Biochemical Pharmacology, Vol. 34, No. 9, pp. 1515–1522, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

CARBON TETRACHLORIDE-INDUCED EICOSANOID SYNTHESIS AND ENZYME RELEASE FROM RAT PERITONEAL LEUCOCYTES

THOMAS J. LYNCH, GEOFFREY J. BLACKWELL and SALVADOR MONCADA
Department of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

(*Received* 13 *August* 1984; *accepted* 13 *November* 1984)

Abstract—When rat peritoneal leucocytes were incubated with carbon tetrachloride, a PLA_2 was activated, eicosanoids were generated and lysosomal and cytoplasmic enzymes were released. The predominant eicosanoid generated was TXB_2 with lesser amounts of PGE_2, 6-keto $PGF_{1\alpha}$ and LTB_4. Preincubation of the cells with two structurally unrelated thromboxane synthetase inhibitors reduced PLA_2 activity and enzyme release and also reduced the total amounts of eicosanoids liberated. An anti-PGI_2 antibody partially reversed the effects of thromboxane synthetase inhibitors indicating a role for endogenous PGI_2 generation in the cytoprotective effects of these agents in this system. Exogenous PGI_2 was also cytoprotective but the timing of its administration was critical. The cytoprotective effect of PGI_2 was potentiated by a phosphodiesterase inhibitor, indicating a possible pivotal role of cAMP in cell protection.

Carbon tetrachloride (CCl_4) has well-documented hepatic toxicity when administered *in vivo* [1]. Although CCl_4 produces its main effects in the liver, other toxic manifestations arise in other organs such as the kidney and lung. Specific hepatotoxicity may require metabolic conversion of CCl_4 to reactive intermediates such as the trichloromethyl radical ($CCl_3^{\cdot}$) [2]. Formation of such reactive species can ultimately lead to the generation of lipid peroxides which have well documented cytolytic effects in many cell systems [3]. This aspect of CCl_4 reactivity together with our interest in the enzymic peroxidation of unsaturated fatty acids which results in the formation of cytotoxic mediators such as thromboxanes and leukotrienes, led us to examine the possible role of eicosanoids in the cytotoxic effects of CCl_4 on rat peritoneal leucocytes and the protective effects of some known inhibitors.

MATERIALS AND METHODS

Preparation of rat peritoneal leucocytes. Adult male Wistar rats 250–350 g were injected intraperitoneally (IP) with 20 ml of 0.2% oyster glycogen in saline. Sixteen hours later the animals were killed by exposure to CO_2 and their peritoneal cavities washed out with a further 20 ml of Hanks solution (buffered with 20 mM Hepes and containing 5 units/ml heparin). The peritoneal fluid was centrifuged at 200 *g* for 10 min at 4° and the resultant cell pellet was reconstituted in fresh Hepes-buffered Hanks solution to a cell concentration of 1×10^7 cells/ml. Aliquots of the cell suspension (1 ml) were dispensed into Linbro 24 well culture vessels which were then placed in an incubator for 2 hr at 37°. After incubation the non-adherent cells and supernatant fluids were removed by aspiration and the adherent cells were overlaid with fresh medium (1 ml).

Experimental procedures. Adherent cell layers on the Linbro plates were prepared as described above. For most experiments all drug groups and controls were prepared in triplicate and all compounds or vehicle controls were preincubated with the cells for 10 min unless otherwise stated. CCl_4 (in DMSO and a final volume of 10 μl) was given at time zero and all further incubations were carried out at 37° for the appropriate times. After incubation the cell supernatants (0.8 ml) were removed (care being taken to avoid removing any cells) and frozen immediately in a dry-ice bath.

Estimation of β-glucuronidase, lactate dehydrogenase and eicosanoids. The estimation of β-glucuronidase activity of the samples was carried out by the method of Dean *et al.* [4]. Briefly, aliquots (0.25 ml) of cell supernatants were incubated with 0.8 mg phenolphthalein β-glucuronide in 0.5 ml acetate buffer (50 mM pH 4.0) for 16 hr at 37°. After incubation 4.25 ml 0.4 M sodium carbonate solution was added, and the absorbance of samples (at 540 nM) were estimated by standard spectrophotometry. The results were expressed as units of activity per 10^7 cells. One unit of enzyme activity was defined as that activity able to hydrolyse one microgram of phenolphthalein β-glucuronide per 16 hr incubation at 37°.

Lactate dehydrogenase (LDH) activities in the samples were determined by the spectrophotometric methods of Wroblewski and La Due [5]. Results were expressed as units of enzyme activity per 10^7 cells.

The following eicosanoids were measured by direct radioimmunoassay techniques [6], TXB_2, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and LTB_4. Results were expressed as ng eicosanoid/10^7 cells.

Pulse labelling of rat peritoneal leucocytes with [1-^{14}C]-*arachidonic acid.* Rat peritoneal leucocytes

were prepared as previously described but before addition to the culture plates the cells (2×10^8) were incubated for 2 hr at 37° with 5 μCi [1-^{14}C]-arachidonic acid. After incubation the cells were sedimented by centrifugation and then washed twice with fresh medium. The aliquots were then placed on the culture plates and treated as described. Following experimental procedures the cell supernatants were extracted and separated by TLC methods previously described. The radioactivity in each zone corresponding to authentic eicosanoid standards was estimated by liquid scintillation counting techniques.

