Biochemical Pharmacology, Vol. 33, No. 8, pp. 1285–1292, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

ARE THE DECREASES IN HEPATIC CYTOCHROME P-450 AND OTHER DRUG-METABOLISING ENZYMES CAUSED BY INDOMETHACIN *IN VIVO* MEDIATED BY INTESTINAL BACTERIAL ENDOTOXINS?

16,16-DIMETHYLPROSTAGLANDIN $F_{2\alpha}$ PREVENTS DECREASES IN HEPATIC DRUG-METABOLISING ENZYMES DUE TO EXOGENOUS ENDOTOXIN

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(*Received* 5 *August* 1983; *accepted* 4 *November* 1983)

Abstract—Administration of either indomethacin (8.5 mg/kg) or *E. coli* endotoxin (3.5 mg/kg) to rats caused significant decreases in a variety of drug-metabolising enzyme activities. Either agent markedly decreased biphenyl 4-hydroxylase by 72–80% and caused lesser decreases (21–64%) in cyt. P-450, aminopyrine *N*-demethylase, ethoxyresorufin *O*-deethylase (EROD), benzyloxyphenoxazone *O*-debenzylase (BPOD), cyt. b5, NADPH-cyt. c reductase, NADH-cyt. b5 reductase, epoxide hydrolase (EH) and glucuronyl transferase (GT). The decreases in GT (21–22%) were significantly less than in cyt. P-450 (45–57%). Sulphotransferase was not affected by either indomethacin or endotoxin. The overall pattern of relative decreases in the different enzymes was similar for either indomethacin or endotoxin. Four activities, however, were affected to a significantly greater extent by indomethacin than by endotoxin at 2–6 mg/kg: EROD, BPOD, cyt. b5 and EH. Additionally, hepatic glutathione was decreased by indomethacin but not by endotoxin. Indomethacin or endotoxin caused similar but not identical decreases in selected protein bands in the "cyt. P-450 region" of microsomal SDS-polyacrylamide gel electrophoretograms. Concomitant administration of 16,16-dimethylprostaglandin $F_{2\alpha}$ afforded significant (50–100%) protection against all the above-mentioned effects of indomethacin or endotoxin. The effects of indomethacin on cyt. P-450 were lessened by concomitant administration of a mixture of neomycin, polymyxin B and bacitracin. Throughout the study there was a close correlation between the extent of decrease in hepatic cyt. P-450 and the degree of intestinal ulceration caused by indomethacin. It was concluded that bacterial endotoxins liberated into the portal blood as a result of indomethacin-induced ulceration of the small intestine probably only partially mediated the effects of indomethacin on hepatic drug-metabolising enzymes. The protection afforded by 16,16-dimethylprostaglandin $F_{2\alpha}$ could have been due to both the prevention of ulceration and to a direct cytoprotective effect on the liver.

High doses of indomethacin in rats cause a decrease in hepatic microsomal cyt. P-450 and cyt. P-450-catalysed monooxygenase reactions [1, 2]. This effect is largely prevented by the concomitant administration of 16,16-dimethylprostaglandin $F_{2\alpha}$ [2]. The present study was designed partly to determine whether indomethacin affects several of the hepatic enzymes associated with drug metabolism or is specific for cyt. P-450. The mechanism of the indomethacin-provoked decrease in cyt. P-450 and of its prevention by 16,16-DMPGF$_{2\alpha}$ is not known. Intestinal ulceration accompanied by bacteraemia is a well-documented consequence of high-dose indomethacin treatment in rats [3–5] and the ulceration was observed in our previous study [2]. The bacteraemia resulting from intestinal ulceration is likely to be accompanied by increased levels of bacterial endotoxins in the portal blood. The administration of endotoxins to rats causes a loss of hepatic cyt. P-450 and microsomal monooxygenases [6, 7]. It is possible that the decreases in cyt. P-450-dependent monooxygenases that followed indomethacin treatment to rats were due to intestinal bacterial endotoxins, reaching the liver in abnormally high concentrations as a result of the intestinal lesions. We have investigated this idea here by comparing the effects of indomethacin and an exogenous endotoxin on hepatic drug-metabolising enzymes. The ulcerogenic effects of indomethacin are diminished by either the antibiotic-suppression of intestinal bacteria [4] or the saccharo 1,4-lactone—inhibition of intestinal bacterial hydrolysis of biliary indomethacin glucuronide [8]. We have further investigated whether there is a correlation between indomethacin-induced intestinal damage and decreased hepatic cyt. P-450 by studying

Abbreviations: cyt. P-450, cytochrome P-450; cyt. b5, cytochrome b5; 16,16-DMPGF$_{2\alpha}$, 16,16-dimethylprostaglandin $F_{2\alpha}$; SDS-PAGE, sodium dodecylsulphate-polyacrylamide gel electrophoresis; EH, epoxide hydrolase.

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the effects of (a) oral antibiotics and (b) an inhibitor of glucuronide conjugation, salicylamide [9]. Prostaglandins prevent indomethacin-induced intestinal ulceration [10]. To investigate the possibility that the mechanism of the protection given by 16,16-DMPGF$_{2\alpha}$ to hepatic cyt. P-450 against indomethacin might primarily be a prevention of ulceration resulting secondarily in prevention of endotoxaemia, we have studied whether 16,16-DMPGF$_{2\alpha}$ can prevent directly the effects of exogenous endotoxin on hepatic cyt. P-450.

