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EFFECT OF INDOMETHACIN ON INCREASES IN LEUKOTRIENE B_4 AND PULMONARY EDEMA IN RESPONSE TO PHORBOL ESTER ADMINISTRATION IN DOGS

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Abstract—The administration of leukotrienes (LTs) into the pulmonary circulation results in edema formation and increased vascular permeability. We reported previously that the administration of phorbol myristate acetate (PMA, 20 μg/kg) to intact anesthetized dogs results in a reduction in circulating white blood cells as well as the development of pulmonary edema concomitant with the appearance of LTs in the lungs. In contrast, when a smaller dose of PMA (10 μg/kg) was administered, neither extravascular lung water nor LTs increased, although there was a similar reduction in circulating white blood cells. In the present study, we used a property of indomethacin, namely, its capacity to augment the formation of LTs, to examine further the relationship between LT generation and pulmonary edema formation in response to PMA administration. In intact pentobarbital-anesthetized dogs pretreated with saline ($N = 9$), the administration of PMA at a dose of 10 μg/kg, i.v., did not result in any change in extravascular lung water or in LTB_4 present in bronchoalveolar lavage fluid (BALF). In contrast, in six animals pretreated with indomethacin (5 mg/kg), the administration of this dose of PMA resulted in increases in both extravascular lung water ($P < 0.05$) and LTB_4 ($P < 0.05$) in BALF. These results provide support for the hypothesis that leukotrienes are requisite for PMA-induced increases in extravascular lung water.

Key words: leukotrienes; acute lung injury; thromboxane; cyclooxygenase; 5-lipoxygenase; PMA

Products of lipoxygenase-mediated arachidonic acid metabolism have been implicated as participants in the enhanced microvascular permeability of PMA†-induced acute lung injury [1–4]. We found, for example, that the administration of PMA (20–30 μg/kg, i.v.) to anesthetized dogs produces acute lung injury characterized not only by an increase in EVLW and a reduction in systemic arterial oxygen tension, but also by the identification of LTB_4 and LTD_4 in BALF, increased arterial concentrations of TXB_2, and a reduction in the number of circulating white blood cells [1, 3]. In contrast, when a smaller dose of PMA (10–15 μg/kg, i.v.) is given, neither EVLW nor leukotrienes in BALF increase, although reductions in the number of circulating white blood cells and increases in the arterial concentrations of TXB_2 are similar to those observed with the larger dose [3]. One interpretation of the latter results is that increased accumulation of EVLW has not occurred because the lipoxygenase pathway of arachidonic acid metabolism has not been activated by the smaller dose of PMA.

In support of the emerging hypothesis that products of 5-lipoxygenase-mediated arachidonic acid metabolism effect the increased EVLW accumulation consequent to PMA administration, we found that the administration of OKY-046, a thromboxane synthase inhibitor, to intact dogs at a dose 10-fold greater than that required to inhibit TX synthesis, prevents the increase in EVLW in response to PMA (20–30 μg/kg, i.v.) [4]. This result could not be attributed to the reduction in arterial TX concentration [4–6], as had been suggested previously [7], but is more likely attributable to a concomitant, but unanticipated, inhibition of lipoxygenase activity [4].

To explore further the possibility that products of lipoxygenase activity are requisite for the PMA-induced increase in EVLW, we took advantage of a less well-recognized property of the cyclooxygenase inhibitor, indomethacin, namely, its capacity to augment lipoxygenase activity. In the human neutrophil, stimulated with a calcium ionophore, indomethacin was reported to increase the formation of LTB_4 [8]. Similarly, in the mouse peritoneal macrophage, stimulated with phorbol ester, indomethacin shifted the arachidonic cascade from cyclooxygenase to lipoxygenase products [9]. Thus,

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† Abbreviations: PMA, phorbol myristate acetate; LT, leukotriene; EVLW, extravascular lung water; BALF, bronchoalveolar lavage fluid; TX, thromboxane; PAP, peak airway pressure; Ppa, mean pulmonary arterial pressure; Psa, mean systemic arterial pressure; ETV, extravascular thermal volume; Pla, mean left arterial pressure; CO, cardiac output; and HETE, hydroxy-eicosatetraenoic acid.

we postulated that, in the presence of indomethacin, the smaller dose of PMA (10–15 μg/kg, i.v.), which had been shown previously [3] to increase thromboxane synthesis in anesthetized dogs, but not leukotriene synthesis or EVLW, would increase lipoxygenase-mediated products of arachidonic acid metabolism, resulting, thereby, in increased lung water accumulation.

