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Modification by steroids of pulmonary oedema and prostaglandin E_2 pharmacokinetics induced by endotoxin in rats

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1 A single i.p. injection of bacterial endotoxin in rats (3.5 mg kg^{-1}) caused lung injury assessed as changes in lung dry : wet weight ratio and leukopaenia over the subsequent 28 h.

2 This treatment also slowed the efflux of ^{14}C from [^{14}C]-prostaglandin E_2 (PGE_2), i.e., increased $t_{1/2}$ and increased the survival of PGE_2 in isolated perfused lungs over the same period.

3 These effects of endotoxin were reversed by methylprednisolone (30 mg kg^{-1}), given 30 min after the endotoxin.

4 Another synthetic corticosteroid, budesonide (1.2 mg kg^{-1}) given 1 h before endotoxin partially prevented the lung injury and leukopaenia but did not affect the increased $t_{1/2}$ for PGE_2 nor its survival.

5 The reversal by methylprednisolone of both the physical signs of lung injury and the changes in PGE_2 pharmacokinetics caused by endotoxin suggests that changes in PGE_2 pharmacokinetics could serve as an index of acute lung injury following sepsis.

Introduction

The Adult Respiratory Distress Syndrome (ARDS) involves acute respiratory failure with non-cardiogenic pulmonary oedema. It occurs in patients with a variety of precipitating causes and continues to carry a mortality rate in excess of 50% (Stevens & Raffin, 1984; Modig, 1986). A frequent precursor to ARDS is sepsis (Rinaldo & Rogers, 1982) and infusion of either live Gram-negative bacteria or purified endotoxin into animals generates a useful model of ARDS, because many features of the clinical condition can be reproduced (Borg *et al.*, 1985a; Brigham & Meyrick, 1986).

In another model of acute lung injury, that induced by α-naphthyl thiourea (ANTU), the physical injury measured as oedema was accompanied by a biochemical disturbance, decreased prostaglandin E_2 (PGE_2) metabolism (Bakhle, 1982). In the present paper, we have studied the effects of endotoxin on the physical state of rat lung and on the pulmonary pharmacokinetics of PGE_2. Furthermore, because there is considerable experimental evidence that corticosteroids antagonize or prevent lung injury due to endotoxaemia (Al-Kaisi *et al.*, 1977; Hinshaw *et al.*, 1980; Brigham *et al.*, 1981; Schumer, 1981; Borg *et al.*, 1985b), we have also looked at the effects of two synthetic corticosteroids, methylprednisolone and budesonide, on our model of endotoxin-induced lung injury. A preliminary account of some of this work has been given to the British Pharmacological Society (Izumi & Bakhle, 1987).

Methods

Male Wistar rats (200–250 g body weight) were used and divided into three treatment groups. Group 1 received endotoxin only; group 2a received endotoxin with methylprednisolone and group 2b received methylprednisolone only; group 3a received endotoxin with budesonide and group 3b received budesonide only. Endotoxin (*E. coli* LPS-B) was given as a single intraperitoneal injection of 3.5 mg kg^{-1} body weight dissolved in sterile saline (1 mg ml^{-1}). A single subcutaneous injection of methylprednisolone (30 mg kg^{-1} body weight) was

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administered 30 min after endotoxin. A similar injection of budesonide (1.2 mg kg^{-1} body weight) was administered 1 h before endotoxin. Both steroids were dissolved in sterile 'Kenacort' vehicle (DRACO AB, Sweden) to give concentrations of methylprednisolone, 15 mg ml^{-1}; budesonide, 1 mg ml^{-1}. Rats in groups 2b and 3b (steroid only) received steroids as in groups 2a and 3a together with the same volume of sterile saline instead of endotoxin. After treatment all rats were returned to their cages with free access to food and water until they were used. At specified times after treatment, rats were anaesthetized with pentobarbitone and the lungs were taken for measurement of physical and pharmacokinetic changes.

Physical measurements

Peripheral white blood cells were counted by conventional methods to give both a total count and a differential count of polymorphonuclear leukocytes (PMNs), using blood from the tail.

Lung weight ratios were determined as follows. Rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.), the thorax opened, and any fluid present in the pleural cavity sucked out and weighed. The lungs and heart were then excised, the heart and other extraneous tissues trimmed off and the lungs rinsed in Krebs solution and blotted dry. The lungs were weighed (wet weight) and then dried in an oven overnight to give the dry weight. The lung dry : wet weight ratios were calculated from these measurements.

