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DECREASED HEPATIC MICROSOMAL CYTOCHROME P450 DUE TO INDOMETHACIN: PROTECTIVE ROLES OF 16,16-DIMETHYLPROSTAGLANDIN $F_{2\alpha}$ AND INDUCING AGENTS

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Abstract—Indomethacin administration to rats caused a dose-dependent decrease in hepatic microsomal cytochrome P450, aminopyrine *N*-demethylase, ethoxyresorufin O-de-ethylase and benzyloxyresorufin O-debenzylase, accompanied by selective alterations in microsomal sodium dodecylsulphate polyacrylamide gel electrophoretograms. High doses (≥8.5 mg/kg) caused the disappearance of certain of the SDS-PAGE proteins tentatively identified as being different forms of cyt. P450, together with either increases, decreases or no change in some of the non-cyt. P450 proteins in the electrophoretogram. Concomitant administration of 16,16-dimethylprostaglandin $F_{2\alpha}$ gave dose-dependent protection against the deleterious effects of indomethacin on the enzymic and electrophoretic parameters of cyt. P450, but did not prevent the changes due to indomethacin in the non-cyt. P450 proteins on the electrophoretogram. In contrast, prior phenobarbitone or 3-methylcholanthrene induction prevented the effects of indomethacin on both cyt. P450 and the other microsomal proteins. Concomitant administration of SKF-525A exacerbated the effects of indomethacin on cyt. P450 and the other proteins. Indomethacin coadministration with 3-methylcholanthrene resulted in the major 3MC-induced putative cyt. P450 apoprotein having a lower mol. wt than usual. Conversely, indomethacin did not prevent the induction by SKF-525A of a different putative cyt. P450 apoprotein, despite causing decreases in cyt. P450 as determined spectrophotometrically and enzymologically. The results indicate that indomethacin rather than one of its metabolites is responsible for the decrease in cyt. P450 and that the mechanisms of protection by prostaglandin and inducing agents are, respectively, different.

Indomethacin is a widely used non-steroidal anti-inflammatory drug. The known effects of indomethacin on the liver are few, although it has been included in a list of drugs of frequent clinical use with known hepatotoxic potential [1]. Abnormal liver function serum tests ascribed to an effect of indomethacin have occasionally been recorded in patients [2] and in rats [3]. In rats indomethacin has been shown to cause decreases in the mitotic index of regenerating liver [4], in hepatic and blood glutathione levels [5] and in hepatic microsomal cytochrome P450 and mono-oxygenase activities [6]. Indomethacin is, by contrast, well-known to cause gastrointestinal ulceration in animals [7–9]. This enterotoxic effect is believed to be dependent upon the metabolism of indomethacin [10–13] and is prevented by coadministration of prostaglandins [14]. We have investigated the effects of 16,16-dimethylprostaglandin $F_{2\alpha}$ and of various modulators of drug metabolism on the decrease in hepatic cyt. P450-dependent mono-oxygenation caused by indomethacin, in an attempt to understand the mechanisms of both the decrease in cyt. P450 and its prevention (as reported here) by prostaglandin.

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† Abbreviations used: 16,16-DMPGF$_{2\alpha}$, 16,16-dimethylprostaglandin $F_{2\alpha}$; PB, sodium phenobarbitone; 3MC, 3-methylcholanthrene; SKF-525A, β-diethylaminoethyl diphenylpropyl acetate; AD, aminopyrine *N*-demethylase; EROD, ethoxyresorufin *O*-de-ethylase; BROD, benzyloxyresorufin *O*-debenzylase; cyt. P450, cytochrome P450; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis (or electrophoretogram).

MATERIALS AND METHODS

Chemicals. Indomethacin was provided by Merck, Sharp & Dohme (Hoddesdon, Herts, U.K.), 16,16-dimethylprostaglandin $F_{2\alpha}$ was a gift from Dr. J. E. Pike of the Upjohn Company, Kalamazoo, U.S.A. and SKF-525A was donated by Smith, Kline & French (Welwyn Garden City, Herts, U.K.). 3-Methylcholanthrene was bought from Fluka AG (CH-9470 Buchs, Switzerland). NADP, DL-isocitric acid, isocitrate dehydrogenase and Coomassie brilliant blue R were obtained from Sigma (Poole, U.K.). Acrylamide, *N*,*N*′-methylene-bisacrylamide, SDS and bromophenol blue were purchased from BDH (Poole, U.K.).

Animals and drug treatments. Adult (220–270 g) male Sprague–Dawley rats were used. The animals were kept for at least 1 week on Lablit bedding and fed a standard diet and water *ad libitum*. Indomethacin was dissolved in an isomolar solution of sodium hydrogen carbonate to give a neutral pH. A stock solution of either 10, 4 or 2 mg indomethacin/ml was prepared depending on the dose and route of administration, such that the maximum vol. given i.v. was 0.2 ml and the maximum vol. given i.p. or p.o. was 1 ml. A stock solution of 16,16-dimethylprostaglandin $F_{2\alpha}$ (16,16-DMPGF$_{2\alpha}$)† (1 mg/ml) was

prepared in methanol. This was then divided into aliquots, evaporated to dryness under N_2 at room temp. and the residue sealed in vials under nitrogen and stored at −20°. The prostaglandin was reconstituted in normal saline just before use.

