Effect of eicosanoid inhibition on the development of pulmonary edema after acute lung injury

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Schuster, Daniel P., Alan H. Stephenson, Sandra Holmberg, and Patrick Sandiford. Effect of eicosanoid inhibition on the development of pulmonary edema after acute lung injury. J. Appl. Physiol. 80(3): 915–923, 1996.—In experimental models of acute lung injury, cyclooxygenase inhibition improves oxygenation, presumably by causing a redistribution of blood flow away from edematous lung regions. This effect on perfusion pattern could also reduce alveolar edema formation. On the other hand, pulmonary pressures usually increase after cyclooxygenase inhibition, an effect that could exacerbate edema accumulation. Therefore we tested the following hypothesis: the total accumulation of pulmonary edema in dogs during a 24- to 28-h period of observation after acute lung injury caused by oleic acid will be less in a group of animals treated with meclofenamate (n = 6) or with the thromboxane-receptor blocker ONO-3708 (n = 5) than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6). Lung water concentrations (LWC), the regional pattern of pulmonary perfusion, and protein permeability were measured with the nuclear medicine imaging technique of positron emission tomography. After 24–28 h, LWC was significantly less (P < 0.05) in the ONO-3708 group than in a group of animals treated with oleic acid alone (placebo, n = 6).

IN AT LEAST TWO ANIMAL models of acute lung injury [one induced by oleic acid (OA) and the other by othchlorvynol administration (27, 28)], inhibitors of arachidonic acid cyclooxygenase activity regularly improve gas exchange. The mechanism could be a redistribution of blood flow away from edematous lung regions (22, 25, 38) or a reduced accumulation of pulmonary edema. On the other hand, increases in pulmonary vascular pressures usually accompany pulmonary cyclooxygenase inhibition, possibly offsetting a beneficial effect on edema accumulation (20, 22, 25, 27). To evaluate the overall effect of eicosanoid inhibition on the development of pulmonary edema after experimentally induced lung injury, we tested the following hypothesis: the total accumulation of pulmonary edema during a 24- to 28-h period of observation after acute lung injury with OA will be less in a group of animals treated with the nonsteroidal anti-inflammatory cyclooxygenase inhibitor meclofenamate or with the thromboxane-receptor blocker ONO-3708 (9) than in a group of animals treated with OA alone.

Our strategy for testing the experimental hypothesis was as follows. First, we developed the ability to maintain animals injured with OA for ≥24–28 h in an anesthetized and ventilated state, mimicking a typical intensive care unit environment. Second, we developed a protocol for maintaining the pulmonary capillary wedge pressure (PCWP) at a relatively steady level throughout the experimental period. Third, we documented that the severity of injury was comparable in the different experimental groups by measuring the rate of transcapillary flux of a radioactively labeled protein as an index of vascular permeability within the lung. Finally, to determine whether drug effects, if any, were due to an effect on the pulmonary perfusion pattern and/or on pulmonary vascular pressures, we measured the regional distribution of pulmonary perfusion repeatedly during the 28-h period of observation and recorded the pulmonary arterial pressure continuously. Lung water concentration (LWC), pulmonary perfusion, and pulmonary vascular permeability were measured with the quantitative nuclear medicine imaging technique of positron emission tomography (PET).

The data show that, in addition to a unique set of new data on the natural history of lung injury caused by OA, the total accumulation of pulmonary edema is different after the use of these two intervention drugs. The data strongly suggest that the beneficial effect of inhibiting thromboxane on edema formation depends on avoiding the simultaneous development of pulmonary hypertension.

MATERIALS AND METHODS

Animal preparation. These studies were approved by Washington University Medical School’s Animal Studies Committee. All dogs weighed 20–25 kg and were anesthetized with pentobarbital sodium (25–30 mg/kg), intubated with auffed endotracheal tube, and ventilated (inspired O₂ fraction = 1.0) with a Harvard pump respirator at a tidal volume of 15 ml/kg and a respiratory rate adjusted to achieve a normal arterial Pco₂. Positive end-expiratory pressure was not used at any time in this study. Additional narcotics were administered, if necessary, to eliminate spontaneous breathing during PET imaging.