MATERIALS AND METHODS

Chemicals. Indomethacin (Sigma Chemical) was dissolved in 0.1 M Na_2CO_3 immediately before its administration. PMA (Sigma Chemical) was dissolved in DMSO as a 2 mg/mL stock solution and stored in aliquots at −80°. Immediately before its administration, the PMA stock solution was diluted with 15 mL of 0.9% NaCl (final concentration of DMSO < 1%).

Preparation of animals. Fifteen heartworm- and microfilaria-free, male, mongrel dogs (25–30 kg), fasted overnight but allowed free access to water, were anesthetized with intravenous pentobarbital sodium (30 mg/kg initial dose, followed by 0.05 $mg \cdot kg^{-1} \cdot min^{-1}$). Pancuronium bromide (0.1 mg/kg initial dose, followed by 0.04 $mg \cdot kg^{-1} \cdot min^{-1}$) was administered intravenously to achieve neuromuscular blockade. Animals were intubated and ventilated (Harvard ventilator) at a tidal volume of 15–18 mL/kg and a rate of 14–16 breaths/min. To prevent atelectasis, positive end-expiratory pressure of 5 cm H_2O was maintained throughout the experiment, and the lungs were hyperinflated to 20 cm H_2O every 10 min, except in the periods immediately before lung water measurements. Ventilatory rate and/or tidal volume were adjusted during the initial stabilization period to ensure that blood gas tensions were within the normal range and were not changed thereafter. PAP was measured throughout the experiment.

A Swan-Ganz catheter was advanced into the main pulmonary artery for continuous measurement of Ppa. A catheter was placed into the aorta via a femoral artery for measurement of Psa and for obtaining aliquots of blood for measurement of arterial blood gas tensions and for assay of TXB_2, the stable metabolite of TXA_2. Isotonic saline (0.9% NaCl) was infused throughout the experiment at 5–7 mL/min, i.v. A catheter was placed into the superior vena cava via a jugular vein for the administration of PMA and as an injection site for determination of cardiac output and ETV by an indicator-dilution technique. At thoracotomy, a catheter was placed into the left atrium via the left atrial appendage for continuous measurement of Pla. Core temperature of the animal was maintained between 37° and 39° by external warming. Vascular blood pressures were measured via catheters filled with heparinized saline attached to strain gauges (P23 ID, Statham) and were recorded on a polygraph (Grass).

Estimates of ETV, EVLW and cardiac output. ETV, an estimate of EVLW, was determined by the double indicator-dilution technique in the following manner. A 5-Fr catheter with attached thermistor (model 96020-5F, Edwards Laboratories) was placed into a femoral artery. A bolus of iced 5% dextrose containing 2 mg of indocyanine green dye was injected rapidly into the superior vena cava. ETV was quantified by a microprocessor-based system (9310 computer, Edwards Laboratories) that simultaneously compares the transit-times of an indicator confined to the vascular space (indocyanine green dye) with one that distributes throughout the entire thermal mass of the lung (iced 5% dextrose) [10]. Cardiac output was calculated by computer integration of the area under the thermal dilution curve. Final thermal-dye estimates of EVLW were confirmed by gravimetric measurements [11]. For gravimetric determination of EVLW, immediately following the final ETV determination, the pulmonary hila, pulmonary artery, and aorta were clamped and the lungs were removed from the chest. Each lung was weighed and then homogenized at 4° with the addition of 500 mL of saline. The homogenates and an aliquot of arterial blood were weighed and dried to constant weight. Hemoglobin concentrations in the blood and homogenate were determined by the cyanmethemoglobin technique [11]. Gravimetric determinations of EVLW were corrected for residual blood volume and for the amount of BALF that was instilled but not recovered.