Estimation of phospholipase A_2 activity. Mononucleate and polymorphonucleate phagocytes possess membrane-bound PLA_2, lysosomal PLA_2 and are also able to secrete a soluble enzyme. In our experiments the PLA_2 activity of the cells/medium was estimated by the double isotope procedures described by Blackwell *et al.* [7]. Adherent cells were incubated for 15 min prior to the desired time point with 8 nmoles 2-[1-^{14}C] oleoyl lecithin, 80 pmoles [N-^{3}H] oleic acid and 10 μmoles unlabelled oleic acid. After incubation the ^{14}C-oleic acid liberated by the PLA_2 action was removed together with the internal standard ^{3}H-oleic acid by hexane extraction. Unhydrolyzed phosphatide and lysophosphatide remained in the aqueous phase. The radioactivity of the hexane extract was estimated by standard double isotope counting techniques and the results quantified as previously described [7].

Purification of Anti-PGI_1 antisera. Rabbit serum containing antibodies to PGI_1 which also cross reacts with PGI_2 [8] was applied (1 ml) to a protein A column. Immunoglobulins were bound to the column matrix allowing albumin to be eluted from the column with 3 column volumes of 0.5 M acetate buffer pH 5. IgG was eluted with 1 ml 1 M acetate buffer pH 5 which was then brought to pH 7.5 with NaOH. The resultant solution was tested for anti-PGI_2 effects using the PGI_2-induced inhibition of ADP-aggregation of washed human platelets [9]. The purified anti-PGI_1 antiserum was then diluted so that 1 μl neutralized the platelet anti-aggregatory effects of 1 ng of PGI_2. A 50 μl aliquot of the antiserum was given to each sample.

Materials. [1-^{14}C]-Arachidonic acid (Batch 31: 56 mCi/mmole) and 2-[1-^{14}C oleoyl] phosphatidyl choline (Batch 12 61 mCi/mmole) were obtained from Amersham International. Phenolphthalein β-glucuronide, β-NADH and pyruvate were purchased from Sigma Chemical Corp. (Dorset, U.K.). Isobutylmethylxanthine was purchased from the Aldrich Chemical Company (Dorset, U.K.). Sterile HEPES buffer and Hanks solutions were prepared by Wellcome Reagents (Beckenham, U.K.) and benzyl imidazole and PGI_2 were made by the Chemical Research Laboratories of Wellcome Research Laboratories. OKY1581 was a generous gift from the ONO Pharmaceutical Company Limited (Osaka, Japan) and the culture well plates were supplied by the Linbro Division of Flow Laboratories (Ayrshire, Scotland).

RESULTS

Effects of CCl_4 on enzyme release and eicosanoid synthesis

Incubation of the peritoneal cells with CCl_4 at a concentration of 0.3 mM for 15 min resulted in the liberation of eicosanoids. CCl_4 could induce non-specific cytotoxicity by virtue of its lipophilicity. However, other lipophilic agents, chloroform and hexane, did not induce TXB_2 synthesis or enzymes at comparable doses. Concentrations in excess of 1 mM caused cell lysis and inactivation of enzymes released without the accompanying eicosanoid generation. Measuring these compounds by specific radioimmunoassay, it was found that the rat peritoneal leucocytes generated predominantly TXB_2 (69% total), smaller amounts of PGE_2 (18%) and 6-keto $PGF_{1\alpha}$ (9%) and very little LTB_4 (4%) (Table 1). Thus for most of the subsequent experiments we measured the formation of the predominant species of eicosanoid namely TXB_2. During a 15 min exposure CCl_4 caused a dose-dependent increase in TXB_2 formation and β-glucuronidase release from the peritoneal cells (Fig. 1). Threshold concentrations of CCl_4 were about 0.2 mM and the highest concentration tested was 1 mM.

In our preparations we observed that the control cell medium contained a background β-glucuronidase activity of $\simeq$80 units/10^7 cells. This confirms previous data and is thought to reflect the response of the cell to plate adherence [10]. Stimulation of this basal level could be achieved with CCl_4 concentrations between 0.2 mM and 1 mM. LDH levels were also measured (data not shown) similar results being obtained to those of β-glucuronidase except that the levels in control cell medium were not detectable.

When rat peritoneal cells were incubated with 0.3 mM CCl_4 there was a time-dependent increase in the synthesis of TXB_2 and the liberation of β-glucuronidase (Fig. 2). There was a rapid initial stimulation (up to 15') of TXB_2 formation which slowed thereafter. β-glucuronidase release continued at an almost linear rate following CCl_4 treatment.

Table 1. Effects of benzyl imidazole on the profile of eicosanoids liberated by CCl_4-treated rat peritoneal cells

	Total eicosanoids generated (%)†			
	TXB_2	6-keto-$PGF_{1\alpha}$	PGE_2	LTB_4
Control	69.2 ± 2.2	9.0 ± 1.0	17.6 ± 2.8	4.8 ± 0.9
BI*	7.5 ± 0.9	79.8 ± 0.3	10.7 ± 0.5	1.9 ± 0.3

* 63 μM.
† Measured by radioimmunoassay.