All surgical procedures were performed under completely sterile conditions with animals in the supine position. 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 visual-
ization. A 17-cm-long 3-mm-ID piece of standard pressure tubing was inserted into the femoral artery for blood sampling; a 5-cm premature infant feeding tube catheter was placed into the external jugular vein for drug and radionuclide administration. Catheter patency was maintained by a continuous infusion of heparinized saline (1 U/ml) at a rate of <3 ml/h. A Foley catheter was used in all animals to drain the urinary bladder.

Cardiac output was measured by the thermodilution technique with use of an Edwards Laboratories cardiac output computer. Transducers (Trandec disposable) were calibrated to the center of the lateral chest and connected to a Mennen model 742 monitor for pulmonary arterial, pulmonary wedge, and systemic arterial pressure recordings. Blood gases were analyzed using an Instrumentation Laboratories model 1306 blood gas analyzer.

PET techniques. All PET measurements were performed with an in-house built “PETT-VT” system. Design features, methods for calibration, corrections for activity decay, and corrections for photo attenuation have been discussed elsewhere (13, 19, 24).

Animals were 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 1.41 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.

The methods used to measure regional pulmonary blood flow (PBF) and LWC and to evaluate pulmonary vascular permeability by measuring the pulmonary transcapillary escape rate (PTCER) for a radiolabeled protein, including supporting validation studies, have been reported previously (12, 13, 19, 23, 24, 29). In general, PET is used to measure the tissue concentration and distribution of a positron-emitting radionuclide. In the current studies the radionuclides were H215O and 68Ga citrate. The activity data measured with PET, when combined with blood activity (used as a reference) and analyzed with appropriate compartmental mathematical models, yield tomographic images representative of PBF or LWC. PET data set collections include (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 H215O (60–80 mCi), (3) a 300-s scan obtained after equilibration of the H215O (for measurement of LWC and the apparent blood-tissue partition coefficient for water), and (4) pulmonary artery, pulmonary wedge, and systemic arterial pressures, cardiac output, and blood gas analysis data. Except during the baseline data set, this data collection also included a 45- to 60-min set of 1.5- to 5-min scans obtained beginning 2 min after the intravenous injection of 68Ga citrate.

A schema of the experimental design is shown in Fig. 1. After all catheters were placed, 500 ml of normal saline were given if the initial wedge pressure was <7 mmHg. Then, in the placebo-treated group, OA (0.08 ml/kg) was given after the first PET data set was obtained. After 1 h, a second PET data set was obtained. A third and fourth PET data set were obtained at 8 and 24–28 h after the administration of OA. The saline placebo was given every 4 h throughout the 24–28-h study period.

In the meclofenamate-treated group, the experimental sequence was identical, except 2 mg/kg meclofenamate was given instead of placebo.

In the ONO 3708 treated group, intravenous drug administration (10 µg·kg⁻¹·min⁻¹) was begun 30 min before the OA was given and then continued throughout the entire 24–24 h period.

Fig. 1. Schematic representation of experimental time line. •, Time of positron emission tomographic data set collections: baseline and 1, 8, and 24–28 h after oleic acid (OA) administration. Dashed line, continuous infusion of thromboxane-receptor antagonist ONO-3708.

Fig. 1. Schematic representation of experimental time line. •, Time of positron emission tomographic data set collections: baseline and 1, 8, and 24–28 h after oleic acid (OA) administration. Dashed line, continuous infusion of thromboxane-receptor antagonist ONO-3708.

Arrows, holus infusions of OA, normal saline placebo, or meclofenamate.
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28-h period. Our rationale for giving this thromboxane-receptor blocker before giving the OA was to prevent any thromboxane-receptor stimulation as the injury developed.

In all groups, arterial and pulmonary arterial blood pressures were recorded continuously using a Macintosh 165B portable computer with Acknowledge 2.0 software (BIOPAC Systems, Goleta, CA). Cardiac output, wedge pressure, and arterial and mixed venous blood gases were recorded every hour. If the wedge pressure was <7 mmHg, NaCl (0.9%) solution was given at a rate of 50 ml/h until the wedge pressure was >7 mmHg, at which time the fluid type was changed to 5% dextrose in water. No attempt was made to reduce elevated wedge pressures with diuretics.

