore the mechanism does not seem to apply to indomethacin. The high correlation among several NSAID and derivatives between the extent of their denaturation of cytochrome P-450 to cytochrome P-420 and their surfactant potencies confirmed the likelihood that the loss of cytochrome P-450 was due to a detergent-like perturbation of the microsomal membrane. This could also explain the non-specific indomethacin-mediated loss of cytochrome b_5, NADPH-cytochrome *c* reductase, NADH-cytochrome b_5 reductase and epoxide hydrolase. If indomethacin was denaturing cytochrome P-450 by acting like a detergent then the most probable explanation for the protection afforded by BHT and SKF-525A is that, by undergoing reversible Type I interactions with the cytochrome P-450 apoprotein (data not shown), these compounds were able either to

deny access of cytochrome P-450 to indomethacin (which reportedly undergoes a Type I interaction with cytochrome P-450 itself [5]) or to stabilize the haemoprotein structure. We believe this to be the first report of a detergent mechanism for the loss of cytochrome P-450 caused by a therapeutic compound, although a surfactant effect has been suggested to explain other biochemical actions of drugs, e.g. cytotoxicity [28, 29].

In addition to a detergent-like action there may also have been a specific effect of a *p*-chlorophenyl moiety against cytochrome P-450. All five of the compounds causing the greatest denaturation of cytochrome P-450 and zomepirac, which caused the seventh greatest denaturation, contained a *p*-chlorophenyl group (Fig. 2). Moreover, whilst benoxaprofen and its three derivatives all had similarly high surfactant potencies, benoxaprofen was the only one of these that contained a *p*-chlorophenyl moiety (Fig. 2) and was the only one that significantly denatured cytochrome P-450. Insufficient surfactant potency explains the failure of *p*-chlorobenzoic acid, *p*-chlorobenzamide and *p*-chlorobenzoylglycine to denature cytochrome P-450.

The abilities of some NSAID to denature cytochrome P-450 may have ramifications not merely for drug metabolism but also for those eicosanoid-metabolising enzymes that are forms of cytochrome P-450, e.g. prostacyclin and thromboxane synthetases [7]. Whether these effects would occur to a significant extent *in vivo* is debatable, however, since the concentration of indomethacin needed to denature hepatic microsomal cytochrome P-450 was far higher than the blood concentration of indomethacin normally achieved during effective anti-inflammatory therapy [30].

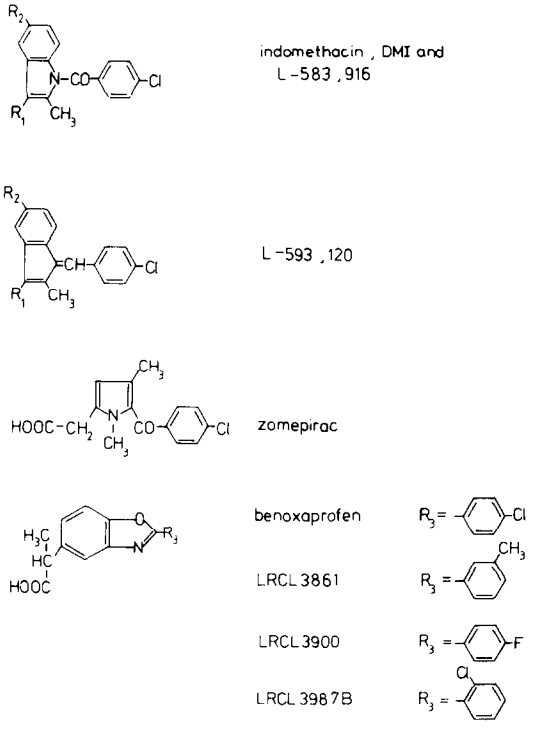

Fig. 2. Structures of selected non-steroidal anti-inflammatory drugs and derivatives. $R_1 = CH_2COOH$ in indomethacin, DMI and L-593,120. $R_1 = C_2H_4COOH$ in L-583,916. $R_2 = OCH_3$ in indomethacin, L-593,120 and L-583,916. $R_2 = OH$ in DMI.

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