MATERIALS AND METHODS

Chemicals. Indomethacin was kindly provided by Merck, Sharp and Dohme Ltd (Hoddesdon, U.K.) and 16,16-dimethylprostaglandin F$_{2\alpha}$ was a generous gift from Dr. J. E. Pike of The Upjohn Company (Kalamazoo, U.S.A.). β-NADH (yeast grade 3), β-NADP (yeast), β-NADPH (chemically reduced type 1), DL-isocitric acid (type 1), isocitrate dehydrogenase (pig heart type 4), cytochrome c (horse heart type 3), adenosine 3′:5′-diphosphate, uridine-5′-diphosphoglucuronic acid, D-saccharic acid 1,4-lactone, p-nitrophenol, p-nitrophenylsulphate, biphenyl, 4-hydroxybiphenyl, reduced glutathione, Coomassie Brilliant Blue R, neomycin sulphate, bacitracin, polymyxin B sulphate, salicylamide and lipopolysaccharide (endotoxin) from *E. coli* (serotype No. 026:B6) were all purchased from the Sigma Chemical Co. Ltd. (Poole, U.K.). Acrylamide, *N,N′*-methylene-bisacrylamide, sodium dodecyl sulphate, bromophenol blue and propane 1,2-diol were purchased from BDH Ltd. (Poole, U.K.). 5,5′-dithiobis-(2-nitrobenzoic acid) was purchased from the Aldrich Chemical Co. Ltd. (Gillingham, U.K.). Benzo(*a*)pyrene 4,5-oxide and benzo(*a*)pyrene *trans*-4,5-dihydrodiol were generous gifts from Dr. H. V. Gelboin, National Cancer Institute, Bethesda, U.S.A. Sterile, nonpyrogenic sodium chloride injection BP 0.9% w/v was bought from Travenol Laboratories Ltd. (Thetford, U.K.)

Animals and drug treatments. Adult (220–270 g) male Sprague–Dawley rats (bred in the University of Aberdeen) were used. They were caged on mineral bedding (Sorbitex from J. Strachan, Perth) and fed Oxoid pasteurised breeding diet and water *ad libitum*. The 0.9% NaCl solution used for dissolving drugs for administration was pyrogen-free. All drug treatments were given between 09.00 and 11.00 hr on three successive days and the rats killed between 09.00 and 09.30 hr on the fourth day. Indomethacin was dissolved in an equimolar solution of sodium hydrogen carbonate to give a neutral pH. A stock solution of either 2 or 4 mg indomethacin/ml was prepared freshly, depending on the dose, so that the maximum volume injected was 1 ml. Indomethacin was administered i.p. at a dose of 8.5 mg/kg except when given jointly with salicylamide, when a dose of 5.0 mg/kg was used for reasons explained in the Results. Antibiotics were given, when appropriate, both intragastrically and in the drinking water [4]. A solution containing 1.3 g neomycin sulphate, 60 mg polymyxin B sulphate and 30,000 U bacitracin per l was provided as the sole source of drinking water from 1 hr before starting treatment with indomethacin until the rats were killed. An additional 20 mg neomycin sulphate, 2 mg polymyxin B and 1000 U bacitracin were given by intragastric intubation as a solution in 1 ml 0.9% NaCl 2 hr after each injection of indomethacin (8.5 mg/kg). Salicylamide was prepared as a 2.5% solution in 70% propane 1,2-diol (propylene glycol). When appropriate, a dose of 200 mg/kg was given i.p. 30 min before indomethacin (5.0 mg/kg). Endotoxin (lipopolysaccharide from *E. coli*, serotype 026:B6) was dissolved in 0.9% NaCl just before use and was injected i.p. at a dose of either 2.0, 3.5 or 6.0 mg/kg. A stock solution of 16,16-DMPGF$_{2\alpha}$ (5 mg/ml) was prepared in methanol. This was then divided into aliquots, evaporated to dryness under nitrogen at room temperature, and the residue sealed in vials under nitrogen and stored at −20°C. The prostaglandin was reconstituted to 1 mg/ml in 0.9% NaCl just before use. 16,16-DMPGF$_{2\alpha}$ in 0.9% NaCl was injected slowly (over approximately 1 min) into the tail vein at a dose of 0.5 mg/kg, either alone or immediately before i.p. administration of either indomethacin (8.5 mg/kg) or endotoxin (3.5 mg/kg). Suitable controls of rats treated with the appropriate injection vehicles were carried out.

