Biochemical Pharmacology, Vol. 33, No. 1, pp. 89–96, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

PROTECTIVE EFFECT OF 16,16-DIMETHYL PROSTAGLANDIN E_2 ON THE HEPATOTOXICITY OF BROMOBENZENE IN MICE

CHRISTIAN FUNCK-BRENTANO, MARINA TINEL, CLAUDE DEGOTT, PHILIPPE LETTERON, GERARD BABANY and DOMINIQUE PESSAYRE*

Unité de Recherches de Physiopathologie Hépatique (INSERM U 24), and Laboratoire Central d'Anatomie et de Cytologie Pathologiques, Hôpital Beaujon, Clichy, France

(*Received* 6 *May* 1983; *accepted* 28 *July* 1983)

Abstract—It has been suggested that 16,16-dimethyl prostaglandin E_2 may have a cytoprotective effect in the liver. To assess this hypothesis, we determined the effects of this prostaglandin on the metabolism and toxicity of bromobenzene in mice. Administration of 16,16-dimethyl prostaglandin E_2 (50 μg/kg s.c., 30 min before, and every 6 hr after, the administration of bromobenzene) did not modify the disappearance curves of unchanged bromobenzene from plasma and liver, and did not modify the amount of bromobenzene metabolites covalently bound to hepatic proteins 1–24 hr after the administration of a toxic dose of bromobenzene (0.36 ml/kg i.p.). The prostaglandin, however, markedly reduced serum alanine aminotransferase activity, the extent of liver cell necrosis, the depletion of glutathione, and the disappearance of cytochrome P-450 after administration of this toxic dose of bromobenzene (0.36 ml/kg i.p.). It also markedly reduced mortality after administration of a lethal dose of bromobenzene (0.43 ml/kg i.p.). We conclude that 16,16-dimethyl prostaglandin E_2 can prevent hepatic necrosis without decreasing the covalent binding of bromobenzene metabolites to hepatic proteins. The mechanism for this dissociation between covalent binding and toxicity remains unknown.

The administration of 16,16-dimethyl prostaglandin E_2 (dm PGE_2), or of several other prostaglandins, has been shown to protect gastric mucosa against a wide variety of necrogenic agents and to prevent intestinal ulcerations induced by indomethacin or corticoids in rats [1, 2]. The prostaglandin was also shown to prevent diet-induced pancreatitis as well as renal lesions produced by several aggressions [2]. This phenomenon, the mechanism of which is still elusive, has been termed 'cytoprotection' [1, 2]. In humans, administration of PGE_2, dm PGE_2, or 15(*R*)-15-methyl prostaglandin E_2 has been shown to prevent the fecal blood loss induced by aspirin or indomethacin, to decrease the luminal shedding of epithelial cells induced by ethanol, and to promote the healing of peptic ulcers [2].

In view of these useful protective effects in extrahepatic organs, it was of interest to see whether dm PGE_2 may also have protective effects in the liver. Recently, it was reported that administration of dm PGE_2 may decrease liver lesions produced by carbon tetrachloride in rats [3, 4], and two abstracts suggested similar protective effects against the hepatotoxicity of acetaminophen, aflatoxin, galactosamine, α-naphthylisothiocyanate (ANIT) and ethanol in rats [5, 6]. It was suggested that dm PGE_2 may have a non-specific, cytoprotective effect in the liver [3]. Whether this is correct or not remains uncertain, however. Firstly, non-specificity must be assessed by studying various other systems. Secondly, a potential effect of the prostaglandin on metabolic activation should be assessed. Indeed, in none of these previous studies was the effect of dm PGE_2 on the metabolism of the hepatotoxins studied [3, 6]. Carbon tetrachloride [7], acetaminophen [8], aflatoxin [9], ANIT [10] and ethanol [11] are transformed in the liver into reactive metabolites which covalently bind to hepatic macromolecules. This metabolic activation is mediated, in part or in totality, by cytochrome P-450. Several prostaglandins have been shown to elicit binding spectra with cytochrome P-450, to be metabolized by this haemoprotein, and to inhibit some monooxygenase activities *in vitro* [12, 13]. Therefore, there was the possibility that dm PGE_2 may inhibit metabolic activation by cytochrome P-450.

Although a definite answer to the question of a cytoprotective effect of dm PGE_2 in the liver may prove difficult to obtain, at least we should know whether dm PGE_2 (a) decreases toxicity and covalent binding (a metabolic effect), or (b) can decrease toxicity without decreasing covalent binding (a finding possibly consistent with a cytoprotective effect).

In this paper, we report the effects of dm PGE_2 on the metabolism and toxicity of bromobenzene, a well-characterized model of metabolite-mediated hepatitis [14–20], in mice.

MATERIALS AND METHODS

Male Crl:CD^R—1 (ICR) BR Swiss mice were purchased from Charles River (Saint Aubin Lès Elbeuf, France). Animals were fed a normal standard diet (M25 biscuits, Extra Labo, Pietrement, Provins, France) given *ad libitum*. Bromobenzene was purchased from Sigma Chemical Co. (St. Louis, MO).