Pharmacokinetic measurements

In another set of animals the lungs were used for perfusion studies. Rats were anaesthetized as before, the chest opened and the pulmonary artery cannulated and perfused with warmed (37°C) and gassed (95% O_2 + 5% CO_2) Krebs solution at a constant rate of 8 ml min^{-1} as described previously (Bakhle *et al.*, 1969). The Krebs solution contained indomethacin (3 μg ml^{-1}) to prevent interference from cyclooxygenase products synthesized by the lungs. After 10 min of perfusion the lungs were used for pharmacokinetic measurements.

The pharmacokinetic variables measured were the time for 50% of injected ^{14}C to appear in the lung effluent perfusate following the injection of ^{14}C-labelled substrates ($t_{1/2}$) and the metabolism of PGE_2 by radioimmunoassay (RIA).

For the $t_{1/2}$ assay, lung effluent was collected in 4-drop fractions (approximately equivalent to 3 s) for about 2 min following an injection (0.1 ml) of [^{14}C]-PGE_2 (500 ng; 0.01 μCi), [^{14}C]-sucrose (0.01 μCi) or [^{14}C]-urea (0.01 μCi) into the pulmonary arterial flow. To each fraction, 4 ml of OptiPhase 'Safe' (LKB, England) was added and radioactivity measured in a liquid scintillation counter (Packard Model 2409). Corrections for quenching were made using external standards (channels ratio method) and radioactivity expressed as d.p.m.

The metabolism of PGE_2 was measured by collecting the lung effluent following the injection of PGE_2 (500 ng, 0.1 ml) in a single fraction for 5 min. A sample of each fraction (0.1 ml) was assayed directly by RIA, with methods (Watts *et al.*, 1982) and antisera (Bakhle & Pankhania, 1987) described previously. The lower limit of detection for PGE_2 was 80 pg ml^{-1} and cross-reactivities were: PGE_1 26%, $PGF_{2\alpha}$ 1.4%, thromboxane B_2 0.02%, 6-oxo-$PGF_{1\alpha}$ 0.03%, 15-oxo-PGE_2 1.2%, 13,14-dihydro-15-oxo-PGE_2 0.6%.

Materials

Endotoxin (lipopolysaccharide B—*E. coli* 0111:B4) was obtained from Difco, sodium pentobarbitone (Sagatal) from May and Baker Ltd, unlabelled PGE_2 and indomethacin from Sigma. We thank Dr R. Brattsand (Draco AB, Sweden) for a generous gift of budesonide and Kenacort solvent, and Upjohn Ltd for methylprednisolone sodium succinate. The radiolabelled substrates: [^{14}C]-urea (58 mCi $mmol^{-1}$), [1-^{14}C]-prostaglandin E_2 (58.4 mCi $mmol^{-1}$) and [U-^{14}C]-sucrose (555 mCi $mmol^{-1}$) were obtained from Amersham International; [5,6,8,10,12,14,15(n)-^{3}H] prostaglandin E_2 (160 Ci $mmol^{-1}$) was obtained from New England Nuclear, Du Pont (U.K.) Ltd. Aqueous liquid scintillation cocktail, 'Optiphase *Safe*', was obtained from LKB. All chemicals for Krebs solution were of Analar grade and were obtained from BDH Chemicals Ltd.

Statistical methods

All values in the paper are expressed as group means ($\pm$ s.e. mean) from the number of experiments shown (n). Statistical analyses were performed by use of Student's unpaired t test and values of $P < 0.05$ were taken to denote a significant difference.

Results

Physical effects of endotoxin treatment

The lung dry : wet weight ratios for 28 h after endotoxin are summarized in Table 1. The dry : wet ratio showed major decreases only at 6 and 16 h.

Table 1 Effects of endotoxin (i.p.) on physical and pharmacokinetic variables of rat lung

	Untreated	*Time after endotoxin* (h) 2	4	6	16	28
Lung dry : wet weight ratio (as %)	20.5 ± 0.3	21.1 ± 0.3	20.8 ± 0.1	19.5 ± 0.3*	19.4 ± 0.3*	20.3 ± 0.2
Peripheral leukocytes ($\times 10^6$ ml^{-1})						
Total	10.9 ± 0.1	5.0 ± 0.2*	3.3 ± 0.3*	6.6 ± 0.5*	6.4 ± 1.0*	10.5 ± 0.1
PMN	2.3 ± 1.0	0.8 ± 0.1*	0.5 ± 0.1*	1.4 ± 0.1*	1.6 ± 0.2*	2.3 ± 0.1
$t_{1/2}$ (s)						
[^{14}C]-PGE_2	34 ± 1	42 ± 2*	65 ± 4*	87 ± 8*	122 ± 4*	38 ± 2
[^{14}C]-sucrose	15 ± 1	16 ± 1	16 ± 1	14 ± 1	16 ± 1	15 ± 1
[^{14}C]-urea	16 ± 1	15 ± 1	17 ± 0.4	14 ± 1	17 ± 2	16 ± 1
n	9	5	8	5	4	8