Microsome preparation and biochemical assays. Rats were killed by cervical dislocation and their livers removed and microsomes prepared as described by Burke *et al.* [2]. Microsomal monooxygenase reactions were carried out at 37° in 0.1 M phosphate buffer, pH 7.6. Aminopyrine *N*-demethylase was measured by the Nash reaction as described by Burke *et al.* [2], using 5 mM aminopyrine, 1 mg microsomal protein and an NADPH-generating system (0.25 mM NADP). Biphenyl 4-hydroxylase was measured fluorimetrically as described by Burke and Prough [11], using 1 mM biphenyl, 2 mg microsomal protein and an NADPH-generating system (0.25 mM NADP) (excitation λ 290 nm, emission λ 330 nm for 4-hydroxybiphenyl). Ethoxyresorufin *O*-deethylase was measured by direct fluorimetry as described by Burke and Mayer [12], and benzyloxyphenoxazone *O*-debenzylase was measured identically, each reaction using 5 μM substrate, 0.25 mM NADPH and 0.4 mg microsomal protein: the reaction was calibrated with 0.3 μM resorufin (excitation λ 530 nm, emission λ 585 nm). NADPH-cyt. c reductase was measured by direct spectrophotometry at 25°, using a modification of the method of Phillips and Langdon [13], with a reaction mixture containing 80 μM cyt. c, 0.3 mM NADPH and 0.02 mg microsomal protein in a total volume of 1 ml 0.3 M phosphate buffer, pH 7.7 and employing an extinction coefficient for reduced cyt. c of 21.1 mM^{-1} cm^{-1} at 550 nm [14]. NADH-cyt. b5 reductase was measured as NADH-ferricyanide reductase by direct spectrophotometry at 25°, using a modification of the the method of Mihara and Sato [15], with a reaction mixture containing 200 μM potassium ferricyanide, 0.2 mM NADH and 0.02 mg microsomal protein in a total volume of 2 ml 0.1 M Tris–HCl buffer, pH 7.5 and employing an extinction coefficient for ferricyanide of 1.02 mM^{-1} cm^{-1} at 420 nm [16]. Epoxide hydrolase was measured at 37° by a modification of the direct fluorimetric method of Dansette *et al.* [17], using a reaction mixture containing 5 μM benzo(*a*)pyrene 4,5-oxide and 0.2 mg microsomal protein in

a total volume of 2 ml 0.015 M Tris–HCl buffer, pH 8.7: the reaction was calibrated with 2.5 μM benzo(*a*) pyrene 4,5-dihydrodiol (excitation λ 310 nm, emission λ 392 nm). Detergent-activated glucuronyl transferase was measured at 37° by a modification of the spectrophotometric method of Mulder and Van Doorn [18], with a reaction mixture containing 0.5 mM p-nitrophenol, 1.5 mM UDP-glucuronic acid, 5 mM D-saccharic acid 1,4-lactone and 4 mg microsomal protein in a total volume of 2 ml 75 mM Tris–HCl buffer, pH 7.3. The microsomes were pre-incubated for 10 min with 200 μl 0.5% Triton X-100 at 25° to activate the glucuronyl transferase. The reaction was stopped with 0.4 ml 16% trichloroacetic acid, protein was centrifuged down, and 0.3 ml of the supernatant was brought to pH 12 with 0.5 ml 0.5 N NaOH then diluted with 2.2 ml of the Tris buffer: unmetabolised p-nitrophenol was measured at 405 nm, using an extinction coefficient of 17.5 $mM^{-1} cm^{-1}$. Sulphotransferase was measured at 37° by the direct spectrophotometric method of Mulder *et al.* [19], using a reaction mixture containing 10 mM p-nitrophenylsulphate, 20 μM 3′,5′-ADP, 0.5 mM phenol and 0.75 mg postmicrosomal supernatant in a total volume of 2 ml 0.1 M Tris–HCl buffer, pH 8.0. Cytochrome P-450 was measured by the method of Omura and Sato [20], cyt. b5 by the method of Strittmatter *et al.* [21] and protein by the method of Lowry *et al.* [22], using bovine serum albumin as standard. The concentration of reduced glutathione in whole liver homogenate was measured by a modification of the Ellman method as described by Mitchell *et al.* [23]. The protein in a 1.0 ml aliquot of homogenate was precipitated with 1.0 ml 4% sulphosalicylic acid and centrifugation. 0.5 ml of the supernatant was diluted 10-fold with 0.1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) [alternative name bis-(3-carboxy-4-nitrophenyl) disulphide] in 0.1 M phosphate buffer, pH 8.0. Reduced glutathione was measured at 412 nm, employing an extinction coefficient of 13.6 $mM^{-1} cm^{-1}$. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of microsomal proteins was carried out using the Laemmli system as described by Burke *et al.* [2].

Statistical analysis was carried out using ANOVA followed by the Bonferroni test [24]. P values < 0.05 were taken as significant.

RESULTS

Effects of indomethacin or endotoxin. Administration of either indomethacin or *E. Coli* endotoxin caused significant decreases in a variety of drug-