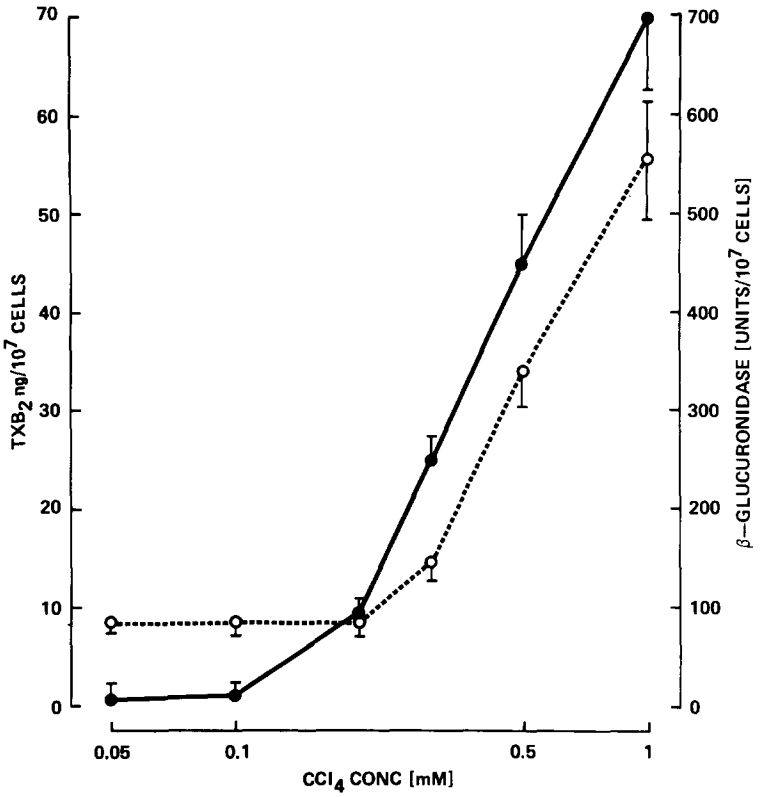

Fig. 1. Stimulation of TXB_2 formation (solid line) and β-glucuronidase release (broken line) from rat peritoneal leucocytes treated with CCl_4 for 2 hr. Each point is a mean value ± S.E.M. of three separate experiments.

Medium obtained from the vehicle-treated cell preparations after 2 hr incubation contained 4.5 ± 0.4 ng/10^7 cells TXB_2 and 124 ± 14 units/10^7 cells β-glucuronidase (N = 5). We therefore selected a 15 min incubation period with 0.3 mM CCl_4 for subsequent experiments unless otherwise stated.

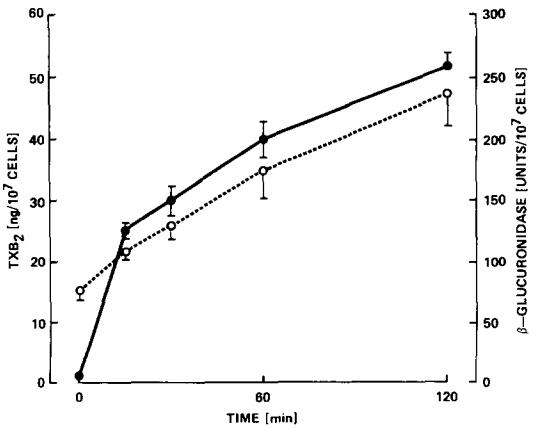

Fig. 2. Time course of CCl_4 (0.3 mM)-induced TXB_2 formation (solid line) and β-glucuronidase release (broken line) from rat peritoneal leucocytes. Each point is the mean ± S.E.M. of three separate experiments.

The effects of benzyl imidazole on TXB_2 formation and β-glucuronidase release from rat peritoneal cells

Substituted imidazoles have been shown to inhibit thromboxane synthetase activity in isolated enzyme preparations [11, 12] and also prevent the generation of thromboxanes in whole cell or organ systems [13, 14]. When pre-incubated with rat peritoneal cells for 10 min prior to the addition of CCl_4 benzyl imidazole (BI) caused a dose-dependent inhibition of TXB_2 formation and β-glucuronidase release (Fig. 3). From the dose–response curves it can be seen that the IC_{50} for TXB_2 formation is 11.5 μM; for β-glucuronidase release 16 μM; and for LDH release 17.5 μM (data not shown).

Similar experiments were performed with OKY1581, where this structurally unrelated compound also caused a dose dependent inhibition of TXB_2 formation [IC_{50} 0.22 μM] and β-glucuronidase release [IC_{50} 0.6 μM] (Fig. 4).

Effects of benzyl imidazole on the liberation of radiolabelled eicosanoids

Experiments performed on cells with ^{14}C-arachidonic acid incorporated into their intracellular lipid stores showed that upon stimulation with 0.3 mM CCl_4 for 2 hr the cells generated a qualitatively and quantitatively similar profile of eicosanoids (Fig. 5) as had seen using radioimmunoassay of products in cells not prelabelled with arachidonate (Table 1).