* To whom correspondence should be addressed at: Unité de Recherches de Physiopathologie Hépatique, INSERM U 24, Hôpital Beaujon, 92118 Clichy, France.

[^{14}C]Bromobenzene (specific activity 50 mCi/mmole) was purchased from Commissariat à l'Energie Atomique (Saclay, France). Its radiochemical purity was checked by gas chromatography and found to be higher than 99%. 16,16-Dimethyl prostaglandin E_2 (dm PGE_2) was a generous gift from the Upjohn Company (Kalamazoo, MI).

Bromobenzene was administered i.p. dissolved in 100 μl of corn oil; control mice received 100 μl of corn oil. Dm PGE_2 was first diluted in ethanol, the last dilution being made in water. The prostaglandin was administered s.c. in 100 μl of an ethanol–water mixture (1.5 μl of ethanol–98.5 μl of water); control mice received 100 μl of an identical ethanol–water mixture. Some mice were pretreated with $CoCl_2 \cdot 6H_2O$, 35 mg/kg s.c. twice daily for 3 days, and were used 17 hr after the last dose of $CoCl_2$.

Serum alanine aminotransferase (ALAT) activity was measured by the method of Reitman and Frankel [21].

Liver fragments were placed in Bouin's fluid and embedded in paraffin 24 hr later; sections were stained with haematoxylin and eosin. The extent of liver cell necrosis was appreciated by a semi-quantitative method as described by Mitchell *et al.* [22].

Glutathione and glutathione disulfide concentrations in the liver were measured at 10 a.m. as previously reported [23].

For the preparation of liver microsomes, liver fragments were minced and homogenized in 3 volumes of 0.154 M KCl, 0.01 M sodium/potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 *g* for 20 min, and the supernatant was centrifuged again at 100,000 *g* for 60 min. The microsomal pellets were stored at −20° until analysed 1–3 days later. Microsomal protein concentration was measured by the method of Lowry *et al.* [24]. Microsomal cytochrome P-450 was measured by the CO-difference spectrum of dithionite-reduced microsomes as described by Omura and Sato [25].

The concentration of unchanged bromobenzene in plasma and liver was measured after the administration of [^{14}C]bromobenzene (1 mCi/kg; 0.36 ml/kg i.p.) by the extraction method described by Zampaglione *et al.* [16].

The amount of bromobenzene metabolites covalently bound to hepatic proteins was measured as follows. Mice were killed at various times after administration of [^{14}C]bromobenzene (1 mCi/kg; 0.22–0.43 ml/kg i.p.). The liver was removed, minced and homogenized in 3 volumes of 0.154 M NaCl. The amount of ^{14}C-labelled metabolites covalently bound to hepatic proteins was then determined as previously described [18]: proteins were precipitated with trichloroacetic acid (TCA), dried, washed three times with TCA, and repeatedly extracted with various solvents of various polarities as previously described [18]. The ^{14}C-labelled material which remained irreversibly attached to hepatic proteins was then counted. We verified that the administration of $CoCl_2$ (which decreased by 60% hepatic cytochrome P-450 concentration) decreased by 61% the amount of bromobenzene metabolites covalently bound to hepatic proteins, 24 hr after administration of [^{14}C]bromobenzene, 0.36 ml/kg i.p. (not shown).

In vitro, [^{14}C]bromobenzene (1 μCi; 0.02 or 1 mM) was added to an incubation mixture (1 ml 0.07 M KCl, 0.13 M sodium/potassium phosphate buffer, pH 7.4) containing microsomes from 125 mg of liver, NADP (0.4 mM), glucose-6-phosphate (8 mM), glucose-6-phosphate dehydrogenase (3 enzyme units) and $MgCl_2$ (6 mM). In some flasks, dm PGE_2 (25–200 μg/l.) was added. In some flasks, cofactors were omitted. The mixtures were incubated, under air, at 37°. After 15 min, microsomal proteins were precipitated with TCA and the amount of ^{14}C-labelled metabolites covalently bound to microsomal proteins was determined as previously described [18], and summarized above. The radioactivity of blanks incubated without cofactors was subtracted from that measured with the complete system.

The *in vitro* peroxidation of microsomal lipids was measured as the formation of thiobarbituric acid reactants as previously described [26]; the absorbance of non-incubated blanks was subtracted from that of the test flasks.

RESULTS

Selection of doses

Twenty-four hours after the administration of various doses of bromobenzene (0.22–0.43 ml/kg i.p.), bromobenzene metabolites were irreversibly bound to hepatic proteins, and serum alanine aminotransferase (ALAT) activity was increased (Fig. 1). A