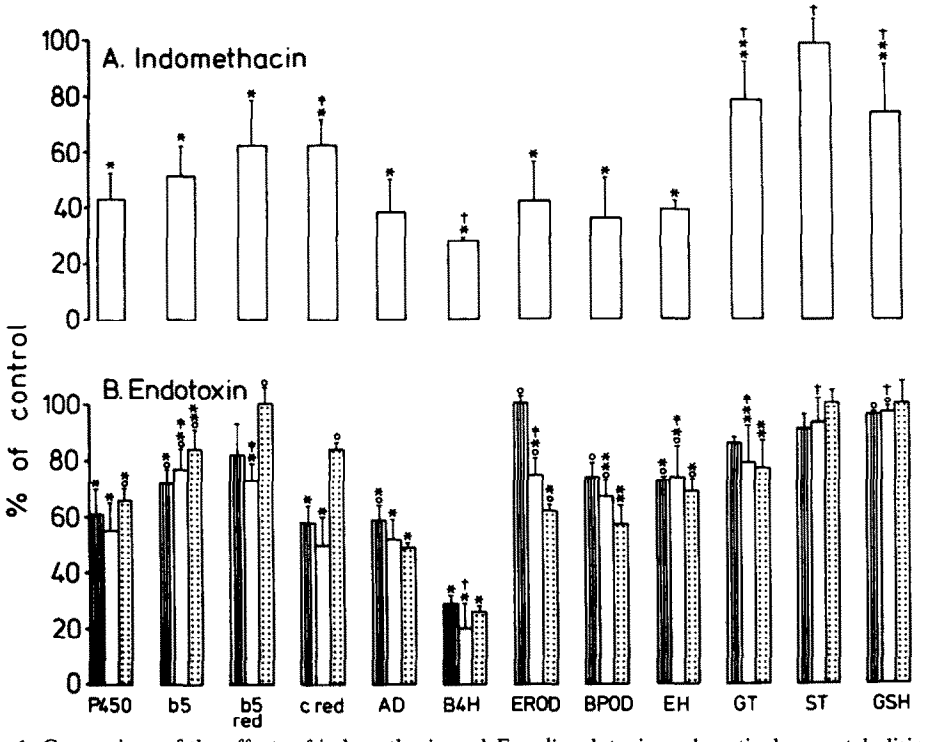

Fig. 1. Comparison of the effects of indomethacin and *E. coli* endotoxin on hepatic drug-metabolising enzyme activities. Drug treatments were once daily for 3 days, i.p. (A) Indomethacin at 8.5 mg/kg. (B) Endotoxin at either 2 mg/kg (■), 3.5 mg/kg (□) or 6 mg/kg (▩). Results are expressed as a percentage of the activity in untreated controls. Control activities were calculated either per mg microsomal protein (P450 and b5), per g liver (GSH) or per min per mg microsomal protein (all the remaining activities). Values are means ± S.D. for 4 rats per treatment. ○ Significantly different ($P < 0.005$) from indomethacin group. * Significantly different ($P < 0.005$) from untreated group. ‡ Significantly different ($P < 0.05$) from untreated group. † Significantly different ($P < 0.005$) from % decrease in cyt. P-450 produced by the same treatment. ‡ Significantly different ($P < 0.05$) from % decrease in cyt. P-450 produced by the same treatment. P450, cyt. P-450; b5, cyt. b5; b5 red, NADH-cyt. b5 reductase; c red, NADPH cyt. c reductase; AD, aminopyrine *N*-demethylase; B4H, biphenyl 4-hydroxylase; EROD, ethoxyresorufin *O*-deethylase; BPOD, benzyloxyphenoxazone *O*-debenzylase; EH, epoxide hydrolase; GT, p-nitrophenol-UDP-glucuronyl transferase; ST, sulphotransferase; GSH, reduced glutathione.

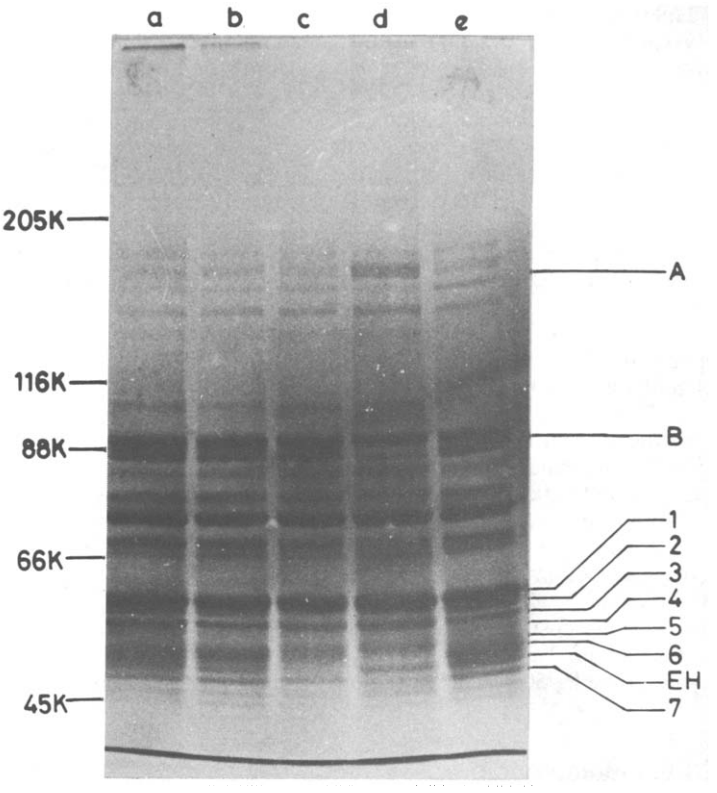

Fig. 2. Sodium dodecylsulphate-polyacrylamide gel electrophoretograms of liver microsomes, showing the effects of indomethacin, endotoxin and 16,16-dimethylprostaglandin $F_{2\alpha}$. Electrophoresis was carried out as described in the Materials and Methods. A quantity of 25 μg microsomal protein from rats pretreated as indicated below was applied to each track, identified as follows: (a) 16,16-DMPGF$_{2\alpha}$; (b) 16,16-DMPGF$_{2\alpha}$ + endotoxin; (c) endotoxin; (d) indomethacin; (e) untreated. Details of pretreatments are given in the Materials and Methods (the 8.5 mg/kg dose of indomethacin and the 3.5 mg/kg dose of endotoxin were used). The numbers beside track (a) indicate the mol. wt. and positions of protein standards. The numbers beside track (e) indicate the putative apoproteins of seven different froms of cyt. P-450, according to Thomas *et al.* [25], plus epoxide hydrolase (EH: located using a sample of purified rat liver EH). The letters beside track (e) identify 2 proteins referred to in the text.