**Image analysis.** From each dog, the four contiguous tomographic slices with the most lung were analyzed from the seven slices reconstructed during each PET scan, encompassing most of the caudal lobes. Regions of interest from the right and left lungs were defined on each transmission scan, as described previously (25, 27). We refer to these regions as "hemislices."

The position of each hemislice 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 and LWC as milliliters of H2O per 100 ml of lung. To normalize the regional PBF data for changes in cardiac output, PBF in each picture element (pixel) was expressed as a fraction of the total blood flow to the hemislice.

*To evaluate the relationship of PBF to anatomic position within a hemislice, the x- and y-coordinates for each pixel, along with the respective fractional PBF values for each pixel, were recorded. By 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 ventral-medial portion of the region and ending with the most dorsal-lateral portion of the region. Each hemislice region contained ~400–500 pixels. We arbitrarily chose to divide the data into 20 "bins" stacked vertically in the ventral-dorsal direction, so each bin contained ~20–25 pixels, which could then be averaged. By keeping the number of bins per region and the number of tomographic slices per dog 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 1/20th of the total number of pixels for the region, beginning with those in the most ventral-medial portion of the region. Bins 1–5 always came 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 pixel locations, the fractional PBF values for all pixels within the bin were averaged, recorded, and plotted. Images that describe this process graphically and schematically have been published previously (25).

We refer to the redistribution of PBF away from injured edematous regions as "perfusion redistribution." To quantify this process, which by large occurs in the most dorsally located positions (22, 25, 27), we determined the difference in fractional PBF between the baseline and subsequent PET data sets in bins 16–20 on each slice. We then summed these differences for those five bins on each slice and averaged these summed values for each dog and then for all dogs within each experimental group at each of the postbaseline PET data sets.

For the PTCER measurement, we averaged the values for each hemislice for each dog and then for all dogs within each experimental group for each of the postbaseline PET data sets.

**Statistical analysis.** Data are presented as means ± SD. Statistical significance was determined with a repeated-measures analysis of variance after verification that data were normally distributed. The General Linear Models procedure of the Statistical Analysis System was used for these analyses. We accepted P < 0.05 as indicating statistical significance. Some data are presented as "box plots" (see Fig. 5). With this type of presentation, the 25th–75th percentile for the variable under review is encompassed by the box, the "whiskers" above and below each box represent the 10th and 90th percentiles. The solid line and the dashed line within each box represent median and mean data, respectively. Symbols above and below the whiskers represent individual data outside the 10th and 90th percentiles.

**RESULTS**

**Baseline data.** There were no differences among the experimental groups in hemodynamic or blood gas data (Tables 1 and 2). Likewise, LWC was similar at baseline (32 ± 6, 31 ± 5, and 33 ± 5 ml/100 ml lung for the placebo, meclofenamate, and ONO-3708 groups, respectively). The distribution of regional PBF at baseline is shown in Fig. 2. Similar to results reported previously (5, 20), fractional regional blood flow increases steadily in these supine dogs along a ventral-dorsal axis, falling off modestly in the most dorsally located regions (Fig. 2). There was no significant difference in the distribution of regional PBF among the different experimental groups at baseline (Fig. 2).

**Effects of lung injury.** The effects of OA-induced acute lung injury on hemodynamics, oxygenation, and PET-derived data over a 24- to 28-h period in the control group of animals are shown in Tables 1–3. Consistent with results from previous studies (20), cardiac output decreased modestly during the first few hours of lung injury but then recovered to baseline values by the end of the study. There were no significant changes in systemic pressure throughout the study, and by design

Table 1. Pulmonary pressures and vascular resistance

<table>
<thead>
<tr>
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<th>Placebo</th>
<th>Meclo</th>
<th>ONO-3708</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>1 h</td>
<td>8 h</td>
</tr>
<tr>
<td>Ppa, mmHg</td>
<td>14 ± 2</td>
<td>15 ± 3</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Ppa</td>
<td>6 ± 1</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
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<tr>
<td>PVR</td>
<td>3 ± 2</td>
<td>5 ± 2</td>
<td>8 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Meclo, meclofenamate; Ppa, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance (Wood's units). *P < 0.05 compared with baseline (B); †P < 0.05 compared with 28-h in placebo or ONO-3708 group for Ppa data but only compared with ONO-3708 for PVR. To simplify presentation, only statistics comparing B with 28 h are shown.
Table 2. Blood gas and ventilation data