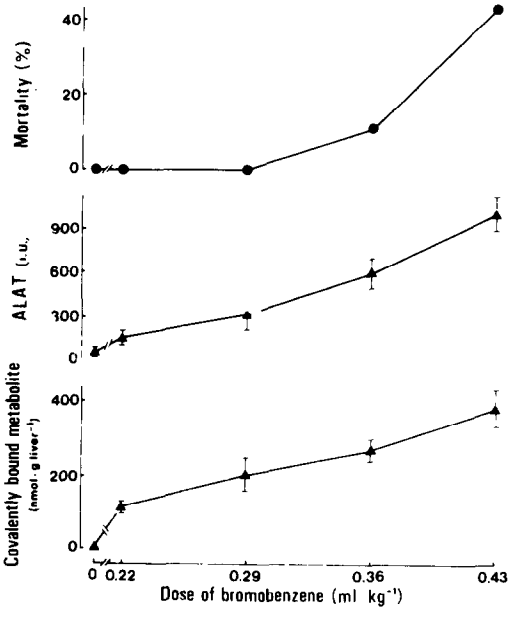

Fig. 1. Bound bromobenzene metabolites, hepatotoxicity and mortality after various doses of bromobenzene. Groups of 20–50 mice received various doses of bromobenzene (0.22–0.43 ml/kg i.p.), and the number of dead animals was counted 24 hr later. Surviving mice were killed at that time and the activity of serum alanine aminotransferase (ALAT) was determined; results are means ± S.E.M. for 20 mice. Other mice were killed 24 hr after administration of [^{14}C]bromobenzene (0.22–0.43 ml/kg i.p.); the amount of bromobenzene metabolites irreversibly bound to hepatic proteins was determined; results are means ± S.E.M. for six mice.

dose of 0.36 ml/kg of bromobenzene produced marked hepatotoxicity with only minimal mortality (Fig. 1); this dose was selected for metabolic and toxicological studies. A dose of 0.43 ml/kg of bromobenzene produced marked mortality (Fig. 1); this dose was selected for mortality studies.

Figure 2 shows the effects of various dm PGE_2 treatments on serum ALAT activity 24 hr after the administration of bromobenzene (0.36 ml/kg i.p.); a significant protective effect required the administration of doses of 50 or 100 μg/kg s.c. of dm PGE_2 given repeatedly (30 min before, and every 6 hr after, the administration of bromobenzene). This treatment schedule was selected for further studies.

Covalent binding and bromobenzene concentrations

In vitro, dm PGE_2 (25–200 μg/l.) could inhibit the covalent binding of bromobenzene metabolites to microsomal proteins (Fig. 3) when this binding was studied with a low concentration of [^{14}C]bromobenzene (0.02 mM) but not with a concentration (1 mM) mimicking that found in the liver 1 hr after the administration of a toxic dose of bromobenzene (Fig. 4A).

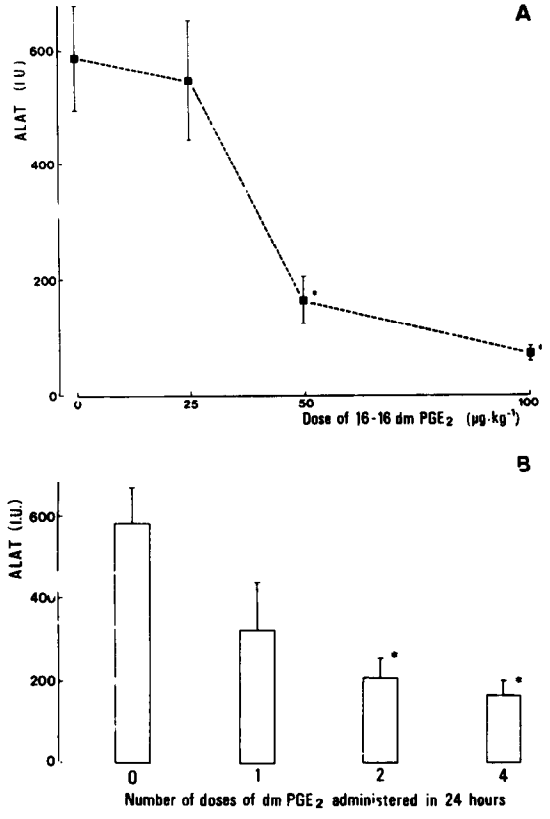

Fig. 2. Effects of various dm PGE_2 treatments on serum alanine aminotransferase (ALAT) activity 24 hr after administration of bromobenzene. ALAT activity was measured 24 hr after administration of bromobenzene 0.36 ml/kg i.p., given either alone or in combination with dm PGE_2. In (A) the dose of dm PGE_2 was varied (0, 25, 50 or 100 μg/kg s.c.) while the treatment schedule was kept constant: dm PGE_2 was given 30 min before, and 6, 12 and 18 hr after, the administration of bromobenzene. In (B) the dose of dm PGE_2 was kept constant (50 μg/kg s.c.) while the treatment schedule was varied: dm PGE_2 was given either once (30 min before bromobenzene), twice (30 min before, and 6 hr after, bromobenzene), or four times (30 min before, and 6, 12 and 18 hr after, bromobenzene). Results are means ± S.E.M. for 18 mice. The asterisks indicate a significant difference from values in mice receiving bromobenzene alone, $P < 0.05$ (Student's *t*-test for independent data).

In vivo, repeated administration of dm PGE_2 (50 μg/kg s.c.) did not significantly modify the concentrations of unchanged bromobenzene in plasma or liver (Fig. 4A) or the amount of bromobenzene metabolites covalently bound to hepatic proteins (Fig. 4B) 1–24 hr after the administration of [^{14}C]bromobenzene. Higher doses of dm PGE_2 (100 μg/kg s.c.) had no effect either on this *in vivo* covalent binding measured 10 hr after the administration of [^{14}C]bromobenzene, 0.36 ml/kg i.p. (not shown).