metabolising enzyme activities (Fig. 1). However, the activities were not all equally affected by either agent and there were noticeable differences between indomethacin and endotoxin. Following indomethacin treatment, the greatest decreases (57–72%) occurred in cyt. P-450, cyt. P-450-dependent monooxygenase activities and epoxide hydrolase, although there were significant differences between the various monooxygenase activities in the actual extent of decrease incurred. More modest losses (38–49%) occurred in cyt. b5 and the NADPH- and NADH-dependent cytochrome reductases, while glucuronyl transferase and the glutathione concentration were even less susceptible (22–26% decreases). Sulphotransferase was not decreased at all.

Although the dose–response relationship was not investigated for the effects of indomethacin (it has been reported previously for the decreases caused in cyt. P-450 and aminopyrine *N*-demethylase [2]), it was studied for the effects of endotoxin and found to be complicated and different for each of the activities measured (Fig. 1). The greatest endotoxin-provoked decrease in the activities overall was seen with the dose of 3.5 mg endotoxin/kg, at which dose the activity suffering the greatest decrease (80%) was biphenyl 4-hydroxylase, with lesser decreases (45–50%) in cyt. P-450, aminopyrine *N*-demethylase and NADPH-cyt. c reductase, and even smaller decreases (21–33%) in the two *O*-dealkylases, cyt. b5, NADH-cyt. b5 reductase, epoxide hydrolase and glucuronyl transferase. Neither the glutathione concentration nor sulphotransferase were decreased at any endotoxin dose. Biphenyl 4-hydroxylase was particularly sensitive to endotoxin at each of the doses tested and also to indomethacin. The general pattern of relative decreases in the activities produced by endotoxin at 3.5 mg/kg was similar to the pattern of relative decreases produced by indomethacin, except that (a) endotoxin affected NADPH-cyt. c reductase significantly more than NADH-cyt. b5 reductase, whereas both were affected equally by indomethacin, and (b) endotoxin had a significantly lesser effect than indomethacin on cyt. b5, ethoxyresorufin *O*-deethylase, benzyloxyphenoxazone *O*-debenzylase and epoxide hydrolase.

As described previously [2], indomethacin pretreatment *in vivo* caused a marked but selective loss of protein bands from the SDS-PAGE electrophoretogram of hepatic microsomes. The proteins affected (bands numbered 2, 4, 6 and 7 in Fig. 2) lay in the region of the electrophoretogram generally

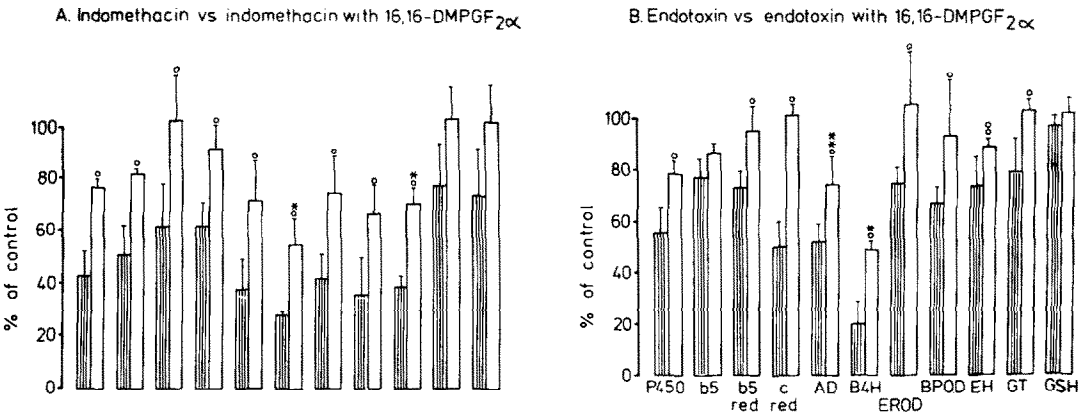

Fig. 3. The effect of 16,16-dimethylprostaglandin $F_{2\alpha}$ on either indomethacin-induced (A) or endotoxin-induced (B) changes in various hepatic microsomal enzyme activities. Details of drug treatments are given in the Materials and Methods (16,16-DMPGF$_{2\alpha}$ 0.5 mg/kg, i.v.; indomethacin 8.5 mg/kg, i.p.; endotoxin 3.5 mg/kg, i.p.). ▥ After pretreatment with indomethacin or endotoxin alone. □ After pretreatment with either indomethacin or endotoxin plus 16,16-DMPGF$_{2\alpha}$. Results are expressed as a percentage of the activity in controls pretreated with 16,16-DMPGF$_{2\alpha}$ alone (16,16-DMPGF$_{2\alpha}$ alone had no significant effect on any of the parameters shown relative to untreated rats, data not shown). The mode of calculation of control activities was as described in Fig. 1. Values are means ± S.D. for 4 rats per treatment. Abbreviations as listed in the legend to Fig. 1. ○ Significantly different from the same parameter after indomethacin or endotoxin treatment alone without concomitant 16,16-DMPGF$_{2\alpha}$ ($p < 0.005$). ⚬ Significantly different as above ($P < 0.05$). * Significantly different from the same parameter after pretreatment with 16,16-DMPGF$_{2\alpha}$ alone (i.e. control value = 100%) ($P < 0.005$). ‡ Significantly different as above ($P < 0.05$).