Indomethacin (i.p., i.v. or p.o. as specified later), or indomethacin (i.p.) and 16,16-DMPGF$_{2\alpha}$ (i.v.) in close succession, were injected in the doses detailed below in the morning on three consecutive days. The rats were killed on the fourth morning. Phenobarbitone (PB, 80 mg/kg in 0.9% NaCl solution) was injected i.p. daily for 3 days prior to indomethacin: it was then either withdrawn 24 hr before the first dose of indomethacin or administered also during the 3 days of indomethacin treatment. In another series of experiments PB was given as a 0.1% solution in drinking water, for 6 days prior to indomethacin: it was then either replaced by ordinary water 24 hr before indomethacin, or continued during the 3 days of indomethacin treatment. A single dose of 3-methylcholanthrene (3MC, 80 mg/kg in olive oil) was injected i.p. 3 days before the first dose of indomethacin. SKF-525A (50 mg/kg in 0.9% NaCl solution) was adminstered i.p. 30 min before each injection of indomethacin.

The rats were killed by cervical dislocation and their livers removed into ice-cold buffer (0.15 M KCl in 0.01 M phosphate buffer, pH 7.6). Liver microsome fractions were prepared as described elsewhere [15], except that homogenizing and washing buffers contained 15% glycerol and the initial homogenization was at 850 rev/min in order to prevent denaturation of cyt. P450 to cyt. P420.

Mono-oxygenase reactions. Microsomal incubations were carried out at 37°, using an NADPH-generating system containing 0.25 mM NADP, 2.5 mM DL-isocitric acid, 0.6 U isocitrate dehydrogenase and 5 mM $MgSO_4$ in 0.1 M phosphate buffer, pH 7.4 and a pre-incubation period of 1.5 min. Aminopyrine *N*-demethylase (AD) activity was measured by incubating 1 mg microsomal protein for 10 min with 5 mM aminopyrine in a total vol. of 2 ml. The reaction was terminated by adding 20% w/v trichloroacetic acid to a final concn in the incubation mixture of 4% w/v. After precipitating the protein by centrifugation, the amount of formaldehyde released was measured in 1.5 ml of supernatant by the method of Nash as described by Werringloer [16]. Ethoxyresorufin *O*-de-ethylase (EROD) was measured as described elsewhere [17], utilizing 5 μM ethoxyresorufin. Benzyloxyresorufin *O*-debenzylase (BROD) was measured similarly, using 5 μM substrate as described by Burke *et al.* (in preparation). In liver microsomes of SKF-525A-treated rats, cyt. P450 and its reactions were measured both before and after dissociation of the stable cyt. P450–SKF-525A complex by ferricyanide *in vitro*. Dissocation, by incubating microsomes with 50 μM potassium ferricyanide at 37° for 3 min, was performed as described by Buening and Franklin [18].

Other assays. Cyt. P450 was measured by the method of Omura and Sato [19] and microsomal protein by the method of Lowry *et al.* [20] with bovine serum albumin as reference. Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli [21] with slight modifications. The running gels contained 7.5% acrylamide and 0.2% *N,N'*-methylene-bisacrylamide and the stacking gels 1.2% acrylamide and 0.06% *N,N'*-methylene-bisacrylamide. Microsomes (2 mg protein) were solubilized in 800 μl of 0.5 M Tris, pH 6.8 containing 20% glycerol, 2% SDS, 12.5% 2-mercaptoethanol and 0.01% bromophenol blue by placing in a boiling water bath for 5 min. A quantity of 25 μg of protein was applied to each sample well of the gel. Electrophoresis was carried out at 30 mA and 110 V for 3.5–4 hr at room temp. (22–24°). The proteins were fixed and stained in the gel overnight with 25% isopropanol–10% acetic acid–0.04% Coomassie brilliant blue R. Initial destaining was carried out in a solution of 10% acetic acid–10% isopropanol–0.004% Coomassie brilliant blue R for 6 hr by gentle agitation in the presence of a strip of visking tubing containing activated charcoal. Final destaining was carried out overnight in 10% acetic acid. The gels were photographed using Kodak Technical Pan film 2415 developed in Kodak HC110 and printed on Ilfospeed grade 4 paper. Statistical analysis was carried out using Student's *t*-test for non-paired samples.

RESULTS

Effects of indomethacin and prostaglandin on cytochrome P450 and mono-oxygenation

Indomethacin administration *in vivo* (8.5 mg/kg i.p., once daily for 3 days) caused significant decreases ($P < 0.005$) in the following parameters relative to saline treated controls: a 46% decrease in the hepatic microsomal concn of cyt. P450, a 58% fall in AD specific activity, a 56% reduction in EROD and a 60% loss of BROD (Table 1). The losses relative to untreated rats were similar, being 53%, 62%, 58% and 64%, respectively, i.e. the vehicle itself did not cause losses in activity. Concomitant administration of the synthetic prostaglandin 16,16-DMPGF$_{2\alpha}$ (0.5 mg/kg i.v.) with the indomethacin partially prevented the activity losses, which were now only 16% (cyt. P450), 19% (AD and EROD) and 24% (BROD). The mean values of cyt. P450 and mono-oxygenation in microsomes of rats treated with indomethacin and 16,16-DMPGF$_{2\alpha}$ were significantly higher ($P < 0.005$) than the values for rats treated with indomethacin alone, but were not significantly different from untreated or saline treated controls. The prostaglandin itself had no significant effect on these parameters, relative to either vehicle-treated or untreated controls ($P > 0.05$).