Bronchoalveolar lavage. Bronchoalveolar lavage was performed before and 60 min after PMA administration via a fiberoptic bronchoscope (Olympus BF, model B3) inserted through the endotracheal tube during continuous ventilation of the animals. The tip of the bronchoscope was wedged into a bronchus in the lower lobe of one lung, and the segment isolated was lavaged with an aliquot of heparinized 0.9% NaCl. After PMA administration, the procedure was repeated in the contralateral lung. BALF was collected for determination of the concentration of LTB_4 as described below. The amount of lavage fluid instilled in the saline and the indomethacin groups was 23 ± 2 and 22 ± 2 mL, respectively, before PMA, and 27 ± 3 and 22 ± 2 mL, respectively, after PMA. The percentage of instilled fluid recovered did not differ between groups and was 60 ± 4% in the saline-pretreated animals and 66 ± 4% in the animals that received indomethacin prior to PMA.

Measurement of immunoreactive TXB_2 and LTB_4. Concentrations of TXB_2 in blood and LTB_4 in BALF were measured as described previously [3]. Briefly, blood samples were collected in plastic syringes containing indomethacin (5 μg/mL) and EDTA (1 mg/mL) and were placed on ice. Blood was centrifuged at 1800 *g* for 20 min at 4°. The plasma was removed and stored at −30° until the assay was performed. For radioimmunoassay of TXB_2, 0.1 mL of the sample was incubated with 0.1 mL tracer (1.6 nCi of [^{3}H]TXB_2, Du Pont-New England Nuclear) and 0.1 mL of antiserum selective for TXB_2 (Advanced Magnetics). Antiserum to TXB_2 cross-reacts 100% with TXB_2 and <0.1% with 6-keto-prostaglandin $F_{1\alpha}$, other prostaglandins and the monohydroxy-6,8,11,14-eicosatetraenoic acids. TXB_2 concentrations were corrected for hematocrit and values are expressed as nanograms per milliliter of blood. The lower limit of detectability for the TXB_2 assay is 27 pg/mL blood.

For determination of LTB_4 concentrations, recovered lavage fluid was placed on ice and diluted with ice-cold absolute methanol to a final concentration of 80% methanol (by vol.) and stored at −20° for 12–14 hr. Precipitated proteins were removed by centrifugation at 1800 *g* for 30 min at −15°, and the supernatant was reduced under vacuum at 30°. The sample was then diluted with 0.01 M sodium phosphate buffer: methanol (80:20, v/v) and passed through a C_{18} SEP-PAK column (Waters). The SEP-PAK column was washed with the phosphate buffer: methanol, and the sample was eluted with 80% methanol. After evaporation to dryness in a vacuum centrifuge (Savant), the residue was dissolved in 0.5 mL H_2O, adjusted to pH 5.7 with 1% acetic acid, and diluted to 1 mL with methanol. LTB_4 was separated by reverse-phase HPLC with an isocratic solvent system of methanol: water: acetic acid (65:35:0.01) on a Nucleosil C_{18}, 5 μm, 4.6 × 250 mm column. The eluate corresponding to the retention time of authentic LTB_4 was collected and evaporated to dryness in a vacuum centrifuge and subjected to radioimmunoassay. Cross-reactivities for LTB_4 antiserum (Advanced Magnetics) were 100% for LTB_4 and <1.0% for LTC_4, LTD_4, LTE_4, 5-, 12-, and 15-HETE, prostaglandins and thromboxane. Each sample was assayed in duplicate. LTB_4 determined by radioimmunoassay is expressed as nanograms per lavage. This value was obtained by determining the concentration of LTB_4 in the total volume of lavage fluid recovered and correcting this value for the amount of lavage fluid instilled [3]. The lower limit of detectability for the LTB_4 assay is 20 pg/mL lavage fluid. The recovery of radiolabeled LTB_4 added to lavage fluid is 66 ± 1%.