considered to be inhabited by the apoprotein subunits of the isozymes of cyt. P-450 [25] (bands 1–7) and epoxide hydrolase (marked EH). A similar but not identical loss of "cyt. P-450 and epoxide hydrolase region" SDS-PAGE bands occurred after pretreatment with endotoxin (3.5 mg/kg): bands 2, 4 and 6 were decreased as with indomethacin, but band 7 was affected less by endotoxin, while the EH band was diminished by endotoxin and not by indomethacin (Fig. 2). In the higher molecular weight region of the electrophoretogram, band B (MW 88K) was decreased by indomethacin but not by endotoxin, while bands A (MW 154 and 175 K) were increased by indomethacin but not by endotoxin.

Administration of indomethacin caused extensive lesions in the lower small intestine, characterised by peritonitis, ulceration and occasional frank necrosis, similar to the lesions described by Brodie *et al.* [5]. Endotoxin, in contrast, was not enterotoxic.

Protective effects of 16,16-dimethylprostaglandin $F_{2\alpha}$. Administration of 16,16-DMPGF$_{2\alpha}$ alone had no significant effect on any of the hepatic enzyme activities measured (data not shown, but given for cyt. P-450 and monooxygenase activities in [2]). However, administration of 16,16-DMPGF$_{2\alpha}$ concomitantly with either indomethacin or endotoxin prevented, to a considerable extent, the decreases in drug-metabolising enzymes seen after either indomethacin or endotoxin alone (Fig. 3). For most of the activities measured, the extent of the indomethacin- or endotoxin- caused decrease was approximately halved by the coadministration of 16,16-DMPGF$_{2\alpha}$. Greater protection was afforded to the cytochrome reductases, glucuronyl transferase, the glutathione concentration and the O-dealkylases (the latter only in the case of endotoxin) than to the other parameters. Injections of 16,16-DMPGF$_{2\alpha}$ alone caused no significant alteration in the microsomal SDS-PAGE electrophoretogram, but the coadministration of 16,16-DMPGF$_{2\alpha}$ largely prevented the decreases in "cyt. P-450 and EH bands" caused by either indomethacin [2] or endotoxin (Fig. 2).

The intestinal lesions caused by indomethacin were also prevented by concomitant administration of 16,16-DMPGF$_{2\alpha}$ in accordance with the literature [10].

Effects of antibiotics or salicylamide on the hepatic consequences of indomethacin. The decreases normally caused by indomethacin in cyt. P-450, its *N*-demethylase and *O*-dealkylase activities (Table 1: other enzyme activities were not measured) and in the "cyt. P-450" SDS-PAGE bands (numbered 3, 4 and 6 in track b of Fig. 4; cf. control track a) were largely prevented by co-treatment with a mixture of antibiotics (neomycin, polymyxin B and bacitracin) given orally (track c in Fig. 4). Treatment with the antibiotics alone had no significant effect, relative to untreated or saline-treated controls, on cyt. P-450 measured either enzymically (Table 1) or electrophoretically (track d in Fig. 4). In the region of the electrophoretogram outside the "cyt. P-450 zone", the antibiotics did not prevent indomethacin from causing increases in band A (MW approx. 160 K) and band C (MW 48 K), but they did prevent the indomethacin-decrease in band B (MW approx. 80 K). Differences between the electrophoretograms shown in Figs. 2 and 4 were probably due to the use of a 6% running gel for Fig. 2 and a 7.5% gel for Fig. 4.

The coadministration of antibiotics also prevented the intestinal ulceration caused by indomethacin alone, which is in agreement with published reports [4].

We did not investigate whether antibiotics prevented the hepatic effects of exogenously administered endotoxin.

Table 1. The effects of either indomethacin alone, antibiotics alone or indomethacin plus antibiotics jointly on hepatic microsomal cyt. P-450 and monooxygenases*

Treatment†	Cyt. P-450 (nmoles/mg protein)	Aminopyrine *N*-demethylase	Ethoxyresorufin *O*-deethylase (nmoles/min/mg protein)	Benzyloxyphenoxazone *O*-debenzylase
Untreated	0.93 ± 0.08	10.1 ± 0.7	0.90 ± 0.18	0.42 ± 0.12
Saline	0.85 ± 0.04	9.3 ± 0.9	0.88 ± 0.05	0.40 ± 0.02
Antibiotics	0.79 ± 0.07	9.9 ± 0.6	0.75 ± 0.08	0.35 ± 0.06
Indomethacin	0.45 ± 0.14‡	3.8 ± 1.1‡	0.38 ± 0.13‡	0.15 ± 0.06‡
Indomethacin plus antibiotics	0.67 ± 0.18	8.6 ± 1.6§	0.69 ± 0.14§	0.30 ± 0.11§

* Determined *in vitro* as described in the Materials and Methods. Values are means ± S.D. for 6 individual rats.