The above effects of indomethacin were dose-dependent for three once-daily i.p. doses (Fig. 1). No marked effect occurred below 5 mg/kg, while 10 mg/kg was lethal to about 33% of the rats, death occurring usually 8–24 hr after the third injection. Maximal effect without mortality occurred with 8.5 mg/kg (3 × i.p.) and this dose was used in all further experiments. Similar potencies for decreasing cyt. P450 were observed irrespective of whether the indomethacin was administered i.p., i.v. or p.o. (Fig. 2). No decrease in cyt. P450 or AD developed at 3 or 6 days after a single 8.5 mg/kg i.p. dose of indomethacin or 3 days after a single 16 mg/kg dose, but

Table 1. Effects of either indomethacin, 16,16-dimethylprostaglandin $F_{2\alpha}$ or indomethacin plus 16,16-DMPGF$_{2\alpha}$ jointly on hepatic microsomal cyt. P450 and mono-oxygenases*

Treatment†	Cyt. P450 (nmoles/mg protein)	Aminopyrine *N*-demethylase	Ethoxyresorufin *O*-de-ethylase (nmoles/min/mg protein)	Benzyloxyresorufin *O*-debenzylase
Untreated	0.93 ± 0.08 (12)	10.1 ± 0.7 (12)	0.90 ± 0.18 (12)	0.42 ± 0.12 (12)
Saline i.p.	0.83 ± 0.05 (12)	9.0 ± 1.0 (12)	0.86 ± 0.04 (3)	0.38 ± 0.01 (3)
Saline i.v.	0.94 ± 0.15 (6)	9.4 ± 1.7 (6)	0.88 ± 0.12 (3)	0.38 ± 0.02 (3)
16,16-DMPGF$_{2\alpha}$	0.90 ± 0.08 (9)	10.2 ± 0.6 (9)	0.93 ± 0.10 (6)	0.44 ± 0.03 (6)
Indomethacin	‡0.45 ± 0.14 (23)	‡3.8 ± 1.1 (15)	‡0.38 ± 0.13 (12)	‡0.15 ± 0.06 (12)
Indomethacin + 16,16,-DMPGF$_{2\alpha}$	0.70 ± 0.17 (23)	7.3 ± 1.7 (22)	0.70 ± 0.23 (9)	0.29 ± 0.10 (9)

* Determined *in vitro* as described in the Materials and Methods. Values are means ± S.D. for the number of individual rats shown in parentheses.

† Each treatment was administered once daily for 3 days. 16,16-DMPGF$_{2\alpha}$ was administered i.v. at 0.5 mg/kg. Indomethacin was administered i.p. at 8.5 mg/kg.

‡ Significantly different from all other groups for this parameter ($P < 0.005$).

Table 2. Effects of indomethacin (8.5 mg/kg), either alone or following treatment *in vivo* with agents that modulate drug metabolism, on hepatic microsomal cyt. P450 and mono-oxygenases*

Treatment†	Cyt. P450 (nmoles/mg protein)	Aminopyrine *N*-demethylase	Ethoxyresorufin *O*-de-ethylase (nmoles/min/mg protein)	Benzyloxyresorufin *O*-debenzylase
Untreated	0.93 ± 0.08 (12)	10.1 ± 0.7 (12)	0.9 ± 0.2 (12)	0.4 ± 0.1 (12)
Saline	0.83 ± 0.05 (12)	9.0 ± 1.0 (12)	0.9 ± 0.04 (3)	0.4 ± 0.1 (3)
Olive oil	0.84 ± 0.07 (3)	10.4 ± 0.8 (3)	1.1 ± 0.2 (3)	ND
Indomethacin	0.45 ± 0.14 (23)	3.8 ± 1.1 (15)	0.4 ± 0.1 (12)	0.15 ± 0.1 (12)
PB	1.92 ± 0.16 (3)	25.3 ± 3.2 (3)	2.3 ± 0.7 (3)	26.8 ± 1.4 (3)
PB + saline	1.54 ± 0.08 (3)	18.6 ± 2.9 (3)	2.2 ± 0.7 (3)	27.8 ± 1.2 (3)
PB + indomethacin	1.70 ± 0.03 (3)	20.6 ± 0.6 (3)	1.5 ± 0.1 (3)	27.1 ± 5.8 (3)
3MC	1.52 ± 0.12 (5)	9.0 ± 1.6 (5)	51.6 ± 11.7 (5)	ND
3MC + saline	1.76 ± 0.01 (3)	8.5 ± 0.5 (3)	51.6 ± 8.9 (3)	ND
3MC + indomethacin	1.45 ± 0.35 (6)	9.1 ± 2.5 (6)	43.2 ± 8.5 (6)	ND
SKF-525A‡	1.00 ± 0.08 (6)	8.4 ± 0.8 (6)	1.3 ± 0.3 (6)	6.4 ± 2.2 (6)
SKF-525A + indomethacin‡	0.48 ± 0.03 (4)	2.6 ± 0.4 (4)	0.15 ± 0.04 (4)	ND

* Determined *in vitro* as described in the Materials and Methods. Values are means ± S.D. for the number of individual rats shown in parentheses (ND, not determined).

