Eicosanoid balance and perfusion redistribution of oleic acid-induced acute lung injury

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STEPHENSON, ALAN H., ANDREW J. LONIGRO, SANDRA W. HOLMBERG, AND DANIEL P. SCHUSTER. *Eicosanoid balance and perfusion redistribution of oleic acid-induced acute lung injury.* J. Appl. Physiol. 73(5): 2126–2134, 1992.—We have proposed that endogenous prostacyclin opposes the vasoconstriction responsible for redistribution of regional pulmonary blood flow (rPBF) away from areas of increased regional lung water concentration (rLWC) in canine oleic acid- (OA) induced acute lung injury (D. P. Schuster and J. Haller. *J. Appl. Physiol.* 69: 353–361, 1990). To test this hypothesis, we related regional lung tissue concentrations of 6-ketoprostaglandin (PG) $F_{1\alpha}$ and thromboxane (Tx) B_2 in tissue samples obtained 2.5 h after administration of OA (0.08 ml/kg iv) to rPBF and rLWC measured by positron emission tomography. After OA only ($n = 16$), rLWC increased in dependent lung regions. Some animals responded to increased rLWC by redistribution of rPBF away from the most edematous regions (OA-R, $n = 6$), whereas others did not (OA-NR, $n = 10$). In another six animals, meclofenamate was administered after OA (OA-meclo). After OA, tissue concentrations of 6-keto-$PGF_{1\alpha}$ were greater than TxB_2 in all groups, but concentrations of 6-keto-$PGF_{1\alpha}$ were not different between OA-R and OA-NR animals. TxB_2 was increased in the dependent regions of animals in both OA-R and OA-NR groups compared with controls (no OA, $n = 4$, $P < 0.05$). The tissue TxB_2/6-keto-$PGF_{1\alpha}$ ratio was smaller in controls and OA-NR in which no perfusion redistribution occurred than in OA-R and OA-meclo in which it did occur. This TxB_2/6-keto-$PGF_{1\alpha}$ ratio correlated significantly with the magnitude of perfusion redistribution. These results suggest that in this model meclofenamate results in perfusion redistribution primarily by inhibition of prostacyclin synthesis, whereas spontaneous redistribution is principally the result of increases in Tx synthesis.

positron emission tomography; pulmonary edema; pulmonary circulation; thromboxane; prostacyclin; dogs

BLOOD FLOW REDISTRIBUTION away from edematous lung regions is common in acute lung injury. Although the impact of perfusion redistribution on the accumulation or resolution of pulmonary edema still remains uncertain, the mechanisms responsible for this phenomenon probably include some combination of regional vasoconstriction, vascular obstruction, or mechanical compression. In the canine oleic acid (OA) model of acute lung injury, recent studies by our group have cast doubt on the importance of either hypoxic vasoconstriction or vascular compression by edema as the principal mechanism for perfusion redistribution in this model (21, 30). Indeed, significant perfusion redistribution only occurs in ~50% of dogs after OA administration, despite equivalent degrees of pulmonary edema (21). However, other recent data have suggested a possible role for eicosanoids as determinants of regional vascular reactivity (1, 13, 19, 21, 27, 28); i.e., treatment with inhibitors of arachidonic acid cyclooxygenase activity promptly results in redistribution of regional pulmonary blood flow (rPBF) away from edematous regions. This redistribution of rPBF is associated with significant improvement in oxygenation, regardless of whether spontaneous redistribution had already occurred (21). These observations, then, suggest that at least some vessels within the injured lung regions remain vasoactive, but they do not indicate whether a similar mechanism is responsible for spontaneous perfusion redistribution.

In the present investigation, we attempted to determine what role, if any, endogenously synthesized prostacyclin, a pulmonary vasodilator prostaglandin (PG), or thromboxane (Tx) A_2, a potent pulmonary vasoconstrictor, might play in the spontaneous perfusion redistribution of OA-induced acute lung injury. We have related positron emission tomography (PET) measurements of rPBF with blood and tissue concentrations of TxB_2, the stable metabolite of TxA_2, and 6-keto-$PGF_{1\alpha}$, a stable metabolite of prostacyclin. Both TxA_2 and 6-keto-$PGF_{1\alpha}$ are products of cyclooxygenase-mediated arachidonic acid metabolism. The data confirm the importance of eicosanoid balance in OA-induced acute lung injury as a major determinant of rPBF, both in the case of spontaneous redistribution and after cyclooxygenase inhibition.

MATERIALS AND METHODS

Animal preparation. Twenty-six healthy adult mongrel dogs weighing between 24 and 30 kg were anesthetized with pentobarbital sodium (25–30 mg/kg), paralyzed with pancuronium bromide (4 mg), intubated with a cuffed endotracheal tube, and ventilated (fractional inspired oxygen content = 1.0) with the use of a Harvard pump ventilator at a tidal volume of 15 ml/kg and a respiratory rate adjusted to achieve a normal arterial P_{CO_2}. Additional barbiturate and muscle relaxant were administered as necessary.

Through bilateral femoral incisions, a balloon-tipped

pulmonary artery catheter and a 100-cm 6.3-Fr pig-tailed catheter were positioned in the pulmonary artery under fluoroscopic visualization. A 7-cm 18-gauge catheter was inserted into the femoral artery for blood sampling; a 5-cm 20-gauge catheter was placed into the external jugular vein for drug and radionuclide administration. Catheter patency was maintained by periodic flushing with heparinized saline (1 U/ml).

Cardiac output (CO) was measured in triplicate by the thermodilution technique with an Edwards Laboratories CO computer. Transducers (Gould P50) were calibrated to the center of the lateral chest and connected to a Mennen model 742 monitor for pulmonary arterial (Ppa), pulmonary wedge, and systemic arterial pressure recordings. Blood gases were analyzed with an Instrumentation Laboratories model 813 blood gas analyzer.