† A mixture of neomycin, polymyxin B and bacitracin were given orally for 3 days. When applicable, indomethacin was given i.p. either alone or 2 hr after an intragastric dose of antibiotics. The dose of indomethacin used in this experiment was 8.5 mg/kg. Details of the drug treatments are given in the Materials and Methods.

‡ Significantly different from saline-treated controls for this parameter ($P < 0.005$).

§ Significantly different from the indomethacin-alone treatment group for this parameter ($P < 0.005$).

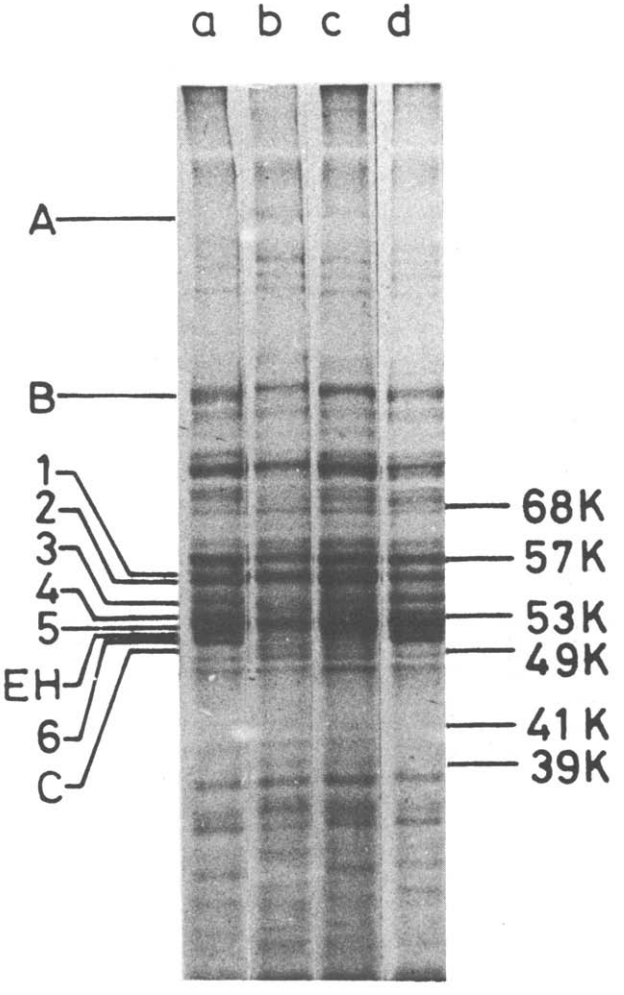

Fig. 4. Sodium dodecylsulphate-polyacrylamide gel electrophoretograms of liver microsomes, showing the effects of indomethacin and antibiotics. Electrophoresis was carried out as described in the Materials and Methods. A quantity of 25 μg microsomal protein from rats pretreated as indicated below was applied to each track, identified as follows: (a) untreated; (b) indomethacin; (c) antibiotics + indomethacin; (d) antibiotics. Details of drug pretreatments are given in the Materials and Methods (the 8.5 mg/kg dose of indomethacin was used). The numbers beside track (d) indicate the mol. wt. and positions of protein standards. The numbers beside track (a) indicate the putative apoproteins of 6 different forms of cyt. P-450, according to Thomas *et al.* [25], plus epoxide hydrolase (EH: located using a sample of purified rat liver EH). The letters beside track (a) identify three proteins referred to in the text.

Since the intestinal ulceration caused by indomethacin in rats is prevented by inhibition of the intestinal bacterial hydrolysis of biliary indomethacin glucuronide [8] and indomethacin does not cause severe intestinal ulceration in species wherein it does not undergo extensive glucuronide conjugation [26], we expected that the coadministration of salicylamide would inhibit the formation of indomethacin glucuronide and prevent the intestinal and hepatic effects of indomethacin. Instead, it was found that concomitant salicylamide greatly potentiated both the ulcerogenic and hepatic effects of indomethacin, although there was no effect of salicylamide alone (data not shown). This part of the study was not pursued further.