† Treatments were once daily, i.p. Saline or indomethacin (8.5 mg/kg) were given for 3 days. PB (80 mg/kg) was given for 6 days and saline or indomethacin were also given for days 4–6 as indicated. Olive oil or 3MC (80 mg/kg) were given once, 3 days before either death or a further 3 days treatment with saline or indomethacin. SKF-525A (50 mg/kg) was given for 3 days either alone or 30 min before indomethacin.

‡ The microsomal parameters were measured without first dissociating the cyt. P450–SKF-525A complex with ferricyanide.

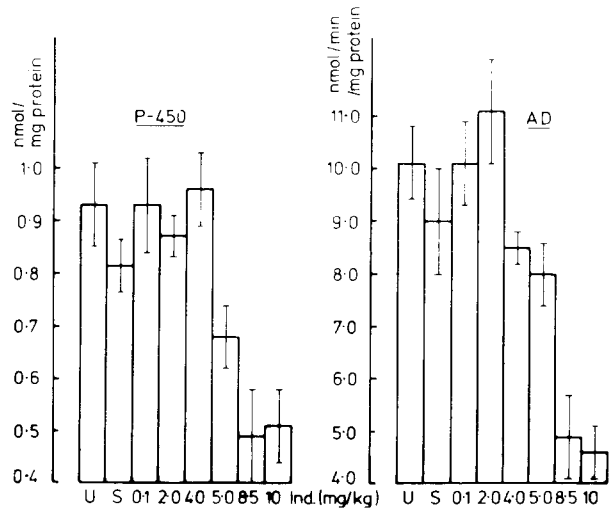

Fig. 1. Dose-responses for indomethacin administered once daily for 3 days i.p.: effects on hepatic microsomal cyt. P450 and aminopyrine *N*-demethylase (AD). Each bar represents a different dose: untreated, U; saline-treated, S; or 0.1–8.5 mg indomethacin/kg. Values are means ± S.D. for five individual rats per dose.

a single dose of 24 mg/kg i.p. did cause a decrease 3 days later, although the interanimal variation in extent of decrease was much greater than after three daily doses. The effect in female rats was comparable to that in male rats, in contrast to the report of Vukoson *et al.* [6], who found female rats to be more susceptible than male rats.

The hepatoprotective effects of 16,16-DMPGF$_{2\alpha}$ were also dose-dependent, with protection increasing up to the highest PG dose tested, 0.5 mg/kg (Fig. 3).

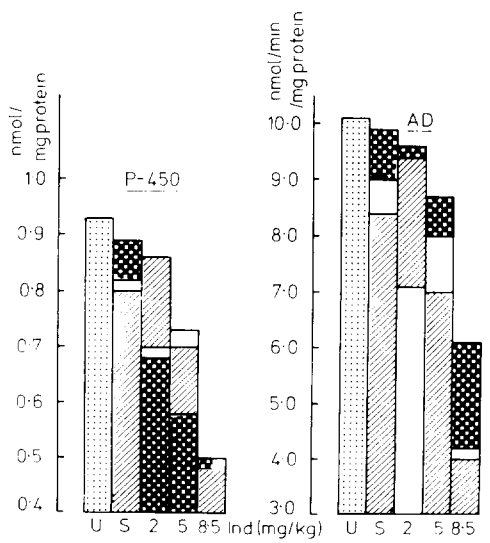

Fig. 2. Dose-responses for indomethacin administered once daily for 3 days by different routes: effects on hepatic microsomal cyt. P450 and aminopyrine *N*-demethylase (AD). Each bar represents a different dose: untreated, U; saline treated, S; or 2–8.5 mg indomethacin/kg, administered either i.p. (□); p.o. (□) or i.v. (□). Values are means ± S.D. for three individual rats per dose–route combination.

Effects of mono-oxygenase inducers and inhibitors on the hepatic effects of indomethacin

The induction of hepatic microsomal cyt. P450, by either PB or 3MC prior to indomethacin administration, was even more hepatoprotective than prostaglandin, in that induction totally prevented the hepatic effects of indomethacin (Table 2). After PB or 3MC induction indomethacin caused no significant loss ($P > 0.05$) in cyt. P450 or its mono-oxygenase activities, while conversely the usual inducing effects of PB or 3MC on mono-oxygenase activities were not significantly modified ($P > 0.05$) by indomethacin. For the results in Table 2, PB was injected i.p. for 6 days, with indomethacin (or saline) being coadministered on the last 3 days. Whether administration of i.p. PB was stopped 24 hr before the first dose of indomethacin, or whether PB was given in the drinking water for the entire 6 days, it had the same preventive action as 6 × once-daily i.p. doses.

In contrast to the consequences of induction, coadministration of the cyt. P450 inhibitor SKF-525A failed to offer any hepatoprotection against indomethacin (Table 2). Whereas the rats were exposed to PB or 3MC for 3 days prior to the first dose of indomethacin, SKF-525A was only administered 30 min before each dose of indomethacin. Although SKF-525A did not increase the detrimental effects to the liver of the usual 8.5 mg/kg dose of indomethacin on cyt. P450, it did increase the mortality (approx. 33% compared with 0% for either indomethacin or SKF-525A alone). Potentiation by SKF-525A of the effect of indomethacin on cyt. P450 was, however, observed using the normally ineffective dose of 5 mg/kg indomethacin (Table 3). Moreover, in rats given the combined treatment both the ascitic fluid and the hepatic soluble fraction (obtained during microsome preparation) contained a green pigment that was not seen in rats treated with either SKF-525A or indomethacin (at either 5 or 8.5 mg/kg) alone. When given alone at 5 mg/kg (3 × once daily i.p.), indomethacin caused no deaths and no