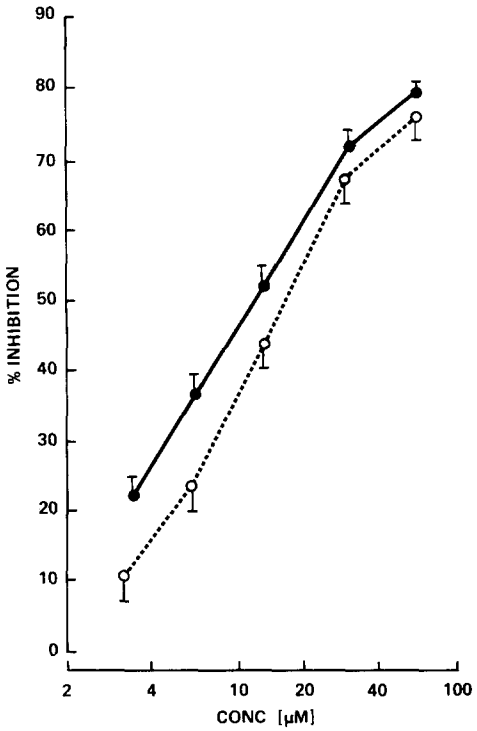

Fig. 3. Dose–response curve of the inhibition of TXB_2 formation (solid line) and β-glucuronidase release (broken line) by benzyl imidazole in CCl_4 (0.3 mM) stimulated rat peritoneal leucocytes. Cells were preincubated with BI for 10 min prior to the addition of CCl_4. The incubations were terminated after 15 min. Each point is a mean value ± S.E.M. of three separate experiments.

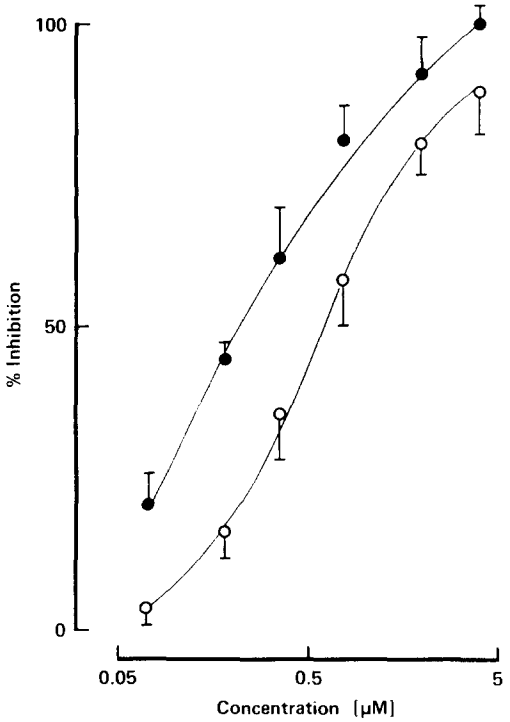

Fig. 4. The effects of OKY 1581 on TXB_2 synthesis (closed circles) and β-glucuronidase release (open circles) following CCl_4 stimulation of rat peritoneal leucocytes. Cells were preincubated with OKY 1581 for 10 min prior to the addition of CCl_4 (0.3 mM). The incubations were terminated after 15 min. Each point is a mean value ± S.E.M. of three separate experiments.

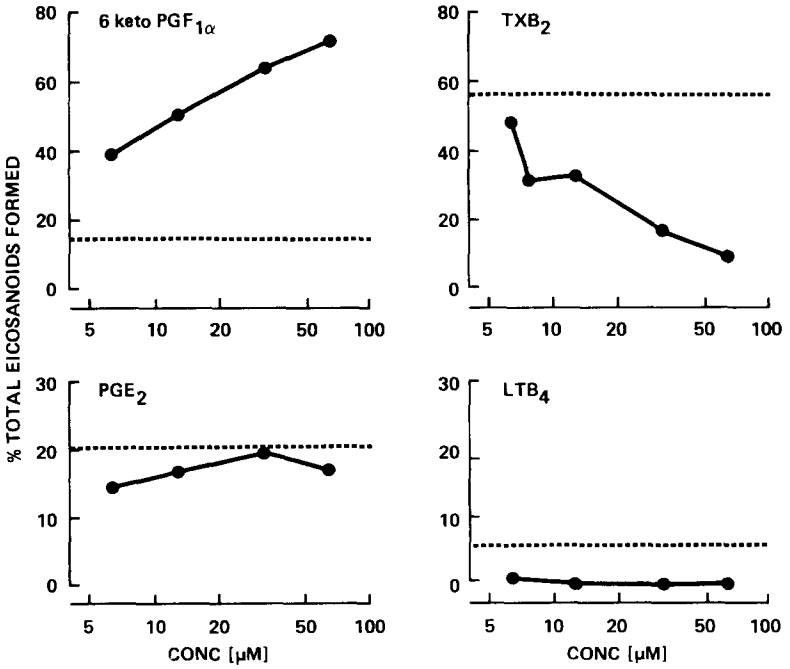

Fig. 5. Generation of eicosanoids from endogenous ^{14}C-arachidonic acid following a 2 hr exposure to 0.3 mM CCl_4 (broken lines) and the effects of BI (solid lines). The results are expressed for each eicosanoid, as a percentage of the total radioactivity associated with each product. Each point represents a mean of three values from one typical experiment.