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Meclo</th>
<th>ONO-3708</th>
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<tbody>
<tr>
<td>PaO₂, Torr</td>
<td>618 ± 50</td>
<td>591 ± 79</td>
<td>591 ± 79</td>
</tr>
<tr>
<td>PaCO₂, Torr</td>
<td>339 ± 106</td>
<td>424 ± 74</td>
<td>424 ± 74</td>
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<tr>
<td>pHₐ</td>
<td>7.32 ± 0.14</td>
<td>7.35 ± 0.03</td>
<td>7.35 ± 0.03</td>
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<tr>
<td>Ve, ml/min</td>
<td>5.8 ± 0.5</td>
<td>5.2 ± 0.7</td>
<td>5.8 ± 1.2</td>
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</table>

Values are means ± SD. PaO₂, PaCO₂, and pHₐ, arterial PaO₂, PaCO₂, and pH, respectively; Ve, minute ventilation. *P < 0.05 compared with B; †P < 0.05 compared with historical B data (20); for technical reasons only, PTCER data were obtained in only 4 of 6 dogs at 28 h; ‡P < 0.05 compared with 1 h.

Table 3. Effect of OA on systemic hemodynamics, oxygenation, and PET-derived data

<table>
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<th></th>
<th>D</th>
<th>1 h</th>
<th>6 h</th>
<th>20 h</th>
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<tbody>
<tr>
<td>CO, l/min</td>
<td>2.95 ± 1.06</td>
<td>2.38 ± 0.80</td>
<td>2.26 ± 0.47</td>
<td>2.68 ± 0.65</td>
</tr>
<tr>
<td>Pa, mmHg</td>
<td>136 ± 7</td>
<td>128 ± 18</td>
<td>130 ± 12</td>
<td>110 ± 13</td>
</tr>
<tr>
<td>LWC, ml/100 ml</td>
<td>531 ± 54</td>
<td>43 ± 2</td>
<td>48 ± 13</td>
<td>54 ± 10‡</td>
</tr>
<tr>
<td>EVLW, ml/100 ml</td>
<td>39 ± 3</td>
<td>30 ± 2</td>
<td>34 ± 12</td>
<td>37 ± 9‡</td>
</tr>
<tr>
<td>PTCER, ×10⁻⁴ min⁻¹</td>
<td>40 ± 10</td>
<td>119 ± 58*</td>
<td>104 ± 59*</td>
<td>75 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SD. OA, oleic acid; PET, positron emission tomography; CO, cardiac output; Pa, mean arterial pressure; LWC, lung water concentration; EVLW, extravascular lung water concentration; PTCER, pulmonary transcapillary escape rate for 68Ga-labeled transferrin. *P < 0.05 compared with B; †P < 0.05 compared with historical B data (20); for technical reasons only, PTCER data were obtained in only 4 of 6 dogs at 28 h; ‡P < 0.05 compared with 1 h.

In summary then, OA-induced acute lung injury was characterized during this 28-h period by transient cardiac depression, delayed but progressive development of pulmonary hypertension, sustained but varying deterioration in oxygenation, steadily increasing pulmonary edema, and a sustained but stable increase in pulmonary vascular permeability. The time variability in oxygenation (improvement at 8 h and deterioration at 24–28 h) may reflect, in part, additional perfusion redistribution at 8 h (reduced PBF relative to edematous dorsal lateral lung units; Fig. 3) and reduced perfusion redistribution at 24—28 h (Fig. 3).

This case there was no evidence of a significant change in PTCER during the observation period.

Changes in the regional pattern of PBF after lung injury are shown in Fig. 3. After OA, there is a reduction in the fractional blood flow to the most dorsally located image bins, as has been reported previously (22,25,27). This effect is even more pronounced at 8 h but returns to the 1-h pattern by 28 h.