PET techniques. All PET measurements were performed with a PETT VI system. Design features, methods for calibration, corrections for activity decay, and corrections for photon attenuation have been discussed elsewhere (15, 23–25).

The animal was placed in the scanner in a supine position with the most caudal PET slice 1–2 cm below the level of the dome of the diaphragm. Data were recorded simultaneously from seven slices with a center-to-center separation of 1.44 cm, an effective slice thickness of 1.39 cm at the center, and an in-plane full-width half-maximum resolution of 1.17 cm.

Our methods for measuring rPBF and lung water concentration (LWC), including microsphere and gravimetric validation studies, have been described in detail previously (15, 23–25). In general, PET is used to measure the tissue concentration and distribution of a positron-emitting radionuclide. In these studies, the radionuclide is $H_2{}^{15}O$. The tissue activity data measured with PET, when combined with blood activity data (used as a reference) and analyzed with an appropriate mathematical model, yield tomographic images representative of PBF or LWC (20).

There are two periods of radioactivity data collection after $H_2{}^{15}O$ administration. A 15-s scan is performed during a 20-s constant intravenous infusion of $H_2{}^{15}O$ to measure the initial distribution of the tracer, which is related to rPBF. Four minutes later (to allow $H_2{}^{15}O$ to equilibrate between blood and extravascular lung tissue), a 300-s data collection is started. Data from this latter scan are used to determine the apparent regional partition coefficient for the tracer (necessary for the calculation of PBF) and to measure regional LWC. During both phases, blood samples are drawn and their radioactivity is determined. The counts in whole blood serve as a reference to convert PET tissue activity measurements into a physiological measurement (PBF or LWC).

Sample collection and processing. Blood samples for measurement of immunoreactive TxB_2 and 6-keto-$PGF_{1\alpha}$ were collected in plastic syringes containing indomethacin (5 μg/ml) and EDTA (1 mg/ml), as reported previously (29). The blood was kept on ice and centrifuged at 1,800 *g* at 4°C for 20 min. The plasma was removed and stored frozen at −30°C until assay. Lung tissue samples were collected and prepared according to the method of Westcott et al. (31). Samples weighing 2.5 ± 0.1 g were collected from dorsal and ventral regions of the right lower (caudal) lobe and the ventral and dorsal regions of the right middle lobe. These locations corresponded to the ventral, middle, and dorsal regions of the slice analyzed on the PET images. Immediately after excision, the regional tissue samples were placed in 40 ml ice-cold methanol and homogenized with a Polytron homogenizer (Brinkman). The tissue homogenate was then cooled to −30°C. Precipitated proteins were removed from the homogenate by centrifugation at 1,800 *g* for 30 min at −15°C. The clear methanolic extract was evaporated to dryness under vacuum at 30°C in a Speed-Vac concentrator (Savant). Samples for eicosanoid assay were solubilized in phosphate buffer (0.1 M, pH 7.4), containing 0.4 M NaCl, 10^{-3} M EDTA, 0.1% bovine serum albumin, and 0.01% sodium azide (assay buffer).

Measurement of immunoreactive TxB_2 and 6-keto-$PGF_{1\alpha}$. Enzyme immunoassay (EIA) of 6-keto-$PGF_{1\alpha}$ and TxB_2 was performed in 96-well microtiter plates precoated with 2 μg/well goat anti-rabbit immunoglobulin G, as described previously (17, 29, 31). Before use, the plates were washed with 10^{-2} M phosphate buffer (pH 7.4) containing 0.05% Tween 20 (wash buffer). The assay was performed in a total volume of 150 μl. In brief, 50 μl of acetylcholinesterase-conjugated eicosanoid tracer (Caymen), 50 μl of antiserum directed against 6-keto-$PGF_{1\alpha}$ or TxB_2 (Advanced Magnetics), and 50 μl of a standard or sample in assay buffer were combined and incubated at 25°C for 18–20 h. After the plates were washed three times with wash buffer, Ellman's reagent (200 μl) was dispensed into each well. Absorbance was recorded at 412 nm in a microtiter plate spectrophotometer (Biotech) when the absorbance for the well containing the "0" standard (B_o) exceeded 0.200 absorbance units. Each sample was assayed in duplicate. A standard curve was generated for each assay. Sample eicosanoid concentrations were determined by comparison to a log-logit transformation of the standard curve as described previously (17, 29). Eicosanoid concentrations were expressed as either picograms per milliliter of blood or nanograms per gram of dried lung tissue.

Experimental protocols. First, a blank ("background") scan was obtained. Then, for these studies, a data set consisted of *1*) a transmission scan, used to correct for photon attenuation during emission scans and for the placement of regions of interest for later image analysis (see below); *2*) a 15-s scan (used for the PBF measurement) obtained during a continuous infusion of $H_2{}^{15}O$ (~60–80 mCi); *3*) a 300-s scan obtained after equilibration of the $H_2{}^{15}O$ (for measurement of LWC and the apparent blood-tissue partition coefficient for water); and *4*) Ppa, pulmonary wedge and systemic arterial pressures, CO, and blood gas analysis data.