Hepatotoxicity and mortality

Administration of repeated doses of dm PGE_2 (50 or 100 μg/kg s.c.) for the first day after the administration of bromobenzene markedly reduced serum ALAT activity (Figs. 2 and 5). Whether the administration of dm PGE_2 was continued or not on the second day did not modify this protective effect (Fig. 5). Repeated administration of dm PGE_2 alone (100 μg/kg s.c.) did not modify serum ALAT activity (not shown).

Hepatic necrosis was not observed in any of the six mice killed 5 hr after the administration of bromobenzene alone (0.36 ml/kg i.p.); at 10 hr necrosis of a few hepatocytes was observed in two of six mice while a few balloon cells were observed in three other mice; 24 or 48 hr after the administration of bromobenzene alone there was usually marked centrilobular necrosis (Table 1). In mice receiving, in addition, repeated doses of dm PGE_2 (50 or 100 μg/kg s.c.), the extent of hepatic necrosis was markedly reduced (Table 1). Administration of dm PGE_2 alone (100 μg/kg s.c., four doses) produced no liver lesion in any of six mice.

Administration of repeated doses of dm PGE_2 (100 μg/kg s.c.) markedly reduced mortality (Fig. 6) after administration of a high dose of bromobenzene (0.43 ml/kg i.p.).

Glutathione and cytochrome P-450

The hepatic concentration of reduced glutathione (GSH) reached a minimum 5 hr after administration of bromobenzene alone (Fig. 7); it then slowly increased to reach about normal values 24 hr after administration of bromobenzene. In mice receiving, in addition, repeated doses of dm PGE_2 (50 μg/kg s.c.), the depletion of GSH at 5 hr was less severe, and a rebound phenomenon was observed 15 hr after the administration of bromobenzene (Fig. 7). Dm PGE_2 given alone had no effect on the hepatic concentration of GSH (Fig. 7).

The concentration of glutathione disulfide (GSSG) in liver was decreased 5 hr after administration of bromobenzene, from 19 ± 2 nmole/g liver in control mice to 4 ± 1 in bromobenzene-treated mice (mean ± S.E.M. for six mice); GSSG remained at higher levels (10 ± 2 nmole/g liver) in mice receiving dm PGE_2 (50 μg/kg s.c.) 30 min before the admin-

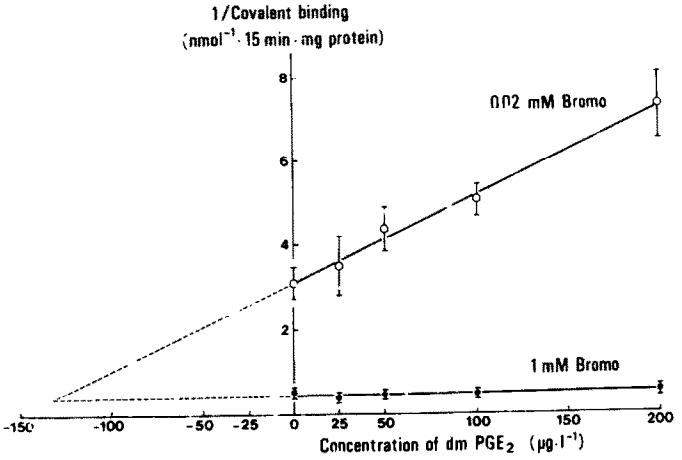

Fig. 3. Effects of dm PGE_2 on the *in vitro* covalent binding of bromobenzene metabolites to microsomal proteins. Untreated mice were killed, and hepatic microsomes were prepared and pooled. [^{14}C]Bromobenzene (Bromo), 0.02 mM or 1 mM, was incubated under air with microsomes from 125 mg of liver and a NADPH-generating system; in some flasks, dm PGE_2, in various concentrations, was added. After 15 min of incubation the amount of ^{14}C-labelled metabolites irreversibly bound to microsomal proteins was determined. The figure shows a Dixon's plot (the reciprocal of the covalent binding rate is plotted against the concentration of the inhibitor, dm PGE_2). Results are means ± S.E.M. for three experiments.