In summary then, OA-induced acute lung injury was characterized during this 28-h period by transient cardiac depression, delayed but progressive development of pulmonary hypertension, sustained but varying deterioration in oxygenation, steadily increasing pulmonary edema, and a sustained but stable increase in pulmonary vascular permeability. The time variability in oxygenation (improvement at 8 h and deterioration at 24—28 h) may reflect, in part, additional perfusion redistribution at 8 h (reduced PBF relative to edematous dorsal lateral lung units; Fig. 3) and reduced perfusion redistribution at 24—28 h (Fig. 3).

Fig. 2. Ventral-dorsal distribution of fractional pulmonary blood flow (PBF) at baseline in all 3 groups of supine dogs. Bin numbers, collections of picture elements on multiple positron emission tomographic images of PBF. Each symbol represents mean value for all dogs in that group. At baseline, there was no significant difference in PBF distribution. Meclo, meclofenamate.

Fig. 3. Ventral-dorsal distribution of fractional PBF at selected times during entire experimental observation period (24–28 h) in placebo animal group. Bin numbers, collections of picture elements on multiple positron emission tomographic images of PBF. Each symbol represents mean value for all dogs in that group. At ~1 h after OA administration, fractional PBF decreases significantly in more dorsally located (higher number) bins. After 8 h, there is an additional redistribution of perfusion away from most dorsally located lung regions. By 24—28 h, perfusion pattern returns to that observed at 1 h.
Effects of interventions. The principal hypothesis in designing this study was that meclofenamate (presumably via cyclooxygenase inhibition of eicosanoid synthesis) or ONO-3708 (as a thromboxane-receptor blocker) would affect the accumulation of pulmonary edema during the experimental observation period. Our principal measure of pulmonary edema was LWC. Mean data for LWC for each of the experimental groups at each PET data-set collection time point are shown in Fig. 4. As already described for the placebo group, LWC also increases 1 h after OA-induced injury in the meclofenamate-treated group and then continues to increase for the duration of the experiment. In the ONO-3708 group, the initial increase in LWC in response to acute injury also occurs but is not followed by further increases. By the end of the experiment, the absolute value for LWC in the meclofenamate group was significantly different (P < 0.05) from that in the ONO-3708 group (P = 0.06 for placebo vs. ONO-3708).

The mean overall change in LWC for each experimental group from baseline to the end of the experiment is shown in Fig. 5. Neither experimental intervention caused a statistically significant difference in LWC accumulation compared with that which developed in the placebo group (mean value of placebo group was different from ONO-3708 group, P = 0.12). However, the difference in overall LWC accumulation between the meclofenamate-treated animals and those treated with the thromboxane-receptor blocker was significantly different.

We reasoned that this apparent difference in pulmonary edema accumulation could be due to the following possibilities: 1) baseline difference among groups, 2) differences in the severity of injury caused by OA, or 3) differences in the physiological response to acute lung injury.

There were no statistically significant differences in any of the baseline variables examined (Table 1). Furthermore, management was similar during the experimental period, as defined by the protocol. As a result, there was no significant difference in the amount of fluid administered, in urine output, or in the pattern of change in cardiac output, systemic arterial pressure, or wedge pressure during the course of the experiments (data not shown for the intervention groups).

Severities of injury were assessed by measuring PTCER. Within each group, there was no significant difference over time (i.e., at 1, 8, or 28 h; data not shown). Also, there was no significant difference among groups when the three data points in each group were averaged (Fig. 6).

In response to injury and the development of pulmonary edema, the pattern of regional PBF changed in each intervention group; this response is similar to that shown in Fig. 3 for the placebo group. The average decrease in regional blood flow to the dorsal lung regions over the entire 24- to 28-h observation period was not different among experimental groups (Fig. 7).

Pulmonary hypertension developed in all three groups after lung injury (Table 1). However, pulmonary hypertension was most pronounced in the group given meclofenamate. The level of pulmonary hypertension at 24–28 h was significantly higher (P < 0.05) in the meclofenamate group than in the other two groups.