The values in the table are the means (±s.e. mean) of results from the number of animals (*n*) shown, except for the sucrose and urea $t_{1/2}$ assays which were performed in 3–4 lungs at each time. The lung dry : wet weight ratio was lower than normal only at 6 and 16 h. The leukocytes either as total leukocytes or as PMNs showed early falls which were maintained up to 16 h. The $t_{1/2}$ values for PGE_2 were increased early and remained high up to 16 h, but those for sucrose or urea were unaffected by endotoxin treatment of the animal.
* Significantly different from value in untreated rats; $P < 0.05$.

The total white blood cell count showed an earlier response to endotoxin (Table 1), falling to about 50% by 2 h with a minimum value of just over 30% at 4 h. The total white blood cell count returned to normal by 28 h. The numbers of PMNs were also severely reduced (less than 25% of normal at 4 h) and remained low until 28 h. The proportion of PMNs in the total white blood cells also fell after endotoxin, from about 22% in untreated rats to 15% at 4 h, showing the relatively greater effect on these leukocytes.

Pharmacokinetic consequences of endotoxin treatment

The $t_{1/2}$ value for [^{14}C]-PGE_2 increased, i.e., efflux of ^{14}C was slower, soon after endotoxin injection (Table 1). This effect is illustrated by the efflux profiles in Figure 1, where results from individual lungs from an untreated rat and one 6 h after endotoxin are shown. The $t_{1/2}$ was increased by about 250% for PGE_2, but that for [^{14}C]-sucrose was not changed nor was that for [^{14}C]-urea (Figure 1 and Table 1). Although the value of $t_{1/2}$ for PGE_2 was increased as early as 2 h after endotoxin, the maximum, more than three times the normal value, was not reached until 16 h and then the $t_{1/2}$ returned to normal by 28 h (Table 1).

Metabolism of PGE_2 was measured at 2 h and at 16 h, the early and late stages of the response to endotoxin. The survival of PGE_2 was clearly increased, i.e., metabolism was decreased, at 2 h to 31 ± 6%, double its normal value (15 ± 2%). Survival was still high at 16 h after endotoxin (43 ± 11%); $n = 4$ in each condition.

Modification by steroids of endotoxin-induced effects

Methylprednisolone Treatment with methylprednisolone, 30 min after endotoxin, completely prevented the fall in lung dry : wet weight ratio between 6 and 28 h. Methylprednisolone only did not change this ratio at any time up to 28 h except for a transient increase in the ratio at 4 h (Figure 2). The leukocytes had a slightly more complex response to methylprednisolone (Figure 3). The steroid only caused a small decrease in total leukocytes (to about 65% of normal) and in PMNs (to 80% of normal) by 4 h. From 6 h onwards, the PMNs never fell below normal level, but total leukocytes were depressed up to 16 h. After endotoxin treatment, the steroid completely prevented the drop in total leukocytes at all times and moderated the early and precipitous fall in PMNs to the levels shown with methylprednisolone alone.

The pharmacokinetic variables were similarly affected by methylprednisolone (Figure 4). Given alone, methylprednisolone caused a small (30–50%) increase in $t_{1/2}$ for PGE_2 at 6 and 16 h only. Given after endotoxin, the early increase (2 h) in $t_{1/2}$ was not affected but, at all subsequent times, the increase in $t_{1/2}$ was clearly diminished though not abolished. Survival of PGE_2, unaffected by methylprednisolone alone, was still increased after endotoxin and methylprednisolone at 2 h. However, at 16 h the increased survival induced by endotoxin was totally prevented by methylprednisolone (Figure 4).

In a smaller series of experiments, the same dose of methylprednisolone was given but at 30 min before the injection of endotoxin, i.e., a total of 1 h