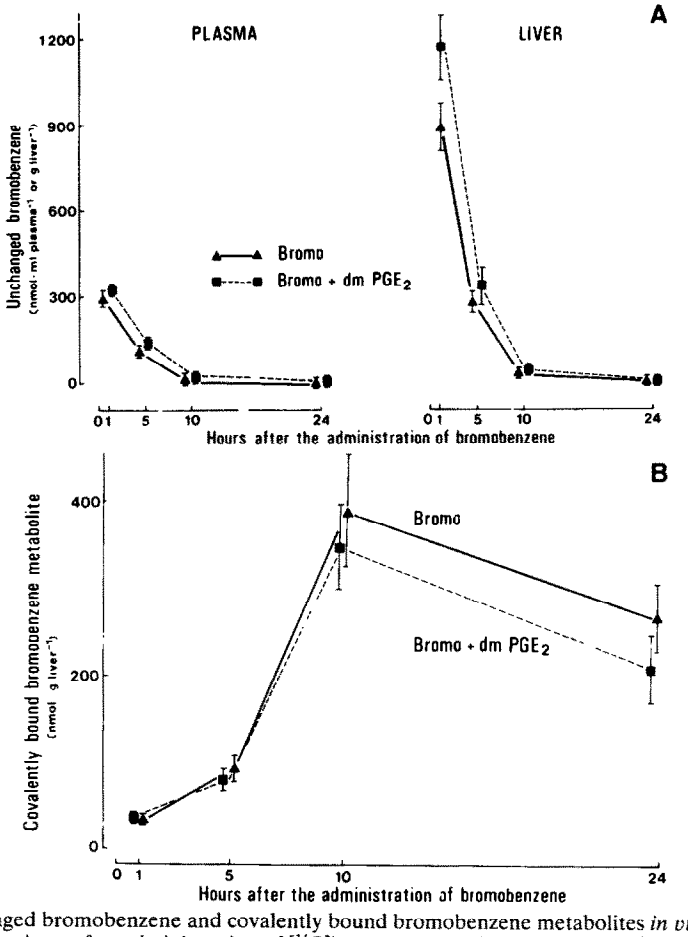

Fig. 4. Unchanged bromobenzene and covalently bound bromobenzene metabolites *in vivo*. Mice were killed at various times after administration of [^{14}C]bromobenzene (Bromo), 0.36 ml/kg i.p., given either alone or in combination with dm PGE_2 (50 µg/kg s.c., 30 min before, and 6, 12 and 18 hr after, the administration of bromobenzene). (A) shows the concentrations of unchanged bromobenzene in plasma and liver. (B) shows the amount of bromobenzene metabolites covalently bound to hepatic proteins. Results are means ± S.E.M. for six mice. None of the differences between mice receiving bromobenzene alone and mice receiving both bromobenzene and dm PGE_2 was statistically significant.

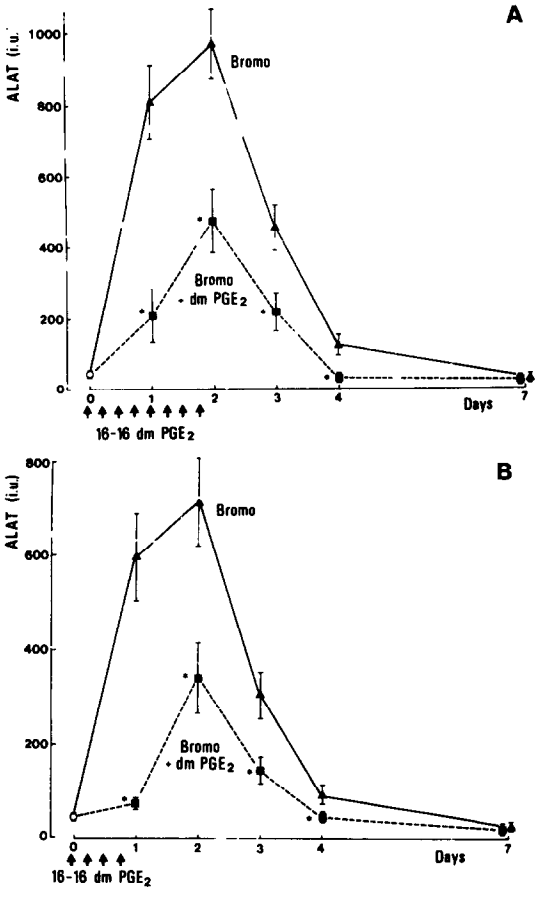

Fig. 5. Effects of dm PGE_2 on serum alanine aminotransferase (ALAT) activity at various times after the administration of bromobenzene. Serum ALAT activity was measured after administration of bromobenzene (Bromo), 0.36 ml/kg i.p., given either alone or in combination with dm PGE_2 (100 μg/kg s.c., 30 min before, and every 6 hr after, the administration of bromobenzene); in (A) the administration of dm PGE_2 was continued for 2 days; in (B) it was stopped after the first day. Results are means ± S.E.M. for 20 mice. The asterisks indicate a significant difference from values in mice receiving bromobenzene alone, $P < 0.05$ (Student's *t*-test for independent data).

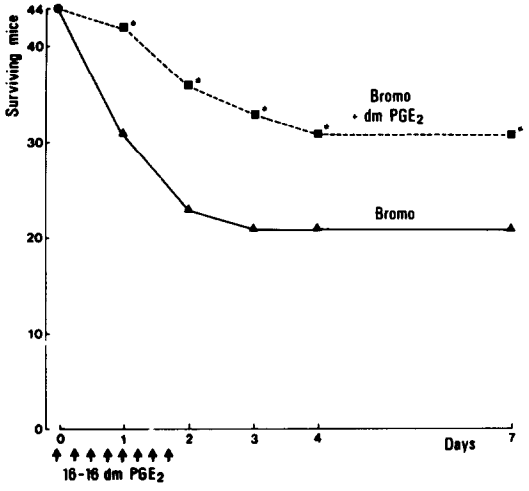

Fig. 6. Survival after administration of a lethal dose of bromobenzene. The figure indicates the number of surviving mice at various times after the administration of bromobenzene (Bromo), 0.43 ml/kg i.p., given either alone or in combination with dm PGE_2 (100 μg/kg s.c., 30 min before, and every 6 hr after, the administration of bromobenzene, for 2 days). The asterisks indicate a significant difference from mice receiving bromobenzene alone, $P < 0.05$ (chi-square test).

istration of bromobenzene; dm PGE_2 given alone did not modify hepatic GSSG concentration.