We studied two experimental groups. In sixteen animals (OA only), a baseline data set was obtained, followed by a right atrial injection of 0.08 ml/kg of OA in three equal boluses, 1 min apart. After each bolus, the catheter was flushed with saline. Approximately 120 min later, a second data set was obtained. In six animals (OA-meclo), the same procedure was performed (except the second data set was obtained ~ 90 min after OA administration), and then meclofenamate (2 mg/kg) was given

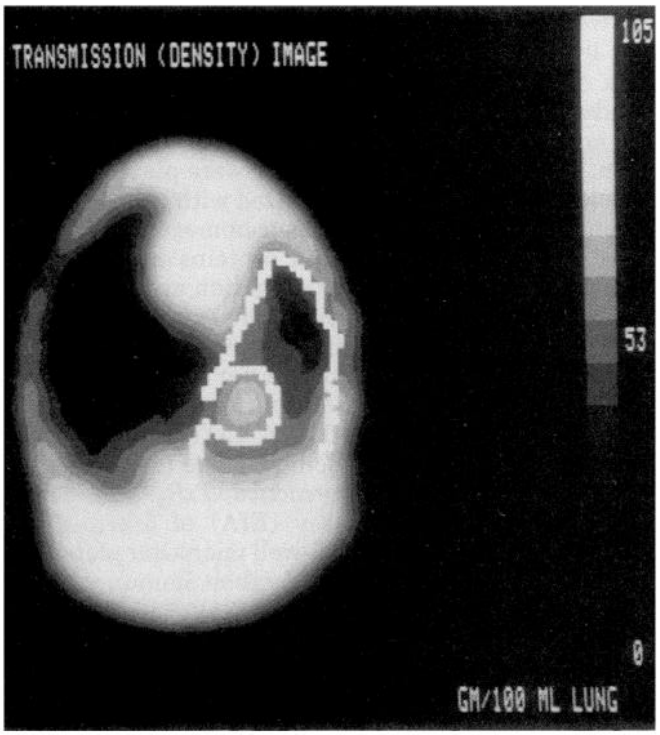

FIG. 1. Transverse tomographic lung density image from 1 animal. Density scale in g/100 ml lung. Animal is supine, and right side of image is right side of animal.

intravenously. After another ~30 min, a third data set was obtained.

After the final data set was obtained in all animals, a right thoracotomy was performed, and the right middle and lower lobes were exposed. The vascular pedicle to each lobe was clamped to prevent KCl from entering the pulmonary or bronchial circulation as the animal was killed with an intravenous infusion of saturated KCl. The lobes were then removed, and lung tissue samples were obtained from areas corresponding to dorsal and ventral regions on the corresponding PET images. In four animals, the same procedure was performed in all respects, except that saline was administered instead of OA and no PET data were obtained. These animals served as the control group for the biochemistry data. Because we have shown previously that no significant change in rPBF or LWC occurs over a similar time period in this animal preparation (15, 24, 25), we used the PET data from four other animals (historic data) in the PET data analysis for the control group only.

Data analysis. From each dog, the single tomographic slice with the most lung was analyzed from the seven slices reconstructed during each scan. Invariably, this was a caudal slice near the dome of the diaphragm. Postmortem examination of dogs in previous experiments has shown that this slice always represents tissue overlapping the middle and caudal lobes. A region of interest from the right lung was defined on the transmission scan, as shown in Fig. 1. We refer to this region of interest as a "hemislice."

The position of each region was kept in computer memory, and mean values for each region were obtained for all PET measurements performed. PBF was measured as milliliters per minute per 100 ml of lung. To normalize the PBF data for changes in CO, PBF in each picture element (pixel) was expressed as a fraction of the total blood flow to the hemislice (frPBF). To evaluate the relationship of PBF to LWC within a hemislice, the image "x"- and "y"-coordinates, along with the respective frPBF and LWC values for each pixel, were recorded.

With the use of the Statistical Analysis System (SAS, Cary, NC), the pixel data were then sorted, first by their y-coordinate. Next, within each value for y, the data were sorted again by their x-coordinate. The result was a listing of the pixels by location, beginning in the most ventrolateral portion of the region and ending with the most dorsomedial portion of the region. Each hemislice region contained ~400–500 pixels. We arbitrarily chose to divide the data into 20 "bins" so that each bin contained ~20–25 pixels worth of data, which could then be averaged. With the number of bins per dog kept constant, bin values could be averaged across dogs, allowing comparisons between experimental groups. The bins were ordered from 1 to 20 such that *bin 1* always contained one-twentieth of the total number of pixels for the region, beginning with those in the most ventrolateral portion of the region. *Bins 1–5* always come from ventral portions of the region of interest, *bins 6–15* from midportions, and *bins 16–20* from dorsal portions. Once the bins were defined by their constituent pixels, the frPBF and LWC values for all pixels within the bin were averaged, recorded, and plotted.

From these data, we calculated a "perfusion redistribution index" (PRI) for each dog by subtracting values of frPBF from like-numbered bins between interventions. For instance, if the value for frPBF from *bin 20* (the most dorsal left-hand bin within the region) was 0.08 before OA and was 0.05 after OA, the difference would be 0.03. In this hypothetical case, the fact that frPBF decreased in the bin indicates perfusion redistribution. We then summed all differences between bin values that were positive (i.e., those bins indicating perfusion redistribution) and took the sum as the PRI.

Statistical analysis. Data are presented as means ± SD. Statistical significance was determined with a repeated measures analysis of variance. For analysis of TxB_2/6-keto-$PGF_{1\alpha}$ data, the data were analyzed following a logarithmic transformation that resulted in a normal distribution for the data. The General Linear Models procedure of the SAS or the Complete Statistical System (CSS, Statsoft) for the IBM-PC computer was used for these analyses. We accepted $P < 0.05$ as indicating statistical significance.

RESULTS

Effect of OA on hemodynamic variables and blood gases. Hemodynamic variables and blood gases recorded before (baseline) and after administration of OA are presented in Table 1. During the baseline measurement period, there were no significant differences between groups in any of these measured variables. In the control group (dogs in which saline was administered instead of OA) there were no changes in these variables over a time course similar to that reported for the groups in which

TABLE 1. *Effect of vehicle (normal saline), OA, or OA and meclofenamate on hemodynamic and blood gas variables*