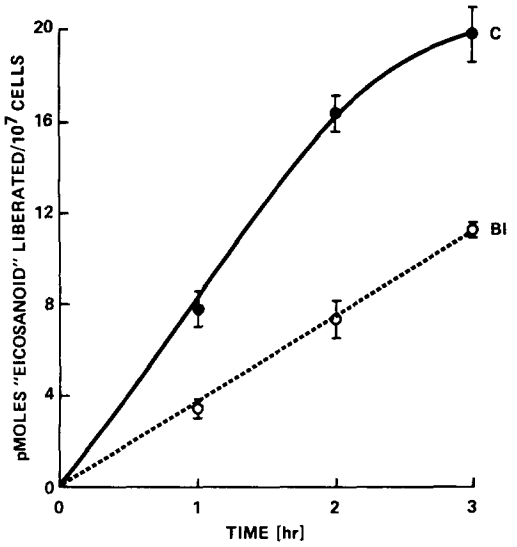

Fig. 6. CCl_4 (0.3 mM)-induced release of ^{14}C-labelled eicosanoids (C) from rat peritoneal leucocytes and its inhibition by BI (63 μM). Each point represents the mean ± S.E.M. of three separate experiments.

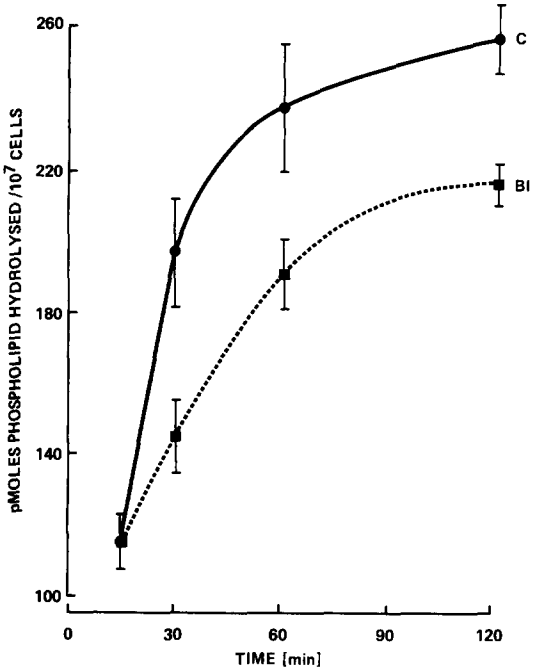

Fig. 7. Inhibition of CCl_4-induced PLA_2 activity (solid line) of rat peritoneal cells by BI (63 μM) (broken line). The PLA_2 activity in the preparation was estimated at each time point by methods described in the text. Each point is a mean ± S.E.M. of values from three separate experiments.

Furthermore, pre-incubation (10 min) of the cells with BI caused a dose-dependent redirection of synthesis of eicosanoids away from TXB_2, and toward 6-keto-$PGF_{1\alpha}$, the stable breakdown product of PGI_2. There was little change in the synthesis of PGE_2 although LTB_4 synthesis was markedly reduced. Similar results were obtained when these eicosanoids were measured by specific radioimmunoassay (Table 1). However, in addition to the labelled products already mentioned we also detected unmetabolized arachidonic acid and some unidentified hydroxy fatty acids, and in cells treated with CCl_4 alone these other products comprised 37% of the total radioactivity recovered. If the total amounts of radioactivity recovered are calculated (expressed as pmoles labelled eicosanoid recovered), then it can be seen (Fig. 6) that BI in a concentration of 63 μM is also able to reduce the total radioactivity released.

Effects of benzyl imidazole on phospholipase A_2 activity of rat peritoneal leucocytes

PLA_2 is a key enzyme involved in mobilising precursor fatty acids for eicosanoid synthesis from membrane phospholipids. Incubating rat peritoneal cells with 0.3 mM CCl_4 results in a time-dependent increase in the PLA_2 activity of the preparation to a maximum of 257 pmoles phospholipid hydrolysed/10^7 cells at 2 hr (Fig. 7). Pre-incubation of the cells with 63 μM BI for 10 min prior to the addition of CCl_4 reduced the expressed PLA_2 activity at all time points and at 2 hr the cells hydrolysed 218 pmoles phospholipid/10^7 cells. Cell preparations treated with vehicle or vehicle plus BI (63 μM) and studied at 2 hr hydrolysed 148 ± 18 pmoles/phospholipid/10^7 cells and 127 ± 16 pmoles phospholipid/10^7 cells respectively. These values represent only a small increase over the zero time point value of 116 ± 7 pmoles phospholipid hydrolysed/10^7 cells.