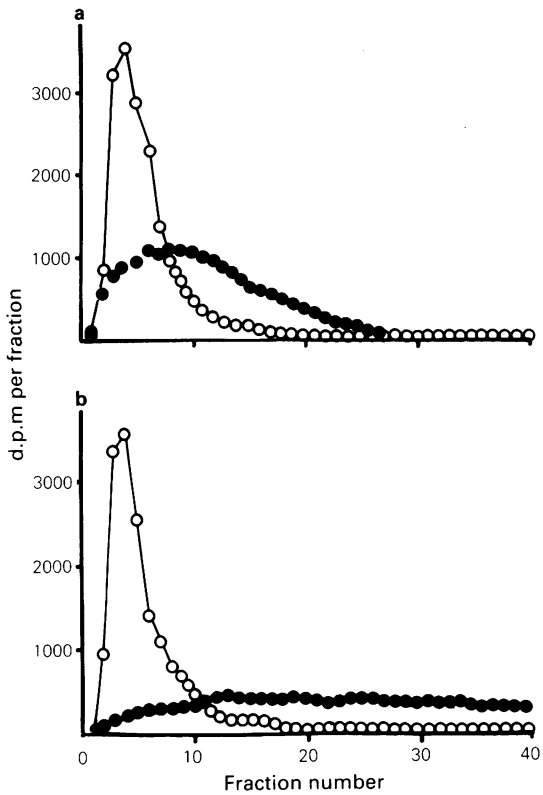

Figure 1 Efflux profile of ^{14}C in lung effluent following bolus injection of ^{14}C-labelled substrates in rat isolated lung. The profiles (from individual lungs) show the ^{14}C in each fraction collected, over 2 min. In (a), the efflux of ^{14}C from [^{14}C]-sucrose (○; $t_{1/2}$ = 15 s) (used as an extracellular marker) is shown to be much faster than that from [^{14}C]-prostaglandin E_2 (PGE_2, ●; $t_{1/2}$ = 34 s) in normal untreated lungs. In (b), at 6 h after endotoxin, the efflux of another extracellular marker, [^{14}C]-urea (○; $t_{1/2}$ = 14 s), is still comparable to that of sucrose in normal lung. However, the efflux derived from [^{14}C]-PGE_2 (●; $t_{1/2}$ = 87 s) is markedly prolonged.

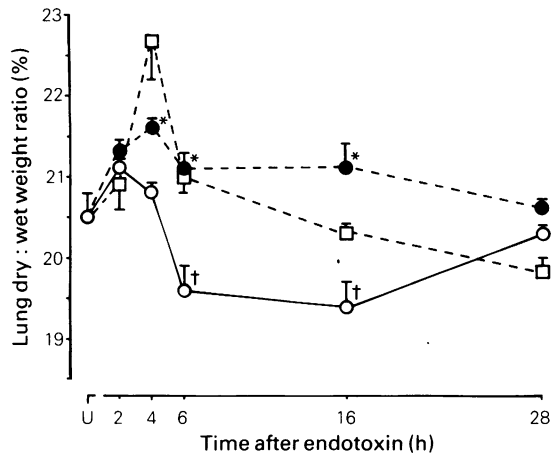

Figure 2 Effect of methylprednisolone on lung weight ratio in rats after endotoxin. The values shown are the means from 4–6 lungs in each condition; vertical lines indicate s.e. The decrease at 6 and 16 h after endotoxin only (○) was completely reversed by treatment with methylprednisolone (●). Note that methylprednisolone only (□) increased the ratio transiently at 4 h. In this and subsequent figures, † denotes a difference from the normal value (U) and * a difference from the value for endotoxin only; $P < 0.05$.

earlier than in the majority of the experiments. The $t_{1/2}$ for [^{14}C]-PGE_2 was measured at 2, 4 and 6 h only (n = 4 at each time). With this earlier treatment, methylprednisolone was able to reverse the early increase in $t_{1/2}$ at 2 h (endotoxin + methylprednisolone, 33 ± 4 s) but not the later values. Thus at 4 h, the $t_{1/2}$ was 73 ± 12 s and at 6 h, 80 ± 8 s.

Budesonide Pretreatment with budesonide prevented the decrease in lung dry : wet weight ratio (Figure 5). However, the steroid alone did increase the weight ratios to above normal values at 6–28 h. The early falls in total leukocytes at 2 and 4 h after endotoxin were not modified by budesonide, although at later times, the leukocyte count had returned to normal values, i.e., earlier than with endotoxin only (Figure 6). A similar picture of delayed protection was seen with the PMNs, no effect at 2 and 4 h, but recovery at 6 h and later.

However, treatment with budesonide was unable to reverse the pharmacokinetic changes at any time (Figure 7). Indeed, budesonide appeared to potentiate the effects of endotoxin on the $t_{1/2}$ value for [^{14}C]-PGE_2 at 4 and 28 h. Furthermore, budesonide did not reverse the increased survival of PGE_2 at either 2 or 16 h (Figure 7).