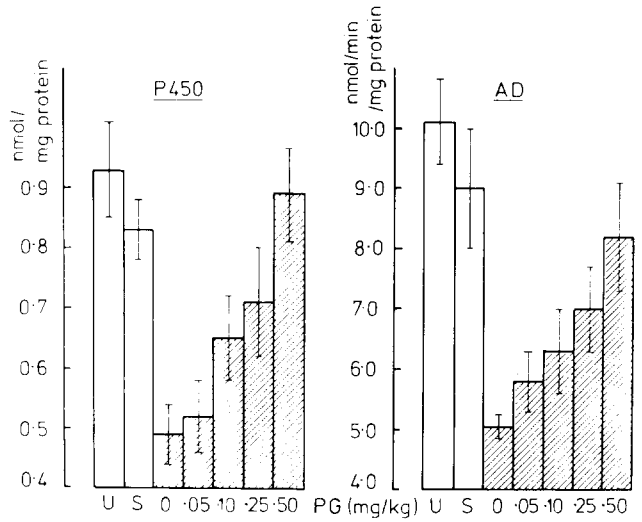

Fig. 3. Dose-responses for 16,16-dimethylprostaglandin $F_{2\alpha}$ administered with a constant dose of indomethacin: hepatoprotective effects on microsomal cyt. P450 and aminopyrine *N*-methylase (AD). Each bar represents a different i.v. dose of 16,16-DMPGF$_{2\alpha}$, 0–0.5 mg/kg, which was administered immediately before an i.p. dose of 8.5 mg indomethacin/kg on each of 3 days; untreated, U; saline-treated, S (the bar marked 0 represents indomethacin alone). Values are means ± S.D. for four individual rats per dose.

significant changes in cyt. P450 or mono-oxygenase activities ($P > 0.05$). When, however, this lower dose schedule of indomethacin was given shortly after SKF-525A approx. 33% of the rats died (a significant increase in mortality, $P < 0.005$) and, compared to the untreated controls, there were significant ($P < 0.005$) losses of 53% in cyt. P450, 68% in AD and 59% in EROD, but an apparent increase of 315% in BROD (all parameters measured in the absence of ferricyanide, which stimulated certain reactions *in vitro* as described below). These results need careful interpretation, however, since although SKF-525A is an inhibitor of cyt. P450 in the short term, it is an inducing agent when administered over several days [22]. In our case SKF-525A selectively induced BROD 14-fold relative to the untreated controls, together with a slight induction of EROD (1.6-fold) but no induction of total cyt. P450 or AD (Table 3). When this induction is taken into account by comparing combined indomethacin (5 mg/kg) and SKF-525A treatment against SKF-525A treatment alone, then it is clear that SKF-525A significantly potentiated ($P < 0.005$) the action of this normally ineffective dose of indomethacin against all four mono-oxygenase parameters measured, the losses being 54% in cyt. P450, 60% in AD, 75% in EROD and 78% in BROD (all parameters measured in the absence of ferricyanide). There is, however, a still further complication to interpreting these results: SKF-525A forms *in vivo* a spectroscopically detectable, stable, inhibitory complex with cyt. P450 that is preserved *in vitro* [18]. Dissociation of this complex by ferricyanide *in vitro* [18] resulted in increases in cyt. P450 and all three mono-oxygenase activities. For AD and EROD (but not cyt. P450 or BROD) the increases were larger (2.5- and 3.8-fold, respectively) in hepatic microsomes from rats pretreated with indomethacin and SKF-525A than in microsomes from rats pretreated with SKF-525A alone (1.8- and 1.4-fold, respectively) (Table 3). Ferricyanide had no effect on these parameters with microsomes of either untreated or indomethacin-alone-treated rats. Because of the differential extent of activation by ferricyanide, some of the microsomal losses due to combined indomethacin plus SKF-525A treatment, relative to SKF-525A-alone-treated controls, were less when measured after, as opposed to before, dissociation of the inhibitory complex. This difference in loss was especially marked for EROD (34% loss after dissociation, cf. 75% loss before dissociation), was much less pronounced for AD (45% loss after, cf. 60% loss before dissociation) and did not occur with cyt. P450 or BROD (55% and 72% loss, respectively after, cf. 54% and 78% loss before dissociation).

Effects on SDS-PAGE

Indomethacin treatment (8.5 mg/kg i.p. 3 × once daily) resulted in an altered hepatic microsomal protein electrophoretic pattern on SDS-PAGE (compared with untreated controls) (Fig. 4). The alterations occurred mainly, but not exclusively, within the region of the gel considered to be populated by variant cyt. P450 apoproteins (bands 1–6, EH being epoxide hydrolase), and were selective as to which proteins were affected. In the 'cyt. P450 region' of the gel several protein bands were diminished by indomethacin but three of the major constitutive proteins (bands 1, 5 and EH of mol. wt 56,250, 50,250 and 49,750, respectively) were not affected. Other than the 'cyt. P450 region' the most notable alterations due to indomethacin were intensification of a very high mol. wt protein band (A), the diminuition of a band (B) of mol. wt 78,000 (possibly NADPH-cyt. *c* reductase), the appearance of a novel protein (band C, mol. wt 47,250), and a general increase in intensity of the low mol. wt region (< 27,000) together with selective band decreases in this