Experimental protocols. A minimum of 30 min was allowed for stabilization after completion of surgical procedures before baseline (pre-indomethacin or pre-saline) measurements of CO and ETV were made. Arterial blood was obtained for determination of blood gases and TXB_2 concentration. Following baseline measurements, dogs received either indomethacin (5 mg/kg, i.v., N = 6) or saline (0.9% NaCl, N = 9). Thirty minutes after the administration of indomethacin or saline, samples of arterial blood were collected and bronchoalveolar lavage was performed. A minimum of 30 min was allowed after bronchoalveolar lavage for hemodynamic and blood gas stability before PMA was administered. All animals received 10 μg/kg of PMA administered over 5 min via a catheter inserted into a jugular vein. Sixty minutes after PMA administration, measurements of CO and ETV were made, and samples of arterial blood were obtained for measurement of blood gases and TXB_2 concentration. Bronchoalveolar lavage was then performed in the contralateral lung. In six experiments in each group, the lungs were removed for gravimetric determination of EVLW.

Statistical analysis. Statistical significance between control and experimental periods was determined with analysis of variance [12]. If the *F* ratio indicated differences among the groups, a Least Significant Difference (LSD) test was performed to identify individual differences. The comparison between the two methods used to measure extravascular lung water after PMA administration was made using Student's *t*-test for paired data. P values of <0.05 were considered statistically significant. Results are reported as means ± SEM.

RESULTS

Effects of the administration of indomethacin or saline and of bronchoalveolar lavage. Neither indomethacin (5 mg/kg) nor saline had any effect on Ppa, Pla, CO, ETV, PAP or arterial oxygen tension (Table 1). In addition, bronchoalveolar lavage, performed before the administration of PMA, did not result in any change in hemodynamic parameters, ETV, PAP or arterial oxygen tension (Table 2). With the exception of the fact that Psa was slightly greater after indomethacin (Tables 1 and 2), the indomethacin and saline groups did not differ before the administration of PMA.

Effects of PMA in the absence of indomethacin. In the animals receiving saline in lieu of indomethacin, PMA administration resulted in significant increases in Ppa and PAP (Table 3). Other hemodynamic parameters, as well as arterial oxygen tension (Table 3) and ETV (Fig. 1), were not altered by PMA administration in these animals. TXB_2 was increased in arterial blood 60 min after PMA administration, indicating that arachidonic acid had been liberated and subsequently metabolized by the cyclooxygenase pathway (Fig. 2). In contrast, LTB_4, measured in BALF, was not increased following PMA administration in the animals pretreated with saline (Fig. 2).

Effects of PMA in the presence of indomethacin. The administration of indomethacin (5 mg/kg) prevented the PMA-induced increase in TXB_2 observed in the saline-pretreated group (Fig. 2), but did not prevent the increase in Ppa (Table 3). In fact, the increase in Ppa was greater in the indomethacin-pretreated group than in the group that had received saline before the administration of PMA (Table 3). In addition, arterial oxygen tension decreased after PMA in the group pretreated with indomethacin. In contrast to the saline-pretreatment group, in animals that received indomethacin, PMA administration resulted in a significant increase in ETV (Fig. 1). In six experiments in each group, estimates of lung water determined by the thermal-indicator technique (ETV) were confirmed by a gravimetric technique (Table 4). The increase in extravascular lung water in the indomethacin group was associated with a 6-fold increase in LTB_4 in BALF (Fig. 2). Sixty minutes after PMA administration, values for LTB_4 in BALF and ETV in the indomethacin group were significantly greater than those in the saline group. Thus, in the presence of indomethacin, this dose of PMA was associated with the development of pulmonary edema and a concomitant increase in a product of 5-lipoxygenase activity, i.e. LTB_4, in the lung.

DISCUSSION

In intact anesthetized dogs, the intravenous

Table 1. Hemodynamic variables, extravascular thermal volume, peak airway pressure and arterial oxygen tension before and after the administration of either saline or indomethacin (5 mg/kg) in anesthetized dogs

Variable	Saline (N = 9)		Indomethacin (N = 6)	
	Before	After	Before	After
Ppa (mm Hg)	13 ± 1	13 ± 1	13 ± 1	16 ± 2
Pla (mm Hg)	2.4 ± 0.5	2.5 ± 0.4	2.3 ± 0.4	2.3 ± 0.4
Psa (mm Hg)	127 ± 4	126 ± 4	138 ± 7	147 ± 7
CO (L/min)	2.8 ± 0.3	2.8 ± 0.3	3.2 ± 0.3	2.3 ± 0.2
ETV (mL/kg)	6.7 ± 0.4	7.0 ± 0.5	6.2 ± 0.3	6.6 ± 0.6
PAP (mm Hg)	7.5 ± 0.2	7.8 ± 0.2	7.4 ± 0.5	7.3 ± 0.4
PO_2 (mm Hg)	87 ± 2	85 ± 2	85 ± 3	84 ± 2

Values are means ± SEM. Abbreviations: Ppa, mean pulmonary arterial pressure; Pla, mean left atrial pressure; Psa, mean systemic arterial pressure; CO, cardiac output; ETV, extravascular thermal volume; PAP, peak airway pressure; and PO_2, arterial oxygen tension.