In another series of experiments, we gave twice the usual dose of budesonide, i.e., 2.4 mg kg^{-1}, at 1 h before endotoxin and measured $t_{1/2}$ for PGE_2 at 2, 4 and 6 h only (n = 4 at each time). At this higher dose, clearer signs of normalization of $t_{1/2}$ values were seen. At 2 h, the $t_{1/2}$ value (42 ± 10 s, n = 4) was not different from the normal value, but at the later times, values of 55 ± 10 s at 4 h and of 76 ± 2 s at 6 h were obtained. These last two values were lower than the corresponding $t_{1/2}$ found using the usual dose of budesonide (1.2 mg kg^{-1}, 94 ± 3 s and 110 ± 11 s respectively, from Figure 7), but not lower than those obtained with endotoxin only (see Table 1).

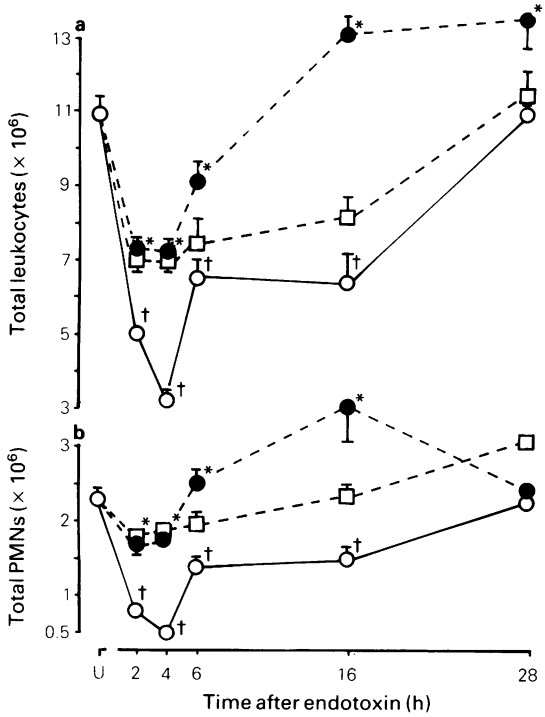

Figure 3 Effect of methylprednisolone on leukocytes after endotoxin. In (a), total peripheral leukocytes are shown to be protected against the precipitous fall caused by endotoxin (○) at all times by methylprednisolone (●). Steroid only has a moderately depressant effect on leukocyte numbers (□). In (b), the same protection was provided to the PMN leukocytes with relatively less effect due to methylprednisolone only. Values shown are the means, $n = 8$–14 in each condition; vertical lines indicate s.e.

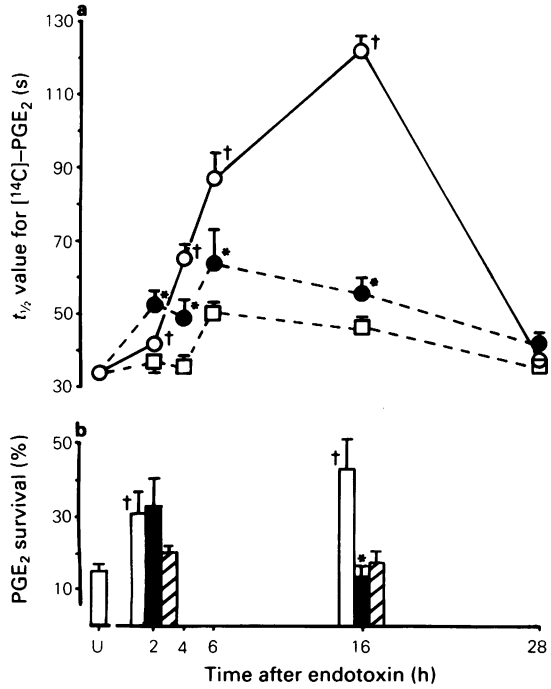

Figure 4 Effect of methylprednisolone on the pharmacokinetics of prostaglandin E_2 (PGE_2) in rat isolated lung after endotoxin. Here also methylprednisolone (●) afforded good protection against the increased $t_{1/2}$ caused by endotoxin only (○) with minimal effects due to methylprednisolone only (□). In (b), survival of PGE_2 in normal, untreated lungs (U) was about 15% and was greatly increased at 2 h and 16 h after endotoxin (open columns). Treatment with methylprednisolone (solid columns) restored survival to normal levels at 16 h only. Methylprednisolone only (hatched columns) had no effect on survival of PGE_2. Values shown are the means, $n = 4$–6 lungs in each condition; vertical lines indicate s.e.

Discussion

Our experiments have shown that bacterial endotoxin injected intraperitoneally will cause pulmonary oedema in rats and that this lung injury is associated with a change in the pulmonary pharmacokinetics of PGE_2. The ability of endotoxin to produce acute lung injury and its consequent use in ARDS models are well established (Borg *et al.*, 1985a; Brigham & Meyrick, 1986). However, most groups of workers have used entotoxin given intravenously and assessed lung injury over the next few hours. We wanted a slower onset of lung injury and for the injury to be non-lethal so that we could study the spontaneous resolution of the oedema and, by implication, repair of the injury. This was achieved by giving endotoxin in a single dose intraperitoneally and the results in Table 1 demonstrate the development of measurable lung oedema by 6 h, its persistence to 16 h and its spontaneous resolution by 28 h.