The depletion of glutathione, 1, 2 and 5 hr after the administration of diethyl maleate (0.5 ml/kg i.p.) was not modified by the previous administration of dm PGE_2 (50 μg/kg s.c., 30 min before the administration of diethyl maleate) (not shown).

The decrease in cytochrome P-450 concentration 24 hr after administration of bromobenzene (Fig. 8) was less severe in mice receiving, in addition, repeated doses of dm PGE_2 (50 μg/kg s.c.). Dm PGE_2 given alone had no effect on cytochrome P-450 concentration (Fig. 8).

Lipid peroxidation

Dm PGE_2 (200 μg/l.), present in the incubation

Table 1. Extent of liver cell necrosis after administration of bromobenzene given either alone or in combination with dm PGE_2*

	Number of mice with necrosis involving:				
	0	5%	5–25%	25–50%	>50%
			of the hepatocytes		
Bromobenzene, 24 hr	0	0	4	6	4
Bromobenzene + dm PGE_2, 24 hr	1	6	7	0	0
Bromobenzene, 48 hr	2	5	5	1	2
Bromobenzene + dm PGE_2, 48 hr	8	5	1	1	0

* In a first experiment, the extent of liver cell necrosis was studied 24 hr after the administration of bromobenzene (0.36 ml/kg i.p.) given either alone or in combination with dm PGE_2 (50 μg/kg s.c., 30 min before, and every 6 hr after the administration of bromobenzene). In a second experiment, necrosis was studied 48 hr after the administration of bromobenzene (0.36 ml/kg i.p.) given either alone or in combination with dm PGE_2 (100 μg/kg s.c., 30 min before, and every 6 hr after, the administration of bromobenzene). In both experiments, the extent of hepatic necrosis was less in mice receiving dm PGE_2, $P < 0.05$ (non-parametric test of Wilcoxon).

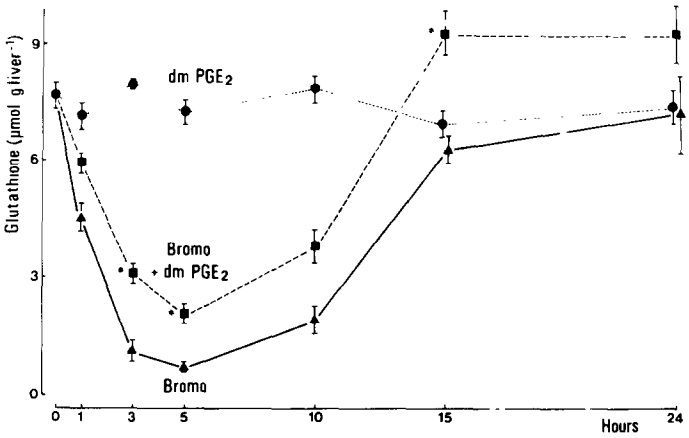

Fig. 7. Effects of bromobenzene and dm PGE_2 on hepatic glutathione concentrations. Mice received bromobenzene (Bromo), 0.36 ml/kg i.p., and/or dm PGE_2 (50 μg/kg s.c., 30 min before, and 6, 12 and 18 hr after, the administration of bromobenzene). The animals were killed at various times after administration of bromobenzene. Results represent means ± S.E.M. for nine mice. The asterisks indicate a significant difference from values in mice receiving bromobenzene alone, $P < 0.05$ (Student's *t*-test for independent data).

mixture, did not modify microsomal lipid peroxidation mediated by 0.2 mM NADPH, or that mediated by 0.2 mM NADPH and 10 μM $FeSO_4$ (not shown); neither did it modify the lipid peroxidation initiated by 12 mM carbon tetrachloride in the presence of 0.2 mM NADPH and 0.05 mM EDTA (not shown). Bromobenzene (12 mM) did not initiate lipid peroxidation in the presence of 0.2 mM NADPH and 0.05 mM EDTA, with or without dm PGE_2 (not shown).

DISCUSSION

Bromobenzene is transformed by cytochrome P-450 into bromobenzene epoxides. These reactive intermediates may then follow four different pathways [27]. They may rearrange to the phenols, be hydrated to the *trans*-dihydrodiols, be conjugated with glutathione, or may covalently bind to hepatic macromolecules, mainly proteins [27]. Under usual conditions, gross relationships are observed between the amount of bromobenzene metabolites covalently bound to hepatic proteins *in vivo* and the extent of liver cell necrosis. Such relationships are apparent when the doses of bromobenzene are varied [14, 15] (Fig. 1), when the activity of cytochrome P-450 is modified [14, 15], or when the conjugation with glutathione is reduced by fasting [18]. We determined the effects of 16,16-dimethyl prostaglandin E_2 (dm PGE_2) on the metabolism and toxicity of bromobenzene in mice.