DISCUSSION

Our results show that the enzyme-decreasing effects of indomethacin were evinced not specifically against cyt. P-450, but that, however, some hepatic drug-metabolising enzymes were considerably more sensitive than others to indomethacin. (We have found a total lack of sensitivity to indomethacin among some hepatic enzymes of endogenous intermediary metabolism, e.g. glucose-6-phosphatase, manuscript in preparation). It is noteworthy that only decreases in enzyme activities, never increases, were noticed as responses to indomethacin. The immediate mechanism of the decreases and the reason for the variations in enzyme sensitivity cannot be deduced, however, from these results. The decreases in monooxygenase activities might appear to be due mainly to an effect against cyt. P-450, rather than against one of the enzymes comprising the microsomal electron transport chain, since although these enzymes also were decreased by indomethacin, cyt. P-450 and its monooxygenase activities were affected more so. However, a relatively small decrease in cyt. b5 or a cytochrome reductase could have a major effect if, as has been suggested, these were rate-limiting for the monooxygenation reactions [27]. The fact that all the indomethacin-sensitive enzymes were partly protected by 16,16-DMPGF$_{2\alpha}$ suggests that they shared at some point a common mechanism for their decreases. The relative lack of sensitivity of glucuronyl transferase to indomethacin might be explained on the basis that, being buried deep in the endoplasmic reticulum membrane [28], it may be less accessible to indomethacin than are either cyt. P-450 or epoxide hydrolase, which are exposed at the cytosolic suface of the membrane [29, 30]. Supporting this, several hepatotoxic chemicals that cause decreases in cyt. P-450 *in vivo* do not affect glucuronyl transferase: however, epoxide hydrolase is not diminished by these chemicals either [31]. Although the "depth" of an enzyme within the endoplasmic reticulum may not be a decisive factor in determining indomethacin sensitivity, a membrane location *per se* may be important, since the cytosolic enzyme, sulphotransferase, was not affected by indomethacin.

The effects of indomethacin on the hepatic drug metabolising enzymes might have been mediated by increased blood and liver concentrations of bacterial endotoxins. A small amount of intestinal bacterial endotoxins reach the portal blood normally and are removed by the reticulo-endothelial cells of the liver [32]. In our experiments we have always found that rats treated with a dose of indomethacin sufficient to cause a decrease in hepatic cyt. P-450 also exhibited severe ulceration of the small intestine. Gastrointestinal ulceration is, in fact, a well-known effect of indomethacin [5]. It is possible that intestinal bacterial endotoxins were liberated excessively into the portal blood as a result of the ulceration, and bacteraemia has indeed been reported as a consequence of indomethacin treatment in rats [4]. Endotoxin administration *in vivo* has been shown to cause a decrease in cyt. P-450 and associated monooxygenase activities, without affecting p-nitrophenol glucuronyl transferase [7] (a similar effect to indomethacin), although there was a decrease in a different glucuronyl transferase isozyme, bilirubin glucuronyl transferase [6]. In this study we have seen that oral antibiotics prevented both the decreases in hepatic enzymes and the intestinal ulceration due to indomethacin. This is evidence of a connection between intestinal bacteria and the ulceration and enzyme decreases. The effect of antibiotics, however, could have been due to either the prevention of bacterial endotoxins or prevention of bacterial hydrolysis of indomethacin glucuronide, which is necessary for the ulcerogenic action of indomethacin [8, 33]. Although the overall pattern of relative decreases in hepatic enzyme activities and electrophoretogram microsomal proteins caused by *E. coli.* endotoxin was similar to that caused by indomethacin, there were significant dose-dependent differences between the effects of indomethacin and endotoxin on 4 to 10 of the parameters measured. The differences between indomethacin and endotoxin might have been due to the fact that we used only one type of endotoxin, whereas a number of different endotoxins might be expected to be released into the blood as a consequence of the intestinal ulceration caused by indomethacin. Alternatively, these differences may indicate that another mechanism for the enzyme decreases was operating in addition to any effect of endotoxins. In this regard, we have preliminary (unpublished) evidence of a direct deleterious effect of indomethacin on cyt. P-450 *in vitro*. It is also possible that the effects seen here on hepatic enzymes were partly caused by interferon, released as a response to the indomethacin-induced ulceration and presumed bacteraemia, since interferon injections cause a decrease in cyt. P-450 [34].

We have shown previously that 16,16-DMPGF$_{2\alpha}$ protects against the hepatic enzyme effects of indomethacin [2]. It is well-known that prostaglandins protect against the gastrointestinal ulcerogenic actions of indomethacin [10] and this was also observed in this and our previous study. In view of the discussion above, it could be considered that the protection afforded by 16,16-DMPGF$_{2\alpha}$ against the indomethacin-provoked decreases in hepatic enzymes might have been due merely to a prevention of ulceration and of the ensuing endotoxaemia. However, we have shown here that 16,16-DMPGF$_{2\alpha}$ also protects the hepatic enzymes more directly against the effects of endotoxin. How this protection against endotoxins is achieved, we do not know. Hepatic

cytoprotection by 16,16-DMPGE$_2$ against the hepatotoxic actions of carbon tetrachloride, α-naphthylisothiocyanate and galactosamine has been reported [35–37]. In this context it is interesting that endotoxins have been suggested to be causative factors in liver disease [32] and in carbon tetrachloride hepatotoxicity [38, 39]. However, the hepatic cytoprotective effects of 16,16-DMPGE$_2$ against carbon tetrachloride may not be restricted to an anti-endotoxin action, since the prostaglandin has also been shown to directly protect isolated hepatocytes against exposure to carbon tetrachloride *in vitro* [40]. It has been suggested by Stachura *et al.* [37] and Ruwart *et al.* [40] that hepatic cytoprotection by prostaglandins may be due to either a stabilisation of cell membranes or an alteration in the metabolic activation of the hepatotoxin.

Acknowledgements—We are grateful to Merck, Sharp and Dohme Ltd. and Dr. J. E. Pike of the Upjohn Company Ltd. for generous gifts of indomethacin and 16,16-dimethyl-prostaglandin $F_{2\alpha}$ respectively. M.F. is a recipient of an Aberdeen University Studentship and an ORS award from the CVCP.

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