We also measured leukocytes in peripheral blood to confirm that endotoxin was entering the circulation from the peritoneum. The rapid and marked fall in total leukocytes and particularly in PMN leukocytes, showed that endotoxin was having the expected effects on circulating leukocytes as early as 2 h post injection. Both leukocyte counts were well on the way back to normal at 6 h, i.e. by the time the first signs of lung oedema were obvious. One possible inference from this temporal correlation is that the leukopaenia was a causal precursor of lung oedema. There is already strong evidence that neutrophils play an important role in the pathophysiological response to endotoxin (Tate & Repine,

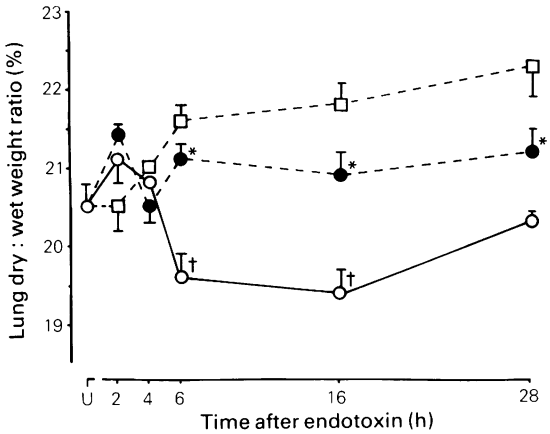

Figure 5 Effect of budesonide on lung weight ratio in rats after endotoxin. The effects of endotoxin (○) were completely prevented by budesonide (●) but budesonide only (□) increased the dry : wet ratio consistently from 6 h onwards. Values shown are the means, $n = 4$ in each condition; vertical lines indicate s.e.

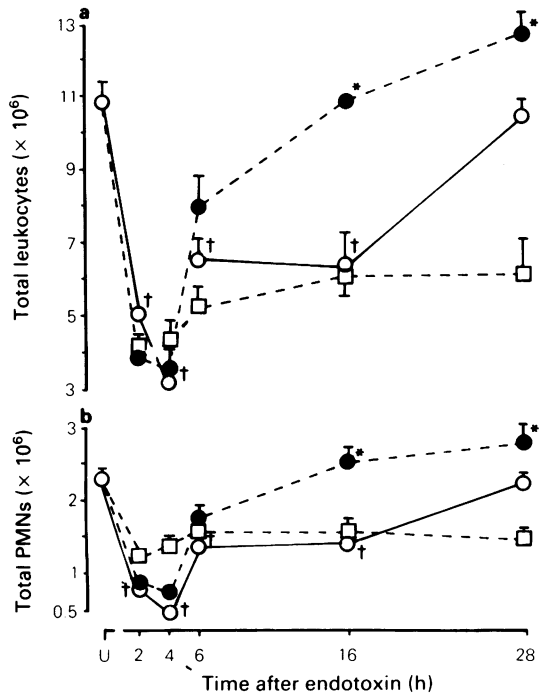

Figure 6 Effect of budesonide on leukocytes after endotoxin. Pretreatment with budesonide (●) did not prevent the effects of endotoxin (○) until 16 h as far as total leukocyte numbers (a) were concerned. (b) The PMNs also did not respond to budesonide until 16 h. Steroid only (□) had a marked effect on leukocytes and on PMNs throughout the experimental period. Values shown are the means, $n = 6$–12 in each condition; vertical lines indicate s.e.

1983; Hammerschmidt, 1983), but they are not essential (Glauser & Fairman, 1985). Neutrophils, for instance, are not the only blood cells involved in endotoxin-induced lung injury (Hinson *et al.*, 1983; Ekstrom *et al.*, 1986) and endotoxin can interact directly with endothelial cells (Meyrick *et al.*, 1986). However, although our model of endotoxin-induced lung injury utilized a less common mode of administration of endotoxin, the effects of endotoxin were comparable with those previously described.