Table 2. Hemodynamic variables, extravascular thermal volume, peak airway pressure and arterial oxygen tension before and after bronchoalveolar lavage in anesthetized dogs

Variable	Saline (N = 9)		Indomethacin (N = 6)	
	Before	After	Before	After
Ppa (mm Hg)	13 ± 1	14 ± 1	16 ± 2	16 ± 2
Pla (mm Hg)	2.5 ± 0.4	2.9 ± 0.5	2.3 ± 0.4	2.6 ± 0.9
Psa (mm Hg)	126 ± 3	134 ± 4	147 ± 7	147 ± 7
CO (L/min)	2.8 ± 0.3	2.5 ± 0.2	2.3 ± 0.3	2.0 ± 0.2
ETV (mL/kg)	7.0 ± 0.5	7.1 ± 0.4	6.6 ± 0.6	6.9 ± 0.6
PAP (mm Hg)	7.8 ± 0.2	7.5 ± 0.2	7.3 ± 0.4	7.2 ± 0.3
PO_2 (mm Hg)	85 ± 2	82 ± 2	84 ± 2	83 ± 3

Values are means ± SEM. See Table 1 for definitions of abbreviations.

Table 3. Hemodynamic variables, peak airway pressure and arterial oxygen tension before and after administration of PMA (10 μg/kg) in anesthetized dogs pretreated with either saline or indomethacin

Variable	Saline (N = 9)		Indomethacin (N = 6)	
	Before	After	Before	After
Ppa (mm Hg)	14 ± 1	23 ± 3*	16 ± 2	38 ± 2*,†
Pla (mm Hg)	2.9 ± 0.5	3.9 ± 0.7	2.6 ± 0.9	2.6 ± 0.9
Psa (mm Hg)	134 ± 4	122 ± 4	147 ± 7	137 ± 7
CO (L/min)	2.5 ± 0.2	2.5 ± 0.2	2.0 ± 0.2	2.1 ± 0.3
PAP (mm Hg)	7.6 ± 0.3	8.7 ± 0.4*	7.2 ± 0.3	8.8 ± 0.4*
PO_2 (mm Hg)	82 ± 2	81 ± 4	83 ± 3	65 ± 6*,†

Values are means ± SEM. See Table 1 for definitions of abbreviations.
* $P < 0.05$ compared with "Before" values.
† $P < 0.05$ compared with "After" values in the saline-pretreated group.

administration of PMA is associated with increased production of products of arachidonic acid metabolism [1–4]. Previously we reported that, when PMA was administered at a dose of 20–30 μg/kg, the resulting lung injury was associated with increases in products of both cyclooxygenase- and 5-lipoxygenase-mediated arachidonic acid metabolism [3, 5]. The proposed roles of the cyclooxygenase products as participants in the altered hemodynamics and microvascular permeability that occur in this model of acute lung injury were called into question by the finding that inhibition of cyclooxygenase activity does not prevent either the pulmonary hypertension [5] or the pulmonary edema [6] produced by PMA administration. In addition, we found that a smaller dose of PMA, namely, 10–

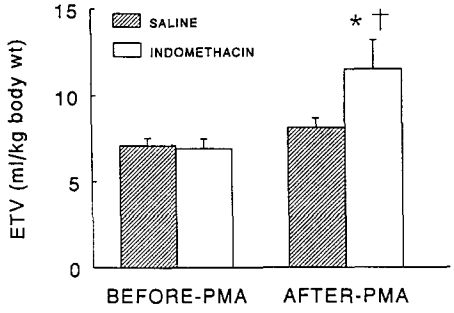

Fig. 1. Extravascular thermal volume (ETV) before and 60 min after the administration of 10 μg/kg PMA into the superior vena cava. Values (means ± SEM) were obtained after pretreatment with either 0.9% NaCl (N = 9) or indomethacin (5 mg/kg, N = 6). Key: (*) $P < 0.05$ compared with the respective value before PMA administration, and (†) $P < 0.05$ compared with the saline group after PMA administration.