	Control (n = 4)		OA Only (n = 16)		OA-Meclo (n = 6)		
	Baseline	120 min	Baseline	120 min	Baseline	90 min	120 min
CO	1.84±0.60	2.09±0.58	1.78±0.51	2.14±0.63	2.39±0.48	2.15±0.39	1.83±0.25*†
Pa	114±29	133±16	129±13	140±19	138±31	137±17	139±24
Ppa	11±3	9±1	9±2	13±4*	11±3	12±4	15±5*
Pwp	6±3	3±1	5±4	4±3	5±1	4±1	4±1
pH	7.36±0.05	7.35±0.04	7.44±0.06	7.34±0.07*	7.40±0.05	7.32±0.03*	7.33±0.03*
Pa_{CO_2}	38±3	43±7	34±5	40±9	36±6	43±3	42±1
Pa_{O_2}	567±27	573±17	602±79	248±172*	639±59	303±89*	518±77†

Values are means ± SD at baseline and at time after OA; n, no. of dogs. OA, oleic acid; OA only, dogs given only OA; OA-meclo, dogs given sodium meclofenamate 90 min after OA; CO, cardiac output; Pa, mean arterial pressure; Ppa, mean pulmonary arterial pressure; Pwp, mean pulmonary wedge pressure; pH, arterial pH; Pa_{CO_2}, arterial Pco_2; Pa_{O_2}, arterial Po_2. *† $P < 0.05$ compared with baseline and OA, respectively.

OA was administered. In the OA-only group (dogs in which measurements were made 120 min after OA administration), Ppa increased, whereas arterial Po_2 (Pa_{O_2}) and blood pH decreased. The Pa_{O_2} of the OA-R and OA-NR subgroups was not significantly different. CO was stable in the OA-only group and in the OA-meclo group 90 min after OA administration (before meclofenamate was administered). Administration of meclofenamate was associated with a decrease in CO and a return of Pa_{O_2} to values not significantly different from baseline measurements (30 min after meclofenamate). Ppa increased only after meclofenamate in this group; however, Ppa was increased from baseline values in both the OA-only and the OA-meclo groups 120 min after OA administration. Therefore, in the OA-meclo group, the effect of time after OA, and not solely the inhibition of cyclooxygenase with meclofenamate, may have also accounted for the increase in Ppa.

Effect of OA on LWC and rPBF. Figure 2 demonstrates the relationships between regional LWC and frPBF. In the control group (n = 4), LWC did not increase over the time interval between the first and second scan (~120 min), and the distribution of frPBF to the distribution of LWC was similar during both measurements. In contrast, the increase in the LWC that occurred after OA is indicated by a shift to the right in the frPBF-LWC curve (average median LWC is indicated by the vertical bar).

As reported in a previous study (21), we found that intravenous OA (OA only) led to the development of two distinct subgroups with regard to the relationship between frPBF and LWC. In both subgroups, LWC increased significantly after OA, mainly in dorsal (dependent) areas (highest bin numbers). However, in the subgroup designated the "redistribution subgroup" (OA-R, n = 6), frPBF also decreased in the dorsal areas; areas that generally exhibited the greatest increase in LWC. In other words, blood flow distributed away from the most edematous areas of the lung (Fig. 2). In the subgroup designated the "no-redistribution subgroup" (OA-NR, n = 10), frPBF did not decrease in the areas of greatest edema. In these animals the frPBF-LWC curve shifted to the right as the median LWC increased after OA administration, but the shape of the curve was nearly the same as that generated during baseline conditions (indicating no perfusion redistribution).

In the OA-meclo group, the frPBF/LWC curve obtained after OA (but before meclofenamate) resembles the curve for the OA-NR subgroup, since frPBF generally does not decrease in the areas of greatest edema. After administration of meclofenamate, however, frPBF decreased in the dorsal areas. Again, these dorsal areas (bins) generally had the greatest LWC. Thus cyclooxygenase inhibition with meclofenamate converted animals with little or no perfusion redistribution to animals with significant perfusion redistribution (Fig. 3).

Among the groups in which OA was administered, the baseline LWC values were not equal. The baseline LWC of the OA-NR subgroup was slightly but significantly less than those of the OA-R and the OA-meclo groups (Fig. 2). More impressive, however, were the differences post-OA (compare median values for LWC in Fig. 2). After OA, LWC was less in the OA-NR subgroup than in the OA-R and OA-meclo groups. Although the increase in LWC after OA was not different between the OA-NR and OA-meclo groups, the increase in LWC in the OA-R group was significantly greater than for either of the other OA groups.

Effect of OA on TxB_2 and 6-keto-$PGF_{1\alpha}$ in blood and lung tissue samples. The concentrations of TxB_2 and 6-keto-$PGF_{1\alpha}$ in systemic arterial or pulmonary arterial plasma did not change from baseline values at 60 or 120 min after the administration of OA within any of the groups studied (Table 2). The only difference observed between groups occurred 120 min after OA when the concentration of 6-keto-$PGF_{1\alpha}$ in the pulmonary arterial plasma of the OA-NR subgroup was significantly higher than in all other groups. A similar trend in the systemic arterial plasma was not quite statistically significant (P = 0.11).

Regional concentrations of TxB_2 and 6-keto-$PGF_{1\alpha}$ in lung tissue samples collected 2.5 h after OA administration are shown in Table 3. Within each group, there were no significant differences in the tissue concentrations of TxB_2 or 6-keto-$PGF_{1\alpha}$ between regions. However, from these data, one sees that, especially in the lower lobe, OA administration resulted in increased tissue concentrations of TxB_2 but not of 6-keto-$PGF_{1\alpha}$ (compared with control animals). The dorsal and ventral regions of the lower lobe show a similar increase in TxB_2 for both subgroups of animals receiving OA only. Meclofenamate prevented the OA-induced increase in TxB_2 and decreased the concentration of 6-keto-$PGF_{1\alpha}$ in these regions compared with control animals. In the lower lobe, the only difference between the OA-only subgroups was