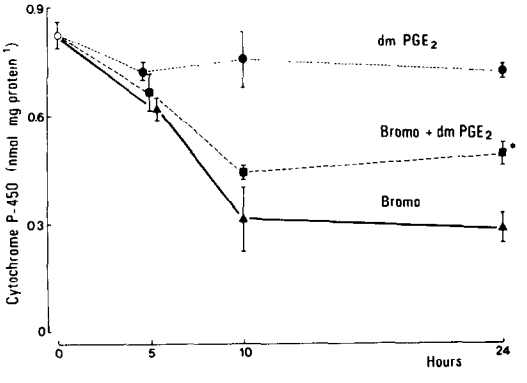

Fig. 8. Effects of bromobenzene and dm PGE_2 on hepatic cytochrome P-450 concentration. Mice received bromobenzene (Bromo), 0.36 ml/kg i.p., and/or dm PGE_2 (50 μg/kg s.c., 30 min before, and 6, 12 and 18 hr after, the administration of bromobenzene). Results represent means ± S.E.M. for nine mice. The asterisk indicates a significant difference from that in mice receiving bromobenzene alone, $P < 0.05$ (Student's *t*-test for independent data).

Prostaglandins have been shown to bind to cytochrome P-450, to be metabolized by this haemoprotein, and to inhibit *in vitro* several monooxygenase activities [12, 13]. Indeed, dm PGE_2 (25–200 μg/l.) was found to inhibit the *in vitro* covalent binding of bromobenzene metabolites to microsomal proteins (Fig. 3) when this binding was studied with a low concentration of bromobenzene (0.02 mM). The rate-limiting step in such covalent binding studies is the enzymatic formation of the reactive metabolites which then quickly react with microsomal proteins. Accordingly, the above-mentioned finding is consistent with the view that dm PGE_2 may competitively inhibit the metabolic activation of bromobenzene by cytochrome P-450. This *in vitro* observation, however, may have little relevance for our *in vivo* studies. *In vivo*, the doses of bromobenzene that were administered (540,000 or 640,000 μg/kg) were several orders of magnitude higher than those of dm PGE_2 (50 pr 100 μg/kg). Accordingly, the concentrations of bromobenzene in the liver far exceeded any possible concentration of dm PGE_2. Under such conditions, dm PGE_2 may exert no competitive effect on the metabolism of bromobenzene. Indeed, dm PGE_2 (25–200 μg/l.) did not inhibit the *in vitro* covalent binding of bromobenzene metabolites to microsomal proteins (Fig. 3) when this binding was studied with a concentration of bromobenzene (1 mM) mimicking the concentration present in the liver 1 hr after administration of a toxic dose of bromobenzene (Fig. 4A). This *in vitro* observation

provided a first indication that the very small doses of dm PGE_2 employed *in vivo* may have only negligible effects on the overall metabolic activation of toxic doses of bromobenzene *in vivo*. This indication was further strengthened by studies on the disappearance of bromobenzene from plasma and liver. The liposoluble bromobenzene is essentially not excreted as such [16]. To leave the body, bromobenzene first has to be metabolized. The first step in this metabolism is the epoxidation of bromobenzene by cytochrome P-450. Therefore, the disappearance curves of bromobenzene from plasma and liver should be a function of the overall epoxidation rate of bromobenzene by cytochrome P-450. *In vivo*, dm PGE_2 did not modify the disappearance curves of unchanged bromobenzene (Fig. 4A), thus providing a second indication that dm PGE_2 did not modify the overall epoxidation rate of bromobenzene *in vivo*. This view was further supported by *in vivo* covalent binding studies. As indicated above, a fraction of the formed bromobenzene epoxides covalently bind to hepatic proteins. Again, dm PGE_2 did not quantitatively modify this *in vivo* covalent binding (Fig. 4B), a third indication that it did not quantitatively modify the overall epoxidation of bromobenzene.

Despite this unchanged covalent binding, dm PGE_2 markedly decreased serum alanine aminotransferase activity (Figs. 2 and 5), liver cell necrosis (Table 1) and mortality (Fig. 6) after the administration of bromobenzene.

The mechanism for this striking dissociation between a quantitatively unchanged covalent binding and a markedly reduced toxicity remains unknown at the present time. A first possibility may be that covalent binding, albeit quantitatively unchanged, may nevertheless be quantitatively modified. This might occur if the prostaglandin somehow prevented the reaction of the epoxides to a few, toxicologically important, target macromolecules. This might also occur if dm PGE_2 exerted some subtle changes on metabolic activation. Bromobenzene is transformed into at least two epoxides in mice: the 3,4-epoxide and the 2,3-epoxide [28]. Both epoxides covalently bind to hepatic proteins [29]. However, the two epoxides may have different reactivity and may bind with different groups in proteins, and with different proteins [29]; accordingly, they may have different toxicities, the 3,4-epoxide being thought to be more toxic than the 2,3-epoxide [30, 31]. A differential effect of dm PGE_2 on the respective formation of these epoxides might, perhaps, result in a qualitatively different, albeit quantitatively unchanged, covalent binding.