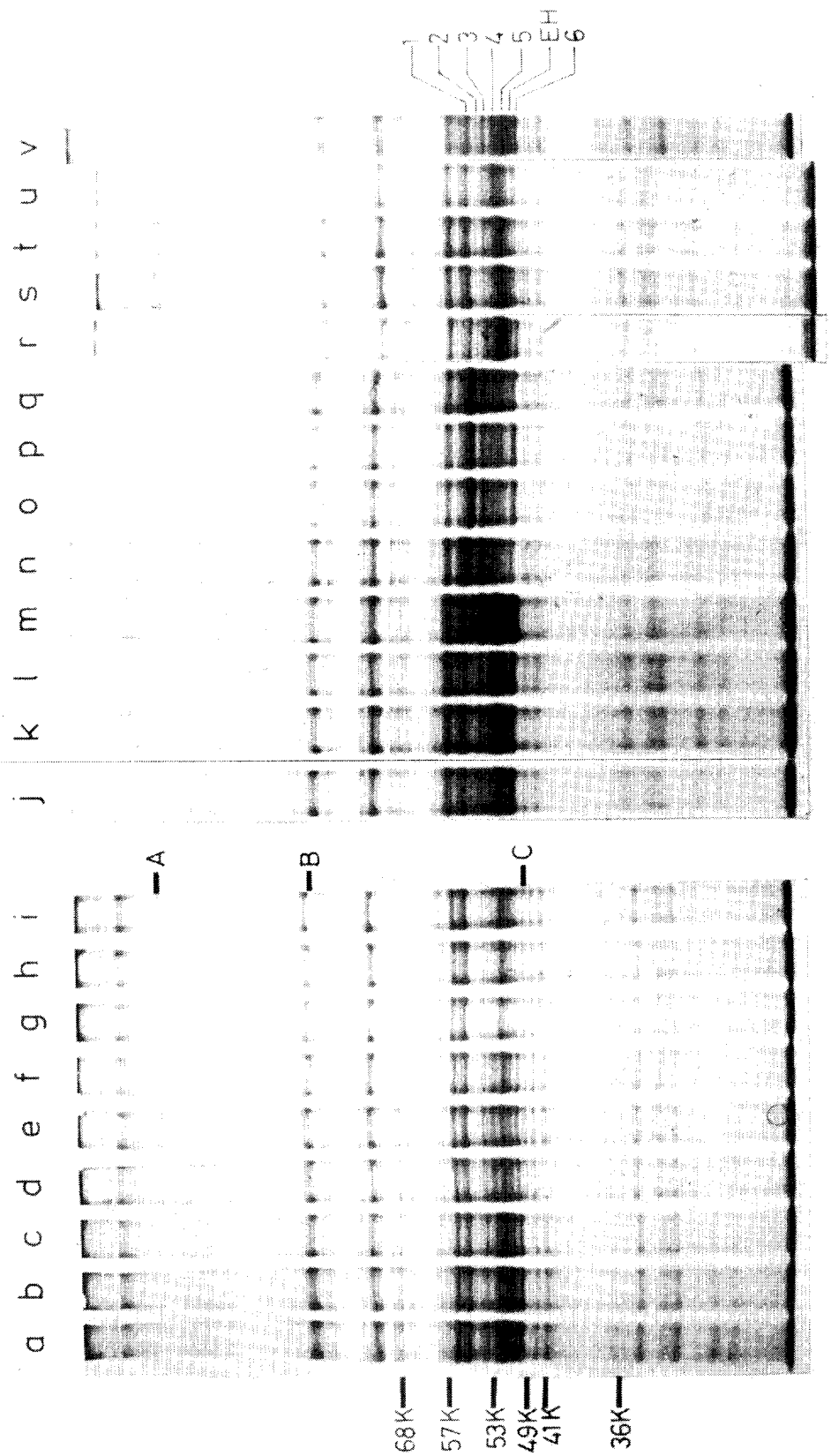

Fig. 4. Sodium dodecylsulphate polyacrylamide gel electrophoretograms of liver microsomes. Electrophoresis was carried out as described in Materials and Methods. A quantity of 25 μg microsomal protein from rats pretreated as indicated below was applied to each sample well, identified by track as follows: (a) and (v) untreated; (b) and (c) 16,16-DMPGF$_{2a}$; (d)–(f) 16,16-DMPGF$_{2a}$ + indomethacin; (g)–(i) indomethacin; (j)–(l) PB − indomethacin; (m) PB; (n) 3MC; (o)–(q) 3MC + indomethacin; (r) SKF-525A; (s)–(u) SKF-525A + indomethacin. Doses for pretreatments are given in Materials and Methods (the 8.5 mg/kg dose of indomethacin was used). The numbers beside track (a) indicate the mol. wt and positions of protein standards. The numbers beside track (v) indicate the putative apoproteins of six different forms of cyt. P450, by reference to Thomas *et al.* [43], plus epoxide hydrolase (EH) as located using a sample of purified rat liver EH. The letters beside track (i) identify three proteins referred to in the text. The figure is a montage of three different gels [(a)–(i), (j)–(q) + (v), (r)–(t)], which have been positioned so that their 'cytochrome P450 regions' (bands 1–6) align.