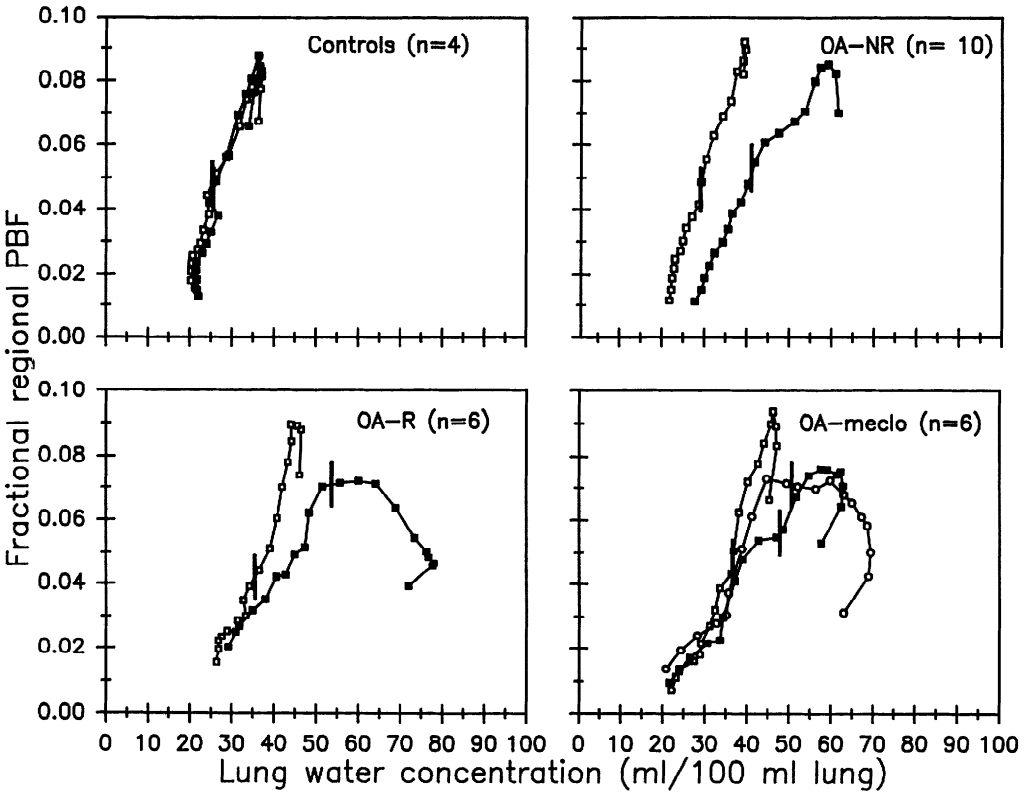

FIG. 2. Fractional regional pulmonary blood flow (PBF) as function of lung water concentration (LWC) in control animals and after oleic acid (OA) administration in animals showing spontaneous perfusion redistribution (OA-R), in animals showing no redistribution (OA-NR), and after meclofenamate (OA-meclo). Open and closed squares, mean values for baseline and 120 min after normal saline (Control) and OA (all other groups), respectively; open circles, 90 min after OA (immediately before meclofenamate). Vertical bar represents average median LWC in image regions analyzed. At each time, each symbol represents 1 average bin value for that group (see MATERIALS AND METHODS). Data are always plotted in order of bin number, from 1 to 20. *Bins 1–5* always come from ventral portions of region of interest, *6–15* from midportions, and *16–20* from dorsal portions (see MATERIALS AND METHODS).

that the 6-keto-$PGF_{1\alpha}$ concentration in the ventral portion of the lower lobe in the OA-R subgroup was not significantly different from the OA-meclo group. Samples from the middle lobe exhibited greater variability between dogs. There were no significant differences in the concentration of 6-keto-$PGF_{1\alpha}$ between any groups in the middle lobe. TxB_2 in the ventral region of the middle lobe was greater in the OA-R subgroup than in the OA-NR subgroup.

The ratio of these metabolites, rather than their actual concentrations, has been used to describe the balance between the vasoconstrictor activity of thromboxane and the vasodilator activity of prostacyclin (3, 14). Indeed, in the present study, the ratio of these metabolites illustrates a more uniform profile among the regions sampled. After the administration of OA, the TxB_2/6-keto-$PGF_{1\alpha}$ ratio was significantly greater compared with the control group in all regions (Table 3). In the OA-meclo group, the regional TxB_2/6-keto-$PGF_{1\alpha}$ ratio was generally greater than in the control group and in both OA-only subgroups. The only difference between the OA-only subgroups was that the TxB_2/6-keto-$PGF_{1\alpha}$ ratio of the OA-R subgroup was not significantly different from the OA-meclo group. Because there were no significant differences between regions within any one group, the regional 6-keto-$PGF_{1\alpha}$ and TxB_2 data were combined and related to the magnitude of perfusion redistribution, expressed as the PRI (Fig. 4). In Fig. 4, the TxB_2/6-keto-$PGF_{1\alpha}$ ratio of the OA-NR subgroup is less than that of the OA-R subgroup, resembling the control group in which the distribution of perfusion to LWC is similar. In contrast, the higher TxB_2/6-keto-$PGF_{1\alpha}$ ratios of the OA-R and OA-meclo subgroups are, in each case, associated with significant perfusion redistribution. In Fig. 5, with the use of linear regression techniques, the PRI data (comparing the last PET scan data before lung tissue sample collection with the baseline PET images) are compared with the ratio of TxB_2/6-keto-$PGF_{1\alpha}$ from the combined regions. A significant correlation exists between the TxB_2/6-keto-$PGF_{1\alpha}$ ratio and the PRI such that greater perfusion redistribution occurs as the TxB_2/6-keto-$PGF_{1\alpha}$ ratio increases.

DISCUSSION

The present study confirms our hypothesis (21) that lung tissue eicosanoid balance is a major determinant of rPBF in OA-induced acute lung injury. Our results strongly suggest that in the presence of edema relatively greater amounts of the vasoconstrictor TxA_2 than of the vasodilator prostacyclin will lead to redistribution of blood flow away from edematous regions, along with salutary effects on blood oxygenation. Apparently, regardless of whether this imbalance in favor of TxA_2 occurs spontaneously or after cyclooxygenase inhibition with drugs like meclofenamate, the net result is an equivalent amount of perfusion redistribution.