15 μg/kg, is associated with increases in pulmonary arterial pressure, but not with the development of pulmonary edema [3]. Moreover, in the latter experiments, no increase in products of 5-lipoxygenase activity was detected in the lung. Thus, leukotrienes in the lung were found to be increased in association with a dose of PMA that resulted in the development of pulmonary edema. Although

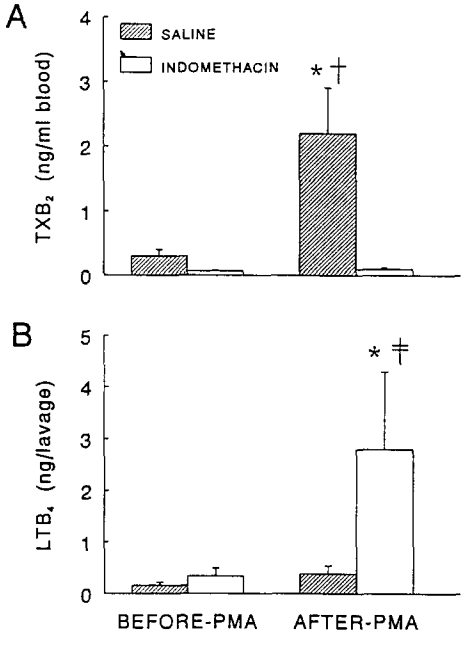

Fig. 2. Concentrations of TXB_2 in arterial blood (A) and LTB_4 in bronchoalveolar lavage fluid (B) before and 60 min after the administration of 10 μg/kg PMA into the superior vena cava. Values (means ± SEM) were obtained after pretreatment with either 0.9% NaCl (N = 9) or indomethacin (5 mg/kg, N = 6). Key: (*) $P < 0.05$ compared with the respective value before PMA administration, (†) $P < 0.05$ compared with the indomethacin group after PMA administration; and (‡) $P < 0.05$ compared with the saline group after PMA administration.

Table 4. Confirmation of double-indicator dilution method (thermal dye) of measurement of extravascular lung water by a gravimetric method 60 min after PMA administration in anesthetized dogs

Pretreatment	Extravascular lung water (mL/kg body wt)	
	Thermal-dye method	Gravimetric method
Saline (N = 6)	8.0 ± 0.4	7.3 ± 0.4
Indomethacin (N = 6)	11.5 ± 1.7*	14.4 ± 2.9*

Values are means ± SEM.
* $P < 0.05$ compared with the saline-pretreatment group.

the finding that increases in EVLW occurred solely when leukotrienes were present supports the hypothesis that these arachidonic acid metabolites contribute to the edema formation of PMA-induced acute lung injury, final establishment of a causal relationship demands a more comprehensive examination.

The results of several studies suggested that inhibitors of either the synthesis or the action of leukotrienes decrease or prevent the formation of pulmonary edema in models of acute lung injury [4, 13–16]. In intact pigs, the LT receptor antagonist LY 203647 prevented the development of *Escherichia coli* lipopolysaccharide-induced pulmonary edema [16]. In addition to studies in intact animals, it was reported that products of 5-lipoxygenase activity are increased in isolated perfused rabbit lungs when either *Pseudomonas aeruginosa* cytotoxin [14] or *E. coli* hemolysin is administered [15]. Moreover, pretreatment with the lipoxygenase inhibitor AA 861 resulted in attenuation of *P. aeruginosa* cytotoxin-induced increases in both edema formation and concentrations of 5-lipoxygenase metabolites [14]. Recently, it was reported that the thromboxane synthase inhibitor OKY-046, when administered at a dose 10-fold in excess of that required to inhibit thromboxane formation, prevents both the increase in EVLW and the increases in amounts of LTB_4 measured in BALF in response to the administration of PMA (20 μg/kg) in intact anesthetized dogs [4]. The results of the aforementioned studies, coupled with the reports that the lung is capable of producing products of 5-lipoxygenase activity [17, 18] and that both the sulfidopeptide leukotrienes [18–20] and LTB_4 [21, 22] are capable of enhancing vascular permeability, provide additional support for the hypothesis that these metabolites of arachidonic acid contribute to the development of pulmonary edema associated with PMA-induced acute lung injury.