Table 3. Effects of indomethacin (5 mg/kg), SKF-525A or indomethacin plus SKF-525A jointly on hepatic microsomal cyt. P450 and mono-oxygenases, showing the additional effect *in vitro* of dissociation of the SKF-525A complex by ferricyanide*

Treatment†	Ferricyanide‡	Cyt. P450 (nmoles/mg protein)	Aminopyrine *N*-demethylase	Ethoxyresorufin *O*-de-ethylase (nmoles/min/mg protein)	Benzyloxyresorufin *O*-debenzylase
Untreated	−	1.03 ± 0.11	9.9 ± 0.7	0.83 ± 0.09	0.34 ± 0.04
	+	1.01 ± 0.02	9.7 ± 1.2	0.83 ± 0.10	0.30 ± 0.03
SKF-525A	−	1.04 ± 0.02	8.0 ± 0.6	1.35 ± 1.96	4.80 ± 0.56
	+	1.73 ± 0.14	14.5 ± 0.5	1.96 ± 0.28	6.63 ± 0.45
Indomethacin	+	0.87 ± 0.18	9.1 ± 1.6	0.89 ± 0.09	0.42 ± 0.16
	−	0.89 ± 0.09	9.8 ± 1.9	0.83 ± 0.07	0.39 ± 0.14
Indomethacin + SKF-525A	−	0.48 ± 0.05	3.2 ± 0.2	0.34 ± 0.06	1.07 ± 0.32
	+	0.77 ± 0.03	8.0 ± 1.2	1.29 ± 0.31	1.82 ± 0.49

* Determined *in vitro* as described in the Materials and Methods. Values are means ± S.D. for three individual rats (four rats for the indomethacin + SKF-525A joint treatment).

† Treatments were i.p., once daily for 3 days. Indomethacin (5 mg/kg) was given either alone or 30 min after SKF-525A (50 mg/kg).

‡ Each parameter was measured using aliquots of the same microsomal suspension, either with or without prior incubation with ferricyanide as described in the Materials and Methods.

area. Equal amounts of microsomal protein were electrophoresed for indomethacin-treated and untreated rats, enabling direct comparisons of relative band intensities. Indomethacin did not alter the total microsomal protein recovered (range = 9.0–11.5 mg/g liver).

Administration of 16,16-DMPGF$_{2\alpha}$ alone did not affect the microsomal electrophoretic pattern. Coadministration of the prostaglandin with indomethacin largely prevented the indomethacin-provoked changes in the 'cyt. P450 region' of the gel, except that the constitutive 52,250 mol. wt band (4) was apparently replaced by a protein of slightly higher mol. wt. Furthermore, prostaglandin did not prevent the appearance of the novel 47,250 mol. wt band (C), nor did it prevent the other changes due to indomethacin outside the 'cyt. P450 region'.

PB induced three proteins in the 'cyt. P450 region' of the microsomal electrophoretogram (bands 3, 5 and 6 of mol. wt 53,500, 50,250 and 48,250, respectively), while 3MC induced one protein (band 1 of mol. wt 56,250) and suppressed the major constitutive 50,250 mol. wt protein (band 5). Administration of either PB or 3MC for 3 days before indomethacin prevented, with three important exceptions, apparently all of the indomethacin-produced changes in the microsomal electrophoretic pattern, both within and outside the 'cyt. P450 region'. The first exception was that, although the effects of PB-induction on the banding pattern of the 'cyt. P450 region' were not modified by coadministration of indomethacin, the degree of PB-induced band intensification was lessened by indomethacin. The second exception was that coadministration of indomethacin resulted in the normally 3MC-induced 56,250 mol. wt protein (band 1) being replaced by a slightly lower mol. wt induced protein, which was not induced by either 3MC or indomethacin alone. The third exception was that coadministration of indomethacin (8.5 mg/kg) and 3MC resulted in apparent induction of the 48,250 mol. wt protein (band 6), which was normally induced by PB but not by indomethacin or 3MC alone.

SKF-525A induced (over 3 days) a single protein in the 'cyt. P450 region' of the microsomal gel: this was apparently the major constitutive 50,250 mol. wt protein (band 5). Coadministration of SKF-525A and indomethacin resulted in electrophoretograms that were both typical of SKF-525A treatment in the 'cyt. P450 region' and typical of indomethacin treatment outside this region (including the appearance of the 47,250 mol. wt protein, band C). Whereas the microsomal electrophoretic pattern for rats treated with 5 mg/kg indomethacin alone was similar to untreated rats, coadministration of SKF-525A and 5 mg/kg indomethacin resulted in a gel pattern similar to rats treated with 8.5 mg/kg indomethacin alone, i.e. there was potentiation of indomethacin by SKF-525A (data not shown).

DISCUSSION

Indomethacin in a once-daily i.p. dose of 8.5 mg/kg for 3 days significantly reduced by between 46% and 60% the levels of microsomal cyt. P450

and three of its mono-oxygenase activities. Similar observations have been reported for a twice-daily i.p. dose of 5 mg/kg [6]. Conversely it has been reported that daily doses of either 6 mg/kg i.p. for 3 days [23] or 3 mg/kg p.o. for 7 days [24] had no effect on hepatic mono-oxygenation in rats. These marked differences in the reported effects of indomethacin are probably due to: (1) variations in indomethacin sensitivity between different strains of rat, as has been reported for the renal toxicity of a nonsteroidal anti-inflammatory drug with a structure similar to indomethacin [25]; and (2) the unusual steepness of the dose-response curve for the effects of indomethacin on mono-oxygenation. Ibuprofen, a nonsteroidal anti-inflammatory drug with a structure largely unrelated to indomethacin, has been reported to cause a similar decrease in hepatic mono-oxygenase activities, although in this case the authors considered that direct inhibition of the enzyme by residual ibuprofen was a more likely explanation than loss of cyt. P450 [26]. It is, however, clear from our electrophoretograms that indomethacin caused a decrease in the amount of hepatic cyt. P450, although we cannot rule out the additional possibility of inhibition by residual indomethacin.