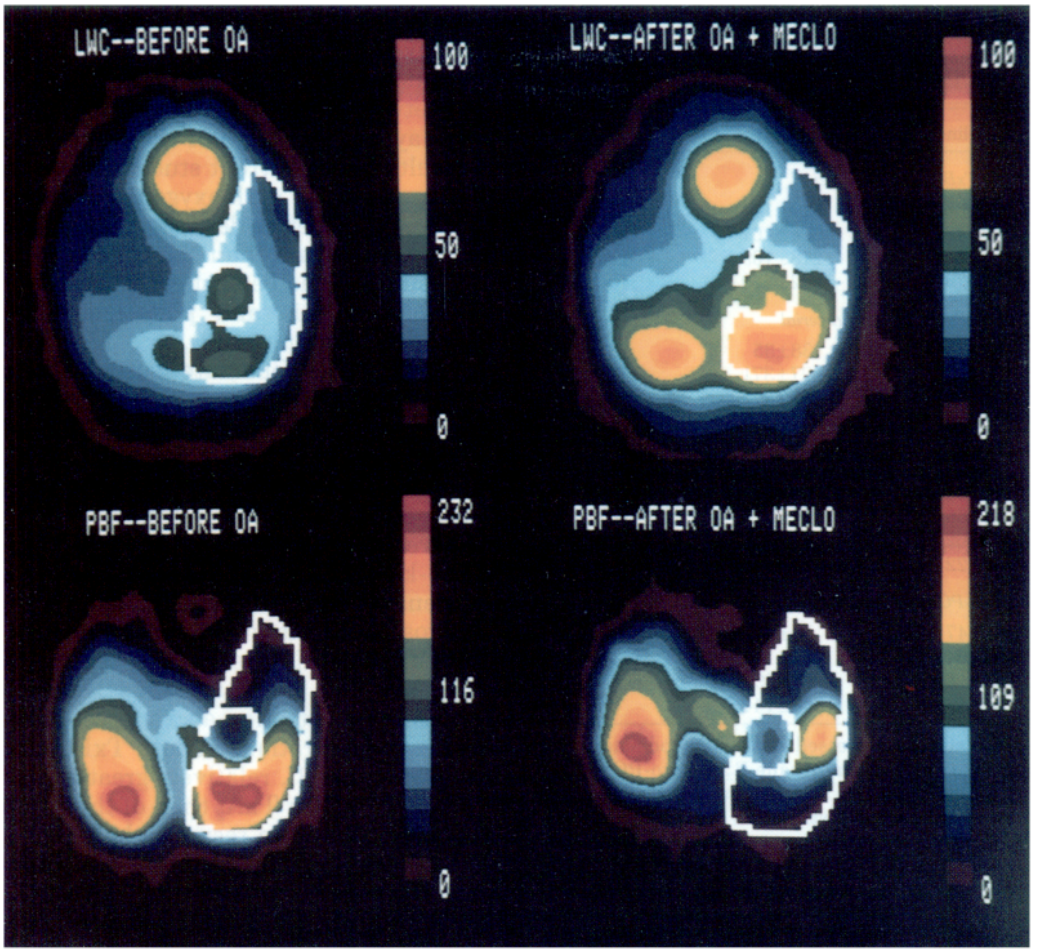

FIG. 3. Single tomographic slice images of LWC and PBF from same animal, at same level and orientation as in Fig. 1. After OA + meclo, LWC increases (mostly dorsally), whereas PBF is markedly redistributed ventrally.

Although the etiology of perfusion redistribution after acute OA-induced lung injury undoubtedly involves a number of different mechanisms, such as vasoconstriction, vascular obstruction, and vascular compression by edema, previous work by our group suggests that edema is necessary but not sufficient to cause perfusion redistribution in this model (21, 22). This conclusion is based on the observation that perfusion redistribution occurs spontaneously only in a subset of animals (designated OA-R in the present study) that develops edema in response to the administration of OA. The remainder (group OA-NR in this study) fail to show perfusion redistribution despite equivalent amounts of edema (21). The importance of edema for perfusion redistribution is also supported by the results of the current study. Although we did not find regional differences in eicosanoid balance when perfusion redistribution occurred, PBF only redistributed away from the most edematous lung regions located in the dorsal (dependent) regions (Figs. 2 and 3). The mechanism by which edema and vasoactive mediators interact to cause perfusion redistribution remains to be determined.

Prostacyclin, a potent pulmonary vasodilator prostaglandin (9, 10) that has been reported to be the major pulmonary product of cyclooxygenase activity in dogs (6, 16), is also the major arachidonic acid metabolite identified in this study (Table 3). In uninjured animals, lung tissue 6-keto-$PGF_{1\alpha}$ concentrations are 14–100 times higher than TxB_2 concentrations (Table 3). Thus, if the tissue concentrations of prostacyclin and TxA_2 reflect the relative vasodilator-to-vasoconstrictor activity in the perfused pulmonary vasculature, the effect should be toward maximal vasodilation under normal conditions.

The major effect of OA injury on lung tissue eicosanoid concentrations is to increase the concentration of TxB_2 with little change in the concentration of 6-keto $PGF_{1\alpha}$ (Table 3). In contrast, OA administration did not result in similar increases in blood TxB_2 concentrations. Instead, the concentration of 6-keto-$PGF_{1\alpha}$ in the blood of those animals that failed to show spontaneous perfusion redistribution (OA-NR) was greater than that in each of the other groups. These data suggest that whether perfusion redistribution occurs depends on the balance between two synergistic effects. On the one hand, a significant increase in tissue TxA_2, without any change in prostacyclin concentrations, results in vasoconstriction and perfusion redistribution away from the edematous regions (as in the OA-R dogs). On the other hand, with a smaller increase in lung tissue TxA_2 and a greater prostacyclin concentration in blood perfusing the lung (pulmonary arterial blood), perfusion redistribution fails to occur (as in the OA-NR group).