In addition to analyzing the pulmonary arterial pressures at the time of each PET data set, we also used the continuous digitized pressure data to more completely characterize the development of pulmonary hypertension. To be certain that recording artifacts were not included in the analysis, we manually in-

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**Fig. 4.** Lung water concentration (LWC) as a function of time and experimental group. Bars, values (means ± SD) from 8 lung regions (2 regions per slice, 4 slices) on multiple positron emission tomographic images. At baseline (B), lung water concentration was similar in all 3 experimental groups. One hour (1) after OA administration, lung water concentration increased (P < 0.05) to a similar extent in all 3 groups, which is consistent with an equivalent degree of edema development. However, lung water concentration continued to increase in placebo (P) and meclofenamate (M) groups during the rest of experimental observation period but not in group given thromboxane-receptor antagonist ONO-3708 (O). *P < 0.05. For clarity, not all statistical comparisons are shown. At 28 h (28), difference between placebo and ONO-3708 groups was different at P = 0.06.

**Fig. 5.** Change in lung water concentration (LWC) between baseline and end of each experiment in each experimental group. Data are presented as box plot (see Statistical analysis for details). Dashed line, mean; solid line, median value for each group. Boxes enclose 25th–75th percentile values; error bars, 10th and 90th percentile limits; O, values for individual animals outside these limits. P, placebo; M, meclofenamate; O, ONO-3708. *M and O groups are significantly different (P < 0.05).
suspected a 2-min period every hour and then recorded the average pulmonary artery diastolic and wedge pressure during that period. The resulting data were plotted as a function of time (Fig. 8). We then integrated the area under each curve, expressed the result as a pressure-time integral, and averaged these integrated data within each group (Fig. 9). The pressure-time integral was greater in the meclofenamate group than in ONO-3708 group ($P = 0.07$).

**Regressions.** With the assumption that we could measure the important variables that might affect the development of pulmonary edema in these studies, we performed a multiple-regression analysis that sought to identify which variables or combination of variables among all 17 experimental animals might best explain the increase in pulmonary edema over the 24- to 28-h period. The following variables were entered into the regression model: pulmonary artery diastolic pressure-time integral, baseline pulmonary arterial pressure, pulmonary wedge pressure-time integral, baseline wedge pressure, PTCER as a measure of severity of injury, and the average decrease in fractional PBF to dorsal lung regions as an index of perfusion redistribution. Only the pulmonary artery diastolic pressure-time integral showed a significant correlation with the change in LWC over the 24- to 28-h time period ($R^2 = 0.39$, Fig. 10). None of the other variables significantly improved the explanatory power of the model, as expressed by the $R^2$.

**DISCUSSION**

Although the mortality from adult respiratory distress syndrome (ARDS) is still unacceptably high, there is reason to believe that survival has recently improved (11, 21). The legitimacy of this apparent improvement can be debated of course. However, if mortality has in fact decreased, the reason(s) is obscure because no prospective randomized controlled study has shown any survival benefit for “specific” forms of treatment (21). This leaves improvements in “supportive” therapy as the most likely explanation (e.g., ventilator strategies, fluid management, steroid use).

One of the most contentious issues relevant to supportive therapy in ARDS has been the continued debate over the value of limiting the development or hastening the resolution of pulmonary edema with efforts to reduce PCWP via fluid restriction or diuresis (18). Although there is a firm theoretical and experimental basis for pursuing this particular therapeutic strategy, supporting clinical evidence has been more difficult to develop. However, several retrospective studies and one prospective study have provided some data to support the contention that such strategies may be associated with improved outcome (14, 17, 26).

If fluid restriction/diuresis can in fact have a favorable effect on the accumulation of pulmonary edema, the mechanism of benefit should involve presumably the reduction of pulmonary capillary hydrostatic pressures or blood flow to injured lung units. However, capillary pressures may not only reflect the patient’s volume state but also the influence of local vasomotor tone. If this is so, pharmacological therapies could be an effective means of reducing local vasoconstriction or regional flow. The present study was designed to test these possibilities.

**OA model.** With the possible exception of the fat embolism syndrome, OA-induced acute lung injury clearly is not a model for the etiology of ARDS. However, as recently reviewed (20), the model does share many pathophysiological similarities with ARDS and so may well be an appropriate model for studying the consequences of lung injury, especially those aspects that are not dependent on unique inflammatory events associated with sepsis or similar conditions (i.e., the “systemic inflammatory response syndrome”).