Another possibility may be that dm PGE_2 does exert a 'cytoprotective' effect in the liver [3]. In the liver, dm PGE_2 has been reported to prevent liver lesions induced by galactosamine [5], a compound whose toxicity does not apparently involve metabolic activation by cytochrome P-450 [32]. In the stomach, dm PGE_2 prevents ulcerations induced by very simple aggressions, such as thermal injury, or exposure to acids, bases, or hypertonic solutions [1]. Dm PGE_2 may also prevent diet-induced pancreatitis, and may decrease renal lesions produced by various aggressions [2]. These pleomorphic protective effects suggest that dm PGE_2 may act not on the initial aggression itself but instead on some intermediate step(s) between initial aggression and ultimate development of tissue necrosis. Our observation of a markedly reduced toxicity despite a quantitatively unchanged covalent binding may be consistent with such a 'cytoprotective' effect of dm PGE_2. Such an effect may also be consistent with our other findings. It was observed that administration of dm PGE_2 partially prevented the decrease in cytochrome P-450 concentration 24 hr after the administration of bromobenzene (Fig. 8). Dm PGE_2 given alone, however, had no effect on the concentration of this haemoprotein. At least two mechanisms may contribute to the decreased cytochrome P-450 concentration observed after administration of toxic doses of bromobenzene: (a) during the period of active formation of the metabolites, the reactive metabolites might covalently bind to cytochrome P-450 and destroy this haemoprotein, as shown for several olefins transformed into reactive intermediates [33]; (b) at later times, necrosis progressively develops and cytochrome P-450 may be digested in necrotic hepatocytes. Dm PGE_2 did not significantly modify the disappearance of cytochrome P-450 during the first 10 hr after the administration of bromobenzene (Fig. 8), a finding consistent with the view that dm PGE_2 does not modify the overall formation of the reactive bromobenzene metabolites. At 24 hr, however, the decrease in cytochrome P-450 concentration was less severe in mice protected by dm PGE_2 (Fig. 8), a finding possibly consistent with the lesser number of necrotic hepatocytes in these mice at that time (table 1). Another effect of dm PGE_2 administration was to prevent partly the depletion of hepatic GSH (Fig. 7) and GSSG 5 hr after administration of bromobenzene. Dm PGE_2 itself, however, had no effect on hepatic GSH (Fig. 7) or GSSG concentration and did not modify the depletion of GSH produced by diethyl maleate. It is thought that the depletion of glutathione observed after administration of toxic doses of bromobenzene may result from two concurrent mechanisms [17, 19]: (a) a first mechanism is the conjugation of GSH with the bromobenzene epoxides, a phenomenon which increases the utilization of glutathione; (b) a second mechanism is a reduced ability of the liver to synthetize glutathione as a possible consequence of alterations in plasma membranes and the uptake of amino acids; interestingly, the latter effects occur very early, much before the appearance of hepatic necrosis; in isolated hepatocytes that had been exposed for 1 hr to bromobenzene, plasma membranes had lost their normal microvilli, the uptake of cysteine and methionine was markedly reduced, as was the ability of the liver to resynthetize glutathione [17, 19]. Conceivably, a cytoprotective effect of dm PGE_2 might prevent early alterations in plasma membranes and preserve the ability of the liver to resynthetize glutathione. In mice treated with dm PGE_2, the liver may normally increase the synthesis of glutathione as a response to its high utilization rate: this might explain why the concentration of GSH was less decreased during the phase of active formation of the epoxides, while a rebound phenomenon occurred at later times (15 hr) (Fig. 7). In mice

receiving bromobenzene alone, the liver may be unable to increase the synthesis of glutathione: this might explain why the concentration of GSH fell to lower levels at early times and why there was no rebound phenomenon at later times (Fig. 7).

The mechanism(s) for the suspected 'cytoprotective' effect of dm PGE_2 in extrahepatic organs, and possibly in the liver, remain(s) totally unknown at the present time. Obviously, it is tempting to speculate some common mechanism(s) for the effects of dm PGE_2 in the various models. Possible avenues for future researches may include potential effects of dm PGE_2 on biological membranes. Can dm PGE_2 unspecifically protect plasma membranes?, can it stabilize lysosomes?, may it somehow prevent lipid peroxidation *in vivo* although it does not prevent it *in vitro*?, may it protect the small, but toxically important, mitochondrial glutathione pool?, or may it prevent alterations in the repartition of potentially toxic ions?—some of these hypotheses are currently under investigation.

We conclude that administration of dm PGE_2 can prevent liver cell necrosis without decreasing the covalent binding of bromobenzene metabolites to hepatic proteins. The mechanism for this protective effect remains unknown at present.

Acknowledgements—This work was supported in part by a grant from the Institut National de Recherche et de Sécurité. We are also very grateful to the Upjohn Co., Kalamazoo, Michigan, for providing us with 16,16-dimethyl prostaglandin E_2.

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