The mechanism by which indomethacin caused the loss of hepatic cyt. P450 remains unclear. One possibility is that indomethacin inhibition of cyclo-oxygenase led to an increase in arachidonic acid [27], which is known to be able to cause a decrease in hepatic microsomal cyt. P450 [28]. It has been suggested that intestinal ulceration by indomethacin, which is preventable by coadministration of prostaglandins, arises from inhibition of the synthesis of endogenous cytoprotective prostaglandins [14, 29]. Our observation that 16,16-DMPGF$_{2\alpha}$ largely prevented the loss of cyt. P450 due to indomethacin suggests that this hepatic effect also may have been due to suppression of endogenous prostaglandins. However, renal toxicity of indomethacin is believed to occur by a direct action on the kidney not involving prostaglandin suppression [30]. Furthermore, exogenous prostaglandins are cytoprotective even when endogenous prostaglandin synthesis is probably not impaired: gastric mucosa is protected against a variety of necrotic agents [14] and liver is protected against the toxic actions of carbon tetrachloride, α-naphthylisothiocyanate and galactosamine [31, 32]. Our observation that the hepatic effects of indomethacin were prevented by PB or 3MC but accentuated by SKF-525A suggests that the decrease in hepatic cyt. P450 was due to parent indomethacin rather than to its metabolites. Similar observations and conclusions have been reported for the gastrointestinal ulcerative actions of indomethacin [11–13, 33–37]. That the enhancement by SKF-525A of the hepatic effects of indomethacin was accompanied by the appearance of a green pigment in the ascitic fluid and in the hepatic soluble fraction suggests that indomethacin stimulated the breakdown of the haem of cyt. P450 [38]. This might have been caused by indomethacin enhancement of lipid peroxidation [39, 40], and might explain why the loss of spectroscopically-detectable cyt. P450 was seemingly greater than the loss of electrophoretically detectable cyt. P450 apoprotein.

Whilst the hepatoprotective action of 16,16-DMPGF$_{2\alpha}$ might have been due to its replacement of depleted cytoprotective prostaglandins, an alternative explanation is that 16,16-DMPGF$_{2\alpha}$ caused an alteration in the metabolism of indomethacin resulting in decreased effects on the liver. Our dosage schedule probably precluded any inducing action of 16,16-DMPGF$_{2\alpha}$ on indomethacin metabolism, especially since three daily doses of the prostaglandin appeared not to induce cyt. P450, but 16,16-DMPGF$_{2\alpha}$ inhibition of indomethacin metabolism was a possibility. PGE$_1$ inhibits drug metabolism in rabbits [41]. However, this seemed not to have occurred in our case, since the known inhibitor SKF-525A increased the hepatic effects of indomethacin, whereas 16,16-DMPGF$_{2\alpha}$ decreased them. Therefore a cytoprotective rather than a pharmacokinetic action of the prostaglandin seems the more probable explanation.

Whatever the mechanism of the hepatic actions of indomethacin however, the effect on microsomal proteins as observed in electrophoretograms was highly selective: neither the total microsomal protein concn per g liver nor the majority of the proteins in the electrophoretogram were affected. Selectivity in the hepatic action of indomethacin is underlined by a report that enterotoxic doses induce hepatic cytosolic metallothionein [42]. The microsomal proteins in the electrophoretogram that were particularly decreased by indomethacin were probably apoprotein sub-units of variant forms of cyt. P450 [43]. However, not all of the putative variant cyt. P450 apoproteins were decreased by indomethacin: that form of cyt. P450 apoprotein which predominates in normal rats (mol. wt 50,250) and that which is pre-eminantly inducible by 3MC (mol. wt 56,250) were particularly indomethacin resistant. It was also noticeable that although SKF-525A increased the deleterious effect of indomethacin on cyt. P450, the resulting electrophoretogram showed no differences in the 'cyt. P450 region' compared to rats treated with SKF-525A alone. Therefore, additional mechanisms are needed to explain the extent of the decrease in the spectroscopically detectable cytochrome and its mono-oxygenase activities. A selective loss of haem, as considered above, offers one explanation, whilst an observed 30% decrease in NADPH-cyt. *c* reductase (data not shown) might explain the losses in mono-oxygenase activities, but not the loss in cyt. P450.

The hepatoprotective actions of, on the one hand PB and 3MC, and on the other hand 16,16-DMPGF$_{2\alpha}$, can be differentiated on the basis of the electrophoretograms. PB and 3MC prevented virtually all the changes caused by indomethacin over the entire electrophoretogram, whereas 16,16-DMPGF$_{2\alpha}$ only prevented the changes in the 'cyt. P450 region'. This would be compatible with the idea that PB and 3MC prevented the effects by decreasing blood and tissue levels of the parent drug, i.e. by inducing indomethacin metabolism, while 16,16-DMPGF$_{2\alpha}$ exerted a more direct cytoprotection specifically toward cyt. P450.

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