The biochemical index of lung injury we chose to study in this model was the pharmacokinetics of PGE_2 in the isolated lung, which we have previously shown to be altered in conjunction with lung oedema induced by ANTU (Bakhle, 1982; Bakhle & Grantham, 1985; Minty *et al.*, 1987). We assessed PGE_2 pharmacokinetics mainly by the $t_{1/2}$ value as this is a relatively quick and easy variable to measure and has been shown to increase early in lung injury (Bakhle, 1982; Bakhle & Grantham, 1985). The $t_{1/2}$ value for PGE_2 increased soon after endotoxin almost in parallel with the leukopaenia but continued to increase well after the leukocyte count had passed its nadir, giving a maximum value at 16 h. Nevertheless, in our experiments, there was a clear correlation between the minimal PMN count (at 4 h) and maximal $t_{1/2}$ value at 16 h, suggesting a link between the two variables.

The $t_{1/2}$ values for sucrose and urea did not change at any time after endotoxin. A similar finding has been reported by Minty *et al.* (1987) with ANTU-induced lung injury and our present results showed that the changes in the $t_{1/2}$ values for PGE_2 were not simply due to changes in the extracellular space available to molecules of this size (mol. wt. = 350 daltons).

The increase in $t_{1/2}$ was accompanied by a decrease in metabolism of PGE_2 on a single passage through the isolated lung, as described earlier for ANTU-induced lung injury (Bakhle, 1982). Increased levels of prostaglandins in pulmonary venous blood after endotoxin *in vivo* have been demonstrated (Anderson *et al.*, 1975; Fletcher & Ramwell, 1977; Coker *et al.*, 1983) and these results have been interpreted as increased pulmonary synthesis of prostaglandins. They could equally be due to decreased inactivation of prostaglandins. The activity of the major prostaglandin metabolizing enzyme, prostaglandin dehydrogenase (PGDH), was reduced in homogenates of rat lung after endotoxin treatment *in vivo* (Nakano & Prancan, 1973) and in man, the metabolism of $PGF_{2\alpha}$ was depressed in septic shock (Oettinger *et al.*, 1983). Furthermore, our assays, per-

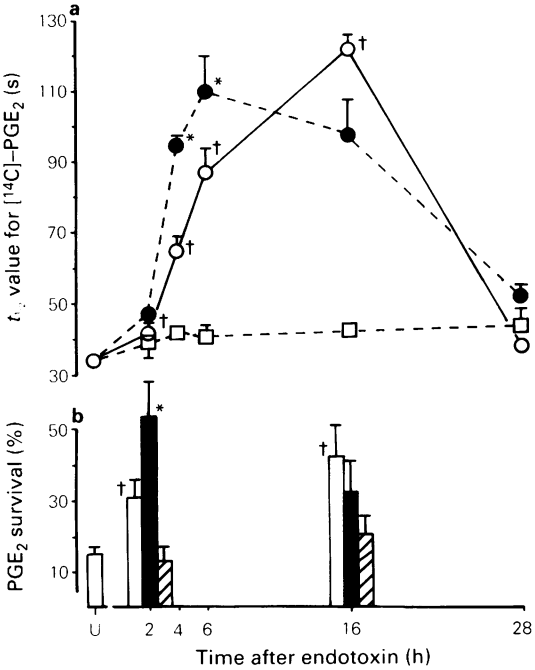

Figure 7 Effect of budesonide on the pharmacokinetics of prostaglandin E_2 (PGE_2) in rat isolated lung after endotoxin. Pretreatment with budesonide (●) did not prevent (a) the increase in $t_{1/2}$ caused by endotoxin (○) at any time, nor did it prevent (b) the increased survival of PGE_2 at either 2 or 16 h (solid columns). Treatment with steroid only did not affect either (a) the $t_{1/2}$ (□) or (b) the survival of PGE_2 (hatched columns). In (b) open columns represent the survival of PGE_2 after treatment with endotoxin only. Values shown are the means, $n = 4$–6 lungs in each condition; vertical lines indicate s.e.

formed in the presence of indomethacin to prevent prostaglandin output from the lung, would support the hypothesis that there is a deficiency in prostaglandin metabolizing activity in lung induced by endotoxin treatment. The early onset of this deficiency, 2 h after treatment, suggests that it may be related to the early leukopaenia which results from sequestration of activated leukocytes in the pulmonary circulation. The activity of PGDH is readily inhibited by a hyperoxic environment (Parkes & Eling, 1975; Klein *et al.*, 1978; Chaudhari *et al.*, 1979; Toivonen *et al.*, 1981) and the generation of oxygen-derived free radicals by activated leukocytes (Freeman & Crapo, 1982; Schraufstatter *et al.*, 1984) may similarly serve to inactivate PGDH.

The biochemical index correlated well in time with leukopaenia and gave an early indication of the oedema to follow. The purpose of using corticosteroids was to determine whether or not their effects on the physical signs (leukopaenia, lung oedema) were accompanied by comparable effects on the biochemical variables.