Effects of PGI_2 on TXB_2 formation and enzyme release

Prostacyclin has been shown to cause inhibition of PLA_2 activity [15] and TXB_2 formation in platelets [16] and also possesses cytoprotective effects in several cell systems [17, 18]. Therefore it seemed possible that the increased synthesis of PGI_2 in this system could possibly account for some of the effects observed following BI treatment of CCl_4-treated rat peritoneal cells.

However, when PGI_2 was administered concomitantly with CCl_4 or preincubated with the cell preparations we could find little evidence of reduced eicosanoid synthesis or enzyme release, in fact there often appeared to be an enhancement of generation or release. Only concentrations in excess of 30 μM reduced both eicosanoid synthesis and enzyme release. If, however, smaller doses (1.4 μM) were employed 4 min after the addition of CCl_4 then some inhibition could be seen (Fig. 8). Furthermore, when we included a standard dose (225 μM) of the phosphodiesterase inhibitor IBMX which in itself has little effect on eicosanoid production together with the PGI_2 (1.4 μM), then PGI_2 exerted inhibitory effects at all times up to 6 min after CCl_4 treatment. Similar effects were obtained when β-glucuronidase or LDH activities were measured.

Effects of anti-PGI_1 antisera on benzyl imidazole inhibition of CCl_4 induced TXB_2 synthesis and enzyme release

In order to investigate further the effects of PGI_2 we incubated the cell preparations with BI in com-

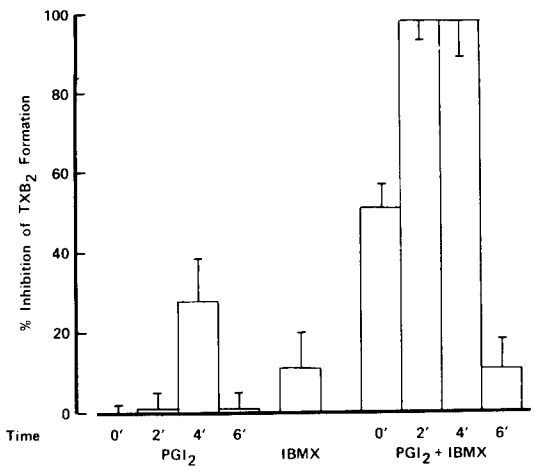

Fig. 8. The effects of PGI_2 (1.4 μM) in the presence and absence of IBMX (225 μM) on TXB_2 formation in rat peritoneal leucocytes following a 15 min exposure to CCl_4 (0.3 mM). IBMX was given 10 min prior to CCl_4 and PGI_2 was given at 0,2,4, or 6 min after CCl_4 administration. Each point is a mean value ± S.E.M. of three separate experiments.

bination with purified anti-PGI_1 antisera before CCl_4 treatment. It can be seen (Table 2) that the antibody can partially reverse the inhibitory effects of BI on β-glucuronidase and LDH release but not on TXB_2 formation thus indicating that part of the observed effects of BI in this system could be due to the synthesis of a cytoprotective PGI_2.

DISCUSSION

We have shown that in rat peritoneal cells CCl_4 can cause the synthesis of eicosanoids and also the release of cytoplasmic and lysosomal enzyme markers. This action of CCl_4 is rapid, within 15 min of exposure to the agent. We have also shown that CCl_4 is able to increase PLA_2 activity in the preparation and also to induce the formation of eicosanoids from cells pre-labelled with ^{14}C-arachidonate. Unfortunately, in these latter studies we had to employ a longer time course, such that the activation of PLA_2 which we measured might not be responsible for mobilizing fatty acid precursors and hence eicosanoid synthesis during the shorter incubation periods. PLA_2 is certainly a key enzyme involved in eicosanoid generation in many cells and tissues, however, there are other enzyme systems which might be activated by CCl_4 (for a review of these systems see Irvine [19]). Moreover, we have not established the exact location of the expressed PLA_2 activity. The most likely source would presumably be the plasma membrane enzyme as it seems unlikely, using the methods employed, that the labelled phospholipid would be transported into the cell, hydrolysed and the labelled products secreted into the medium. However, both PMN leucocytes [20] and macrophages [21] are able to secrete a neutrally active Ca^{2+} dependent PLA_2 which therefore could cause the hydrolysis of our labelled substrate. Nevertheless, it is clear that CCl_4 is able to induce PLA_2 activity and the generation of eicosanoids, and, as it is likely that both events are interrelated, the actual process of activation must be considered. We have already alluded to the role of lipid peroxidation in CCl_4-induced cytotoxicity and it is accepted that this process is violently destructive for cell membranes. Thus a positive feedback role at least for lipid peroxides, thromboxanes and leukotrienes on PLA_2 activity is certainly attractive. However, recent information indicates that this process may only play a minor role, especially in the early stages of CCl_4 interaction, and the causative mechanism may involve the liberation of Ca^{2+}. CCl_4 has been shown to inhibit ATP-dependent Ca^{2+} pumps which leads to the liberation of Ca^{2+} from intracellular stores, possibly the endoplasmic reticulum and the dense tubular network [22]. Recently Ca^{2+} flux has been shown to be directly related to cell injury [23]. Moreover, it is widely accepted that the catalytic activity of PLA_2 is also dependent upon Ca^{2+} and several workers have postulated that Ca^{2+} (possibly via calmodulin) might directly control the catalytic capacity of PLA_2 [24]. Similarly, the role for Ca^{2+} in lysosomal enzyme release from phagocytic cells is also well-documented [25]. Therefore, it is possible to suggest that local increases in cytoplasmic free Ca^{2+}, dependent, at least partially, on the formation of lipid molecules such as thromboxanes, lysophosphatides and leukotrienes play a role in CCl_4-induced tissue damage. Indeed, some of these mediators have been suggested to act as Ca^{2+} ionophores [26, 27].