The vast majority of studies that have used OA to cause acute lung injury, however, have been performed over a very short period of 3–5 h. Derks and Jacobowitz-
Derks (4) and Schoene et al. (16) studied the evolution of OA-induced injury over ≥1 wk, but these studies were performed in unanesthetized spontaneously breathing dogs. Thus the experimental conditions did not mimic the clinical conditions under which ARDS patients are supported and cared for. Johanson et al. (8) also studied the long-term effects of OA-induced injury, this time in anesthetized baboons mechanically ventilated at an inspired O₂ fraction of 0.4. In aggregate, these studies (4, 8, 16) showed that the pathological and pathophysiological evolution of OA-induced injury was quite similar to that reported for ARDS, although the development of fibrosis was infrequent or repeated insults were required.

The present study adds to this database in several significant ways. First, in a setting that is directly applicable to conventional intensive care unit support, we have shown that a single dose of OA produces a stable form of lung injury, at least during the first 24-28 h of observation. This inference is based on our finding that the rate of transpulmonary-capillary protein flux (i.e., PTCER), while distinctly abnormal, did not change significantly during repeated measurements within the study period. Second, whereas this injury is associated with a very substantial increase in the amount of pulmonary edema (assessed as LWC or extravascular lung water) during the 1st h after the onset of injury, a nearly equal but more slowly developing increase occurs during the rest of the observation period (Fig. 4). Third, as noted in previous studies (22, 25, 36), this model of lung injury is associated with a significant redistribution of PBF ("perfusion redistribution") away from edematous dorsally located lung regions in these supine dogs (Fig. 3). This effect, while sustained during the 24- to 28-h experimental period, appears to reach a maximum at ~8 h and then becomes less pronounced by 24–28 h. Although these changes seem to correlate with an effect on oxygenation (i.e., more perfusion redistribution was associated with improved oxygenation), how these changes will affect the accumulation of pulmonary edema are less clear.

![Fig. 8. Examples of pulmonary artery diastolic (PAd) or pulmonary capillary wedge pressure (PCWP) plots as a function of time in individual animals from meclofenamate-treated group (A) and placebo group (B). First point in each case represents data obtained at baseline before OA administration. Each subsequent point represents a 2-min average of digitized data obtained at hourly intervals. Cross-hatched areas, area under PAd curve (PAd-time integral). Note obvious dramatic rise in pulmonary arterial pressure in meclofenamate-treated animal compared with placebo-treated animal. Average wedge is 3 mmHg in A and 5 mmHg in B.](https://example.com/fig8.png)

![Fig. 9. Pulmonary artery diastolic pressure-time integral (PAd * time) in each experimental group. Mean value for meclofenamate-treated group was different from that for group treated with ONO-3708, P = 0.07.](https://example.com/fig9.png)

![Fig. 10. Scatter plot of change in LWC for each animal between baseline and end of each experiment vs. PAd * time (see Figs. 5 and 9). Solid line, line of regression. Analysis shows that 39% of variation in change in LWC among experimental animals can be explained by differences in PAd * time integral. Melo, meclofenamate; ONO, ONO-3708.](https://example.com/fig10.png)
Effect of eicosanoid inhibition. Thromboxane and prostacyclin are potent vasoactive substances that clearly seem to affect the pathophysiology of OA-induced lung injury (20). This type of injury is associated with increased lung tissue thromboxane B2 levels without demonstrable increases in the tissue levels of 6-ketoprostaglandin F1α, (the stable metabolite of prostacyclin) (25, 27). Meclofenamate returns the thromboxane B2 levels to baseline and markedly reduces the 6-ketoprostaglandin F1α levels below baseline. Accordingly, the regional distribution of PBF in this model seems to be strongly, although not completely, dependent on the separate effects of these two mediators or on their inhibition (25, 27). Specifically, increased lung thromboxane concentrations are associated with enhanced perfusion redistribution, whereas lung prostacyclin appears to interfere with this effect. With meclofenamate, perfusion redistribution is maximized. Because studies by others suggested that a reduction in blood flow per se could affect edema accumulation during lung injury (1, 22), the effect of meclofenamate on perfusion redistribution suggested a means by which we could pharmacologically reduce perfusion to injured lung units without causing systemic changes in hemodynamics.