Both methylprednisolone and budesonide moderated the endotoxin-induced leukopaenia and lung oedema. Steroid effects on the leukopaenia were somewhat obscured by their intrinsic ability to lower the total leukocyte count (see 'steroid only' values) but, since endotoxin induced a greater leukopaenia, recovery of the leukocytes and PMN numbers towards the 'steroid only' value was taken as evidence of the protection by the steroid against this effect of endotoxin. Lung oedema (dry : wet weight ratios) was more clearly reversed by the steroids. Such antagonism of the effects of endotoxin are well known for methylprednisolone (Al-Kaisi *et al.*, 1977; Brigham *et al.*, 1981; Borg *et al.*, 1985b) and, although relatively less substantiated for budesonide (Ottosson *et al.*, 1986), it would be expected from budesonide's generally anti-inflammatory profile of activities (Kallstrom *et al.*, 1985).

The effects of the steroids on PGE_2 pharmacokinetics were not quite as we had expected. Methylprednisolone reversed the increases in $t_{1/2}$ after 4 h and the survival of PGE_2 at 16 h, although it did not affect the early increase in prostaglandin survival. Direct effects of methylprednisolone on PGE_2 pharmacokinetics were not as marked as its effects on the leukocytes. The relevant enzyme, PGDH, appears to turn over quite rapidly (Blackwell *et al.*, 1975) and it may thus be equally susceptible to the stimulation of protein synthesis associated with corticosteroids. This may be one mechanism by which PGE_2 metabolism is restored to normal by methylprednisolone after endotoxin treatment although, if the sequestration of activated leukocytes were prevented or reversed, there would also be less opportunity for oxidative attack on lung PGDH.

With budesonide pretreatment, practically no changes in PGE_2 pharmacokinetics after endotoxin were seen. The effects on leukocytes and lung oedema of this steroid were obvious comparatively late (after 16 h). If the process leading to changed PGE_2 pharmacokinetics are initiated early after endotoxin, then the failure of budesonide to normalize PGE_2 pharmacokinetics could reflect its slower onset of action, relative to methylprednisolone, or insufficient dosage rather than a fundamentally different mode of action. This possibility received some support from the experiments where budesonide was given at a higher dose than in most of the experiments. Under these conditions, $t_{1/2}$ values for PGE_2 following endotoxin treatment were improved, but not completely reversed, at 2, 4, and 6 h. Our choice of 1.2 mg kg^{-1} was based on the results of Kallstrom

et al. (1985), who used a different inflammatory stimulus in rat lung.

The crucial nature of the timing of steroid treatment was emphasized by the earlier onset but later loss of methylprednisolone's effects when this steroid was given in the same dose but one hour earlier, i.e. 30 min before endotoxin. From this limited series of experiments it seems that the steroids need to be at effective concentrations between 1 and 4 h after endotoxin, a period coincident with the onset of leukopenia. This would be compatible with our suggestion that the altered pharmacokinetics are related to the sequestration of activated leukocytes in lung and perhaps to their generation of oxygen-derived free radicals.

The clinical efficacy of steroids in treatment of ARDS (Schumer, 1976; 1981; Sibbald *et al.*, 1981), although not universally accepted (Blaisedell, 1981; Weigelt *et al.*, 1985), is also known to be related to the duration of the condition. The best results are achieved by large doses of steroid given as early as possible (Sprung *et al.*, 1984; Sibbald *et al.*, 1981; see also Hinshaw *et al.*, 1980; Borg *et al.*, 1985b). It is clearly not possible, given the present means of diagnosis of ARDS – abnormalities in chest X-rays or in blood gases – to identify and treat the 'pre-ARDS' patient as early as we can in our experimental study. One of the criteria in our search for a biochemical index was to identify a biochemical property that would change early in lung injury and that would correlate with the intensity of injury. Furthermore, this property should also respond to treatment as the injury itself.

In conclusion, we feel our results show that, in this model, the pharmacokinetics of PGE_2, assessed by the $t_{1/2}$ measurement, fulfil most of the requirements of a biochemical index of lung injury. The $t_{1/2}$ value changed early after endotoxin, its maximal change and recovery paralleled the oedema and clearly, with methylprednisolone, it responded as well as the oedema and leukocyte changes. The other advantages of PGE_2 pharmacokinetics are that the $t_{1/2}$ value is simply measured from assay of the total ^{14}C in lung effluent (or arterial blood *in vivo*) and that PGE_2 is pharmacologically relatively inactive in the pulmonary circulation or on blood cells, e.g. platelets or PMNs. However, there is evidence that PGE_2 pharmacokinetics may not be as suitable an index in other models of acute lung injury (Bakhle & Grantham, 1987).

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