Whether these dual effects of tissue and blood eicosanoids are truly linked remains to be determined, as does

TABLE 2. *Effect of OA on concentrations of TxB_2 and 6-keto-$PGF_{1\alpha}$ in systemic arterial and pulmonary arterial blood*

Group	Baseline	60 min	120 min
	TxB_2: systemic arterial		
Control	54±35	119±134	133±133
OA-NR	182±182	224±161	260±228
OA-R	251±244	218±169	144±116
OA-meclo	194±73	282±222	104±47
	TxB_2: pulmonary arterial		
Control	80±53	38±35	131±148
OA-NR	150±91	427±371	314±302
OA-R	221±168	256±273	214±179
OA-meclo	236±231	337±140	115±95
	6-Keto-$PGF_{1\alpha}$: systemic arterial		
Control	42±34	38±13	54±28
OA-NR	56±55	167±174	167±145
OA-R	49±62	62±52	76±65
OA-meclo	43±22	111±75	50±25
	6-Keto-$PGF_{1\alpha}$: pulmonary arterial		
Control	62±47	59±23	71±53*
OA-NR	84±64	164±143	148±72
OA-R	34±48	67±69	61±44*
OA-meclo	54±39	143±144	63±36*

Values are means ± SD and are expressed as pg/ml blood plasma at baseline and time after OA. TxB_2, thromboxane B_2; 6-keto-$PGF_{1\alpha}$, 6-ketoprostaglandin $F_{1\alpha}$; Control, no OA; OA-NR, subgroup given OA only in which spontaneous perfusion redistribution did not occur; OA-R, subgroup given OA in which spontaneous perfusion redistribution did occur; OA-meclo, group given sodium meclofenamate 60 min after OA. * $P < 0.05$ compared with no-redistribution group.

the source of the increases in either eicosanoid. The smaller TxB_2/6-keto-$PGF_{1\alpha}$ ratio measured in lung tissue samples from the OA-NR group may be due to increased blood concentrations of 6-keto-$PGF_{1\alpha}$ subsequently trapped in tissue at the time of its collection, or it may represent differences in pulmonary synthesis.

The absence of a significant increase in blood TxB_2 concentrations after OA administration in the present study is somewhat at variance with work reported by others in intact dogs (5, 12), intact sheep (18), or in isolated lungs from rabbits (2, 11) or guinea pigs (26). Higher doses of OA, species differences, and the differences between intact animals and isolated lung preparations may explain some of these differences. However, the results of the current study suggest that lung tissue concentrations of cyclooxygenase products of arachidonic acid may better describe their activity on the pulmonary vasculature than the concentrations present in the plasma, as has been suggested previously for endotoxin-induced (31) and platelet-activating factor-induced acute lung injury (4).

Although meclofenamate caused a similar degree of perfusion redistribution after OA (compared with the OA-R group), there were marked differences in eicosanoid balance. Tissue concentrations of both TxB_2 and 6-keto-$PGF_{1\alpha}$ were markedly reduced in the OA-meclo group, yet the reduction in 6-keto-$PGF_{1\alpha}$ was relatively much greater than the reduction in TxB_2. As a result, the TxB_2/6-keto-$PGF_{1\alpha}$ ratio was markedly elevated after meclofenamate (Figs. 4 and 5), supporting the concept that prostacyclin is an important mediator of the low pulmonary vascular resistance present under normal conditions. This high tissue TxB_2/6-keto-$PGF_{1\alpha}$ ratio after OA administration also suggests that although the absolute concentrations of both TxB_2 and 6-keto-$PGF_{1\alpha}$ were low (Table 3), relatively greater concentrations of the vasoconstrictor TxA_2 than of the vasodilator prostacyclin may have been responsible for the increased Ppa in the OA-meclo group after administration of meclofenamate. Together, these results suggest that meclofenamate may bring about perfusion redistribution in this model primarily by inhibiting prostacyclin synthesis, whereas spontaneous redistribution (when it occurs) is principally the result of increased Tx synthesis.

Pa_{O_2} improved markedly after meclofenamate (Table 1) and was markedly higher than in dogs given OA alone, a finding frequently reported by others (1, 5, 12, 13, 18, 19, 21) in this and other models of lung injury (7, 8, 27, 28) when meclofenamate, indomethacin, acetylsalicylic acid, or ibuprofen were used for inhibition of cyclooxy-

TABLE 3. *Effect of OA on concentrations of TxB_2 and 6-keto-$PGF_{1\alpha}$ in lung tissue samples*

Group	Sample Location: Lower lobe-dorsal	Lower lobe-ventral	Middle lobe-dorsal	Middle lobe-ventral
		TxB_2, ng/g dry tissue		
Control	4.0±3.1	1.9±0.7	6.7±7.3	2.0±1.4
OA-NR	16.3±6.9*†	28.2±12.2*†	35.7±67.1	19.5±20.8‡
OA-R	23.0±3.0*†	31.8±5.2*†	19.2±8.2	60.5±52.8*†
OA-meclo	5.0±6.9	5.8±4.5	3.9±2.6	21.5±18.3
		6-Keto-$PGF_{1\alpha}$, ng/g dry tissue		
Control	115.6±84.6†	205.1±144.8†	206.3±259.2	76.6±59.2
OA-NR	128.6±28.0†	254.8±55.2†	147.1±123.5	207.1±225.9
OA-R	101.0±29.0†	126.6±76.9	129.8±125.2	175.8±116.1
OA-meclo	9.7±13.8	4.2±4.7	5.2±6.3	76.8±120.8
		TxB_2/6-keto-$PGF_{1\alpha}$ ratio		
Control	0.07±0.10†	0.01±0.01†	0.04±0.05†	0.05±0.06†
OA-NR	0.13±0.05*†	0.14±0.10*†	0.18±0.12*†	0.10±0.05*†
OA-R	0.25±0.11*†	0.35±0.26*†	0.25±0.17*†	0.31±0.09*
OA-meclo	2.29±2.10*	4.91±6.83*	2.37±2.58*	0.95±0.86*