These previous studies (25, 27), however, also showed that, despite a similar degree of perfusion redistribution, edema accumulation could actually be exacerbated if enhanced redistribution was mediated by thromboxane (or a synthetic analogue). Presumably, this effect was the consequence of thromboxane-induced pulmonary venous hypertension. To dissociate the effect of thromboxane per se from that of perfusion redistribution, we used the thromboxane-receptor antagonist ONO-3708. We initiated the antagonist infusion 30 min before giving the OA in a dose previously shown to effectively block a thromboxane-receptor agonist (28). Although it would have been desirable to compare thromboxane-receptor blockade with prostacyclin-receptor blockade, to our knowledge no agent is currently available that acts solely as a prostacyclin-receptor antagonist.

The timing of the two interventions also was different: ONO-3708 was given 30 min before OA, and meclofenamate was given 1 h after OA. We chose this protocol because it conformed to that used in a previous study by Schuster et al. (25). Whereas it might be argued that both drugs should have been given at the same time, it seems unlikely that this 90-min difference was important to the overall accumulation of pulmonary edema during the 24- to 28-h period. Furthermore, administering meclofenamate earlier should, if anything, have exacerbated the difference between the meclofenamate- and ONO-3708-treated groups by further prolonging the pressure difference between the two groups.

As shown in Fig. 5, we found less edema accumulation over 24–28 h in the ONO-3708 group than in the placebo or the meclofenamate-treated groups. Statistically, this difference achieved significance compared with the meclofenamate-treated group. Because severity of injury (as assessed by the rate of pulmonary transcapillary protein flux) was similar, magnitude and temporal changes in the regional pattern of perfusion were similar, there was no significant baseline difference in PCWP or change in wedge pressure after onset of injury (Table 2), and fluid balance and other elements of care in the intensive care unit were similar in the three groups, none of these factors provides a potential explanation for this observed effect.

The variable that was different among the groups and is also relevant to the pathogenesis of pulmonary edema was the level of pulmonary hypertension after the onset of lung injury. In a previous study by Schuster et al. (25), in which eicosanoid effects were evaluated only for 2 h after lung injury, the administration of the thromboxane mimetic U-46619 was associated with a marked increase in pulmonary arterial pressure and a marked worsening of edema accumulation compared with a group treated with meclofenamate despite similar effects on perfusion pattern after lung injury. In the present study, by contrast, the continuous administration of ONO-3708 was associated with less pulmonary hypertension (at least compared with the meclofenamate-treated group) as well as less edema accumulation. This interaction could be mediated by blockade of thromboxane effects on pulmonary venous pressures (10, 30), but this inference is speculative. Although several groups have reported techniques for dissociating the effects of pulmonary arterial vs. pulmonary venous vasoconstriction on pulmonary arterial pressures (2, 3), these methods were not employed in the present study. Therefore, additional work would be necessary to verify that the salutary effect of thromboxane blockade was specifically associated with an effect on pulmonary venous pressures.

Summary. The present study builds on previous experimental and clinical work that underscores the importance of controlling pulmonary capillary pressures in the pathogenesis of pulmonary edema after acute lung injury. Whereas many studies have evaluated the effects of eicosanoids on pulmonary hemodynamics or edema formation after acute lung injury, such studies have almost always been conducted in isolated lung models or in intact animals studied for relatively brief periods of time (2–6 h). We believe this study to be the first to show a beneficial effect on the accumulation of pulmonary edema produced by a drug that was not specifically given to affect cardiac filling pressures during a time interval and in an experimental preparation that are relevant to the clinical management of ARDS. Our interpretation of the data is that thromboxane blockade alone results in less pulmonary venous hypertension and less pulmonary edema. This effect can be offset by concomitant prostacyclin inhibition if the net effect is an increase in pulmonary pressures. Interestingly, others have reached a similar conclusion in an isolated noninjured lung preparation (32). In the present study, we blocked thromboxane with the specific receptor antagonist ONO-3708, but it is interesting to speculate that similar effects might be achieved with a simple thromboxane-synthetase inhibitor like ketoconazole (33) or even with low-dose aspirin.
Finally, although the present study provided no evidence that perfusion redistribution would also affect edema accumulation, a formal test of this hypothesis must await an experimental design that actually results in differences in perfusion pattern after lung injury among the different experimental groups.

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