We have also examined the effects of thromboxane synthetase inhibitors in this model of cell damage. We have shown that two structurally unrelated compounds BI & OKY1581 are able to prevent thromboxane generation and lysosomal enzyme release. Inhibition of TXB_2 formation and lysosomal enzyme release parallel each other but a positive role for thromboxane induction of enzyme release or vice

Table 2. CCL_4-induced β-glucuronidase and lactic dehydrogenase release and TXB_2 formation: Reversal of benzyl imidazole inhibition by purified anti-PGI_1 antibody

	TXB_2 form^n (ng/10^7 cells)	%I	β-glucuronidase (units/10^7 cells)	%I	LDH (units/10^7 cells)	%I
Control	18.7	—	325.8	—	225.4	—
BI*	2.1	88.8	157.3	51.8	112.1	49.7
BI + Anti-PGI_1	2.7	85.9	276.3	16.8	181.9	19.3
BI + Anti-PGI_1	19.1	+2.1	351.9	+8.0	269.7	+19.6

* Benzyl imidazole concentration was 63 μM.

versa has not been proven. In preliminary experiments the cyclooxygenase inhibitors aspirin and indomethacin could completely abolish TXB_2 synthesis but with little effect on enzyme release. Indeed, other workers have reported augmented lysosomal enzyme release with these agents [28]. Thus, the precise relationship between TXA_2 synthesis and enzyme release is not clear, however, it is likely that both events are the result of an activation process common to both pathways. In addition to these effects we have shown that BI is able to reduce PLA_2 activity and the total release of eicosanoids. BI, at the concentrations employed in this study has no direct effect on PLA_2 activity (data not shown). It seems likely that this effect is achieved by BI preventing thromboxane generation leading to an overall increase in the synthesis of the cytoprotective agent PGI_2. The redirection certainly occurs following BI treatment of CCl_4 stimulated cells (Fig. 5 and Table 1). Thus, the involvement of PGI_2 in the cytoprotective effects of BI in this system seems possible, although the actual source of this compound in our experiments is not clear. The adherent cell population comprises 62% PMN leucocytes, 36% macrophages and <2% lymphocytes and mast cells. The PMN leucocyte produces predominantly TXB_2 and PGE_2 in response to stimuli which cause prostanoid synthesis [29]. Macrophages, however, in addition to these prostanoids also generate PGI_2 [30]. Therefore from our results we may suggest that the production of substantial quantities of PGI_2 following thromboxane synthetase inhibition is due to a rapid transformation by macrophages of the endoperoxide derived from PMN leucocytes. This diversion of synthesis from TXB_2 to PGI_2 following thromboxane synthetase inhibition has been shown to occur in isolated rabbit pulmonary arteries [31] and perfused hydronephrotic kidneys [32]. Furthermore, the inter-cell conversion which we believe takes place between PMN leucocytes and macrophages has been shown to occur between platelets and leucocytes in whole blood [33]. Nevertheless, the production of cytoprotective PGI_2 cannot account for all the cytoprotective effects of BI in this system (Table 2) which therefore implies that TXA_2 or B_2 might function as cytotoxic agents. The question of how PGI_2 is able to exert its cytoprotective effect is unclear, in the context of our speculation of the role of free Ca^{2+} in the response of cells to CCl_4, PGI_2 is able to prevent increases in intracellular free Ca^{2+} via increases in cellular cyclic AMP [34]. However, the critical timing of the administration of PGI_2 is curious yet has been reported previously [18]. This phenomenon is overcome only by the use of large concentrations of PGI_2 or by the addition of a phosphodiesterase inhibitor. Perhaps, as in other sytems, a stimulation of adenylate cyclase leads to an induction of a cAMP phosphodiesterase [35]. Furthermore, lipid peroxides and lysophospholipids have been shown to increase the activity of cAMP phosphodiesterase [36] and these compounds might be formed in large quantities during CCl_4 intoxication. This explanation might explain the results obtained with a phosphodiesterase inhibitor together with PGI_2 which clearly potentiates the effects of PGI_2.

In conclusion we have demonstrated that CCl_4 can induce eicosanoid generation and enzyme release from rat peritoneal leucocytes. Benzyl imidazole can reduce this release by reducing thromboxane generation, via the generation of PGI_2. The mechanisms for CCl_4-induced cytotoxicity in this system, possibly involving Ca^{2+} liberation, are now under investigation in our laboratory.