Values are means ± SD. *†‡ $P < 0.05$, compared with control, OA-meclo, and OA-R, respectively.

genase. Interestingly, there was no significant difference in Pa_{O_2} between the OA-R and OA-NR dogs. This difference in meclofenamate and nonmeclofenamate-treated animals may be explained in several ways. First, it may be that our PET measurements of rPBF, although accurate compared with microsphere techniques (15), still underestimate perfusion redistribution at the acinar level, a level of resolution below that of current PET technology. Second, differences in the amount of alveolar edema between the OA-NR and OA-R subgroups (quantified as differences in LWC) may have been important. Thus the effects of perfusion redistribution on Pa_{O_2} in the OA-R group were probably offset by the significantly greater LWC (Fig. 2). However, Pa_{O_2} increased markedly in the OA-meclo group after meclofenamate was administered at a time when LWC was similar to that in the OA-R group. This difference in LWC may also have been the result of a greater TxA_2/6-keto-$PFG_{1\alpha}$ ratio in both the OA-R and OA-meclo groups compared with the OA-NR group, resulting in higher microvascular pressures and increased alveolar edema (26). These speculations, however, remain to be proven by future investigations.

In conclusion, the results of this study are consistent with our previously proposed concept that in the presence of edema, perfusion redistribution will occur if vasoconstriction from Tx predominates over the vasodilation caused by prostacyclin (21). In contrast, with lesser increases in Tx, and perhaps with increased prostacyclin concentrations in blood perfusing the injured lung, perfusion redistribution fails to occur. Finally, cyclooxygenase inhibition with meclofenamate decreases the tissue concentrations of prostacyclin to a greater extent than Tx, resulting in perfusion redistribution from the vasoconstrictor activity of the remaining TxA_2 or from noncyclooxygenase-derived vasoconstrictors present in the edematous lung regions.

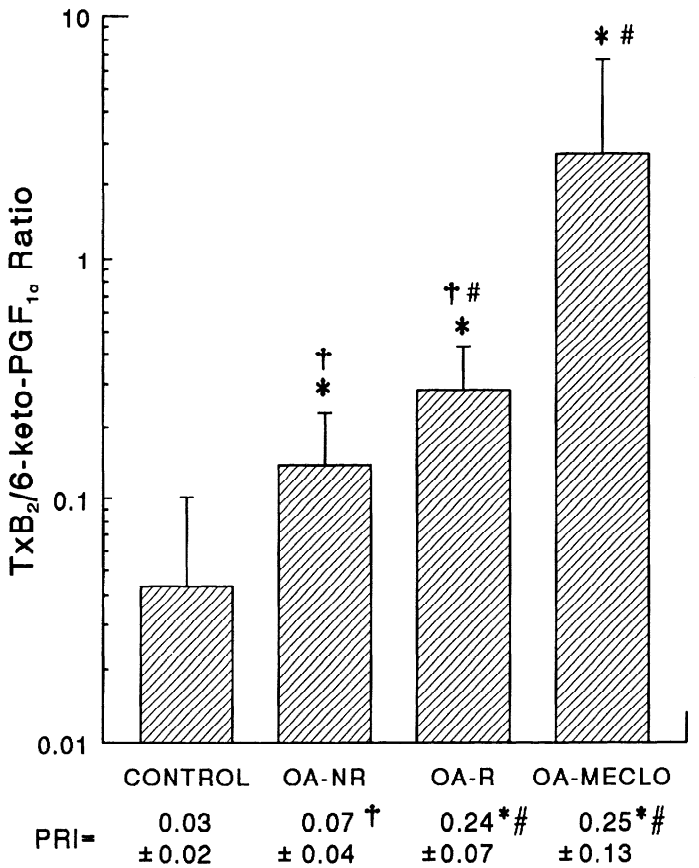

FIG. 4. Log thromboxane (Tx) B_2/6-ketoprostaglandin (PG) $F_{1\alpha}$ ratio of lung tissue samples in control animals, 150 min after OA, and 60 min after meclofenamate (same groups shown in Fig. 2). Perfusion redistribution index (PRI) compares positron emission tomography data obtained 120 min after saline (Control) or OA with baseline data. *†# $P < 0.05$, compared with control, OA-meclo, and OA-NR, respectively.

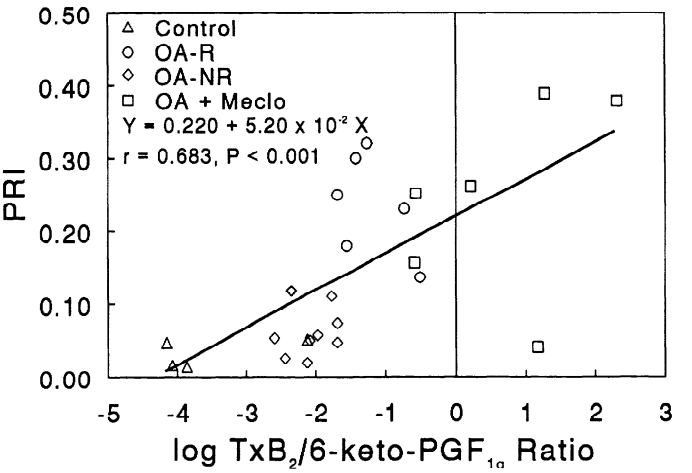

FIG. 5. Linear regression relationship of PRI and log TxB_2/6-keto-$PGF_{1\alpha}$ ratio of lung tissue samples in control animals, 150 min after OA, and 60 min after meclofenamate (same groups shown in Fig. 2).

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