In the present work, the effect of indomethacin on products of cyclooxygenase (TXB_2) and 5-lipoxygenase (LTB_4) activity, as well as its effect on the accumulation of EVLW in anesthetized dogs treated with PMA, was examined. Although indomethacin is clearly an inhibitor of cyclooxygenase activity, its administration may, under some experimental conditions, also result in increased formation of products of 5-lipoxygenase activity [8, 9]. In human neutrophils stimulated with

the calcium ionophore A23187, indomethacin administration was reported to result in a 300% increase in the formation of LTB_4, as well as smaller increments in the concentrations of 5-, 12- and 15-HETE [8]. This effect of indomethacin was not shared by either acetylsalicylic acid or ibuprofen. This latter result suggests that the indomethacin-induced increase in LTB_4 involved more than simple redirection of substrate from the cyclooxygenase to the 5-lipoxygenase pathway of arachidonic acid metabolism. A similar result, i.e. increased production of a product of 5-lipoxygenase activity in the presence of indomethacin, was reported to occur in mouse peritoneal macrophages stimulated with zymosan or a combination of phorbol ester and calcium ionophore [9]. That indomethacin-induced stimulation of the formation of 5-lipoxygenase-mediated metabolites of arachidonic acid can occur in the lung was shown by the report that indomethacin pretreatment caused an increase in total amounts of sulfidopeptide leukotrienes produced in response to antigen stimulation of sensitized guinea pig trachea [23].

The results presented here demonstrate that indomethacin is capable of altering the profile of arachidonic acid products that are formed in response to the administration of PMA in intact dogs. In the absence of indomethacin, the dose of PMA administered in this study (10 μg/kg) resulted in pulmonary hypertension and an increase in TXB_2 concentration in arterial blood. The latter finding indicates that, in response to the administration of this dose of PMA, arachidonic acid is released and is metabolized via cyclooxygenase and thromboxane synthase to form TXA_2. That there was no increase in EVLW in this experimental group suggests that neither the PMA-induced release of arachidonic acid nor the formation of thromboxane is responsible for the pulmonary edema associated with PMA administration.

The administration of indomethacin, in the absence of PMA, resulted in no change in either pulmonary arterial pressure or EVLW. However, the response to PMA was altered dramatically in the presence of indomethacin. The PMA-induced increase in pulmonary arterial pressure was greater than that in the saline-pretreated group despite the indomethacin-associated inhibition of the thromboxane formation. More importantly, in the presence of indomethacin, PMA administration resulted in an increase in EVLW as determined by two independent measurement techniques. This pulmonary edema formation was associated with increased amounts of LTB_4 in the lung. One interpretation of these findings is that, in the presence of indomethacin, arachidonic acid released in response to PMA administration was metabolized via 5-lipoxygenase to form LTB_4. Moreover, these results support the hypothesis that products of 5-lipoxygenase activity are responsible, at least in part, for the edema formation that occurs in PMA-induced acute lung injury. In addition, the finding that inhibition of thromboxane synthesis by indomethacin did not prevent the PMA-induced increase in pulmonary arterial pressure suggests that this metabolite of arachidonic acid is not responsible for the associated pulmonary hypertension.

In summary, the results of the present work provide support for the hypothesis that products of arachidonic acid metabolism produced via the action of 5-lipoxygenase, but not via the action of cyclooxygenase, contribute to the pulmonary edema formation associated with the administration of PMA in intact anesthetized dogs. In addition, the finding that indomethacin pretreatment resulted in the enhanced formation of a product of 5-lipoxygenase activity in response to the administration of PMA demands that care be exercised in the interpretation of studies in which indomethacin is used to prevent the formation of products of cyclooxygenase-mediated metabolism of arachidonic acid in experimental models of acute lung injury.

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