REFERENCES

1. L. S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, Vol. 5, pp. 908–912. MacMillan, New York (1975).
2. T. F. Slater, K. Cheeseman and C. Benedetto, in *Recent Trends in Chemical Carcinogenesis* Vol. 1, (Eds. P. Pani, F. Feo and A. Columbano), p. 260 (1983).
3. R. O. Recknagel and E. A. Glende, *Crit. Rev. Toxicol.* **2**, 263 (1973).
4. R. T. Dean, W. Hylton and A. C. Allison, *Biochim. biophys. Acta* **584**, 57 (1979).
5. F. Wroblewski and J. S. La Due, *Proc. Soc. exp. Biol. Med.* **90**, 210 (1955).
6. P. M. Simmons, J. A. Salmon and S. Moncada, *Biochem. Pharmac.* **32**, 1353 (1983).
7. G. J. Blackwell, R. J. Flower, F. P. Nijkamp and J. R. Vane, *Br. J. Pharmac.* **62**, 79 (1978).
8. S. Bunting, S. Moncada, P. Reed, J. A. Salmon and J. R. Vane, *Prostaglandins* **15**, 565 (1978).
9. S. Bunting, S. Moncada, P. Reed, J. A. Salmon and J. R. Vane, *Prostaglandins* **15**, 537 (1978).
10. J. Schnyder and M. J. Baggiolini, *Exp. Med.* **22**, 435 (1978).
11. G. J. Blackwell, R. J. Flower N. Russell-Smith, J. A. Salmon P. B. Thorogood and J. R. Vane, *Br. J. Pharmac.* **64**, 435P (1978).
12. S. E. Burke, D. J. Lefer and A. M. Lefer, *Archs. int. Pharmacodyn. Ther.* **265**, 76 (1983).
13. B. J. R. Whittle, G. L. Kauffman and S. Moncada, *Nature* **292**, 472 (1983).
14. E. F. Smith, A. M. Lefer and J. B. Smith, *Can. J. Physiol. Pharmac.* **58**, 294 (1980).
15. E. G. Lapetina, C. J. Schmitges, K. Chandrabose and P. Cuatrecasas, *Biochem. biophys. Res. Commun.* **76**, 828 (1978).
16. M. Minkes, N. Stanford, M. M-Y. Chi, G. J. Roth, A. Raz, P. Needleman and P. W. Majerus, *J. clin. Invest.* **59**, 449 (1977).
17. G. J. Blackwell, M. Radomski, J. R. Vargas, and S. Moncada, *Biochim. biophys. Acta* **718**, 60 (1982).
18. F. Guarner, M. Fremont-Smith, J. Corzo, J. Quiroga, J. L. Rodriguez and J. Prieto, *Adv. Prostaglandin, Thromboxane Leukotriene Res.* **1**, 75 (1983).
19. R. F. Irvine, *Biochem. J.* **204** 3, (1982).
20. P. Elsbach, J. Weiss, R. C. Franson, S. Beckerdite-Quagliata, A. Schneider and L. Harris, *J. biol. Chem.* **254**, 11000 (1979).
21. P. D. Wightman, M. E. Dahlgren, P. Davies and R. J. Bonney, *Biochem. J.* **200**, 441 (1981).
22. L. Moore, *Biochem. Pharmac.* **29**, 2505 (1980).
23. B. F. Trump, I. K. Berezesky, U. Laikok, A. R. Osornio, W. J. Mergner and M. W. Smith, *Scanning E. M.* **II**, 437 (1980).
24. N. Moskowitz, L. Shapiro, W. Schook and S. Puszkin, *Biochem. biophys. Res. Commun.* **115**, 94 (1983).
25. L. J. Ignarro, in *Lysosomes Biol. Path.* **4**, 481 (1974).
26. E. G. Lapetina, M. M. Billah and P. Cuatrecasas, *J. biol. Chem.* **256**, 11984 (1981).
27. C. N. Serhan, I. Fridovich, E. J. Goetzl, P. B. Dunham, and G. Weissman, *J. biol. Chem.* 4746, (1982).
28. T. Okimura, H. Ohmori, Y. Kubota and I. Yamamoto, *Biochem. Pharmac.* **28**, 2729 (1979).

29. R. M. J. Palmer and J. A. Salmon, *Immunol.* **50**, 65 (1983).
30. R. J. Bonney, P. Davies, F. A. Kuehl and J. L. Humes, *J. Reticuloendothelial Soc.* **28**, 113S (1980).
31. S. Bunting, S. Moncada and J. R. Vane, *Br. Med. Bull.* **39**, 271 (1983).
32. A. R. Morrison, K. Nishikawa and P. Needleman, *J. Pharm. exp. Ther.* **205**, 1 (1978).
33. G. J. Blackwell, R. J. Flower, N. Russell-Smith, J. R. Salmon, P. B. Thorogood, and J. R. Vane, *Br. J. Pharmac.* **64**, 436P (1978).
34. D. E. Knight and M. C. Scrutton, *Nature, Lond.* **309**, 66 (1984).
35. R. Alvarez, A. Taylor, J. J. Fazzari, and J. R. Jacobs, *Molec. Pharmac.* **20**, 302 (1981).
36. H-H. Tai and C-L. Tai, *Archs. Biochem. Biophys.* **214**, 622 (1982).