Effect of defibrinogenation on lung fluid and protein exchange after glass bead embolization

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JOHNSON, ARNOLD, AND ASRAR D. MALIK. Effect of defibrinogenation on lung fluid and protein exchange after glass bead embolization. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 53(4): 895-900, 1982.—We examined the basis of the protective effect of defibrinogenation in dogs in preventing pulmonary edema following embolization with glass beads (A. Johnson and A. B. Malik, J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49: 841-845, 1980). Studies were made in four groups of sheep prepared with the lung lymph fistulas. Group I were normal animals in which pulmonary lymph flow (Qlym) was increased by inflation of a Foley balloon catheter in the left atrium. Group II were control animals that were embolized with plain glass beads (mean ± SE, 0.46 ± 0.04 g/kg) to raise pulmonary arterial pressure (Ppa) threefold above base line. Group III were defibrinogenated with ancrod (0.70 NIH U·kg⁻¹·day⁻¹) for 3 days, and then the pulmonary vascular bed was embolized with the same dose of beads as group II. Group IV were also defibrinogenated but received enough beads (0.78 ± 0.07 g/kg) to raise the Ppa to the same level (3-fold) as group II. The Qlym increased without a change in the lymph-to-plasma protein concentration ratio (L/P) after embolization in groups II, III, and IV, whereas the increase in Qlym in group I was associated with a decrease in L/P. Therefore, defibrinogenation did not prevent the glass bead microembolization-induced increase in lung vascular permeability. Group III had smaller increases in $P_{pa}$ than both groups II and IV following embolization. The extravascular lung water/g lung wt was less in group III compared with the control value, but the value in group IV was not different from control. It is concluded that defibrinogenation does not prevent the increase in permeability following embolization with glass beads; however, defibrinogenation prevents pulmonary edema if the same number of glass beads are injected as in control group. The protective effect may be due to a smaller increase in the pulmonary microvascular pressure in the defibrinogenated animals.

pulmonary lymph flow; coagulation; ancrod; fibrinogen; PVR; precapillary constriction, extravascular lung water, vascular permeability

FIBRIN ENTRAPMENT in the pulmonary microcirculation has been implicated in mediating the increase in pulmonary extravascular lung water following microembolization (11, 15), but its role is controversial (1). Our data in dogs indicated that fibrinogen depletion prevented the accumulation of extravascular water content in the lung after glass bead microembolization (7), suggesting the involvement of fibrin. Because only the extravascular lung water content was measured (7), the basis for the protective effect was not apparent. It was concluded that it "was due to inhibition of the increase in lung vascular permeability or to a time-dependent reduction in pulmonary microvascular pressure" (7). In the present study, we reexamined the question in sheep in which we collected pulmonary lymph to assess the basis of the previously observed protective effect in dogs. Because Binder et al. (1) have found that defibrinogenation in sheep did not prevent the increase in lung vascular permeability after microembolization induced by siliconized glass beads, in this study we also wished to confirm this observation using nonsiliconized beads. We have used nonsiliconized beads in dogs (7) because they cause secondary thrombosis after pulmonary microembolization (7, 9) and thus may be of value in examining the role of coagulation factors in the pathogenesis of pulmonary edema.

METHODS

Sheep were anesthetized with thiopental sodium (20 mg/kg) and were maintained with nitrous oxide supplemented with 1% halothane. The animals were intubated and paralyzed with pancuronium bromide (0.04 mg/kg). The respirator rate and tidal volume were adjusted at the start of the experiment so that arterial $P_{O_2}$, $P_{CO_2}$, and pH were in the normal range. The ventilation was held constant during the experiment. Large-bore catheters (10-F) were placed in the femoral artery and the jugular vein. A Swan-Ganz thermodilution catheter (7-F) was positioned in the pulmonary artery via a femoral vein.

The pulmonary arterial ($P_{pa}$) and pulmonary arterial wedge pressures ($P_{pw}$) were monitored using a Statham P23Db transducer. Pulmonary blood flow ($Q_l$) was determined in triplicate using the thermodilution technique (Edwards Laboratory 9520 cardiac output computer). Pulmonary vascular resistance (PVR) was calculated as ($P_{pa} - P_{pw}$)/$Q_l$.

We cannulated the effluent duct of the caudal mediastinal node as described previously (10, 16). The procedure involved ligation of the midportion of the caudal mediastinal lymph node to enable collection of the lymph from its effluent duct. The pulmonary origin of lymph from this preparation has been tested previously by others and us (10, 17). The lymph and blood samples were collected every 30 min for determination of lymph flow ($Q_{lym}$), lymph protein concentration ($L$), and plasma protein concentration ($P$). The protein concentration of the lymph and plasma were measured using the biuret method (AutoAnalyzer, Technicon Instruments). Pulmonary transvascular lymph protein clearance was calculated by $L/P \times Q_{lym}$. The steady state was defined as...
at least three consecutive Qlym values that did not differ by more than 0.25 ml/h.

Studies were made in four groups.

**Group I (n = 10).** These were normal sheep (body wt 25.0 ± 1.2 kg) that had a Foley balloon catheter (12-F) sutured into the left atrium via the left atrial appendage (13). After a base-line steady state the catheter was inflated to increase left atrial pressure by 10–15 Torr from base line. The purpose of left atrial hypertension was to increase the pulmonary microvascular pressure. The data in groups II, III, and IV were compared with the data from group I.

**Group II (n = 6).** These were control sheep (body wt 25.6 ± 1.3 kg) receiving glass bead microemboli. Measurements were made every 15 min until a base-line steady state was attained, after which a sufficient number of nonsiliconized glass beads (200 μm diam) were injected to raise the Ppa to about threefold the base-line value. The beads (0.46 ± 0.04 g/kg body wt) were injected via the jugular vein, so that proper mixing in the right ventricle was assured. Measurements were made until a postembolic steady state was reached, usually within 2 h after embolization.

**Group III (n = 5).** These sheep (body wt 25.3 ± 0.8 kg) were defibrinogenated by treatment with ancrod. The animals received ancrod at a dose of 0.70 NIH U·kg⁻¹·day⁻¹ for 3 days. Defibrinogenation was verified prior to the experiment by the inability of the blood to clot. The dosage of beads in group III was matched to that in group II.

**Group IV (n = 4).** These sheep (body wt 23.3 ± 0.7 kg) were defibrinogenated as sheep in group III, but these animals received a higher dose of beads (0.78 ± 0.07 g/kg) to raise Ppa to levels comparable with the control group, i.e., threefold increase from the base line.

Arterial blood samples were taken at base line (just before embolization), at 5 min postembolization (PE), and then at 30 min intervals for determination of leukocyte (2), platelet (2), and fibrinogen levels (15). The extravascular lung water content-to-bloodless dry lung weight ratio was determined by the method of Pearce et al. (14) after rehydration of the dried inflated lung (6, 7). Unpaired data were analyzed using the one-way analysis of variance, and significance of changes from base line was assessed using two-way analysis of variance. Significance was accepted at P < 0.05.

**RESULTS**

Figure 1 shows Ql, Ppa, Ppw, and PVR for groups II, III, and IV. The base-line pulmonary hemodynamic values were not significantly different from each other. Ql

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**FIG. 1.** Pulmonary blood flow (Ql), pulmonary arterial pressure (Ppa), pulmonary wedge pressure (Ppw), and pulmonary vascular resistance (PVR) in control (group II) and defibrinogenated-embolized groups (groups III and IV) during base line and following embolization. Values are expressed as means ± SE. Significantly different from base line (*), control (†), and 5' postembolization (PE) (‡), respectively, at P < 0.05.
did not change in any group PE, although there were decreasing trends in both defibrinogenated groups (Fig. 1). Ppa increased in all the groups after embolization, but the values in group III following embolization were less than the values in the control group throughout the study (Fig. 1). PVR also increased in all groups after microembolization but to a higher value at 5 min PE in groups II and IV than in group III. Moreover, there were steady increases in the PVR in both defibrinogenated groups (groups III and IV) after embolization such that the PVR values in both groups at 120 min PE were greater (P < 0.05) than the 5 min PE values (Fig. 1). At 120 min PE, the PVR in group IV was also greater (P < 0.05) than the control value, but PVR values in groups II and III at 120 min PE were the same (Fig. 1). The changes in aortic pressure in the three groups after glass bead embolization are indicated in Table 1. The aortic pressure decreased significantly only in the defibrinogenated embolized groups.

Figure 2 shows the changes of white blood cell and platelet counts and fibrinogen concentration following embolization. The fibrinogen concentration decreased following embolization in the control group. The fibrinogen concentrations in ancrond-treated groups III and IV were significantly lower than the control values throughout the experimental period (Fig. 2). The base line platelet count in group IV was greater than the value in group II; however, platelet levels decreased significantly and remained low throughout the study following embolization in all groups. There were no significant differences in the base-line leukocyte counts among groups II, III, and IV. The leukocyte count did not change significantly from base line following embolization in any group, although there was a trend for the leukocyte levels to decrease in all groups.

Table 2 shows absolute and experimental-baseline data for groups I, II, III, and IV. Fig. 3, A and B, shows the time course of changes in Qlym, lymph-to-plasma protein concentration ratio (L/P), and transvascular protein clearance (C1) after glass bead microembolization. Qlym increased and L/P did not change significantly from base line after embolization in all groups (Fig. 3, A and B, and Table 1). The increases in C1 were also comparable in all groups following embolization (Fig. 3, A and B, and Table 2). There was, however, a trend for the Qlym and C1 to be lower in group III than in group II, although the magnitude of the increases from base line were not dif-

### Table 1. Changes in aortic pressure after glass bead embolization

<table>
<thead>
<tr>
<th>Base Line</th>
<th>Postembolization, min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Control-embolized</td>
<td>97.6 ± 7.7</td>
</tr>
<tr>
<td>Defibrinogenated-embolized, 0.50 g/kg</td>
<td>90.7 ± 4.6</td>
</tr>
<tr>
<td>Defibrinogenated-embolized, 0.78 g/kg</td>
<td>85.4 ± 7.1</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmHg. * Different from base line.
Table 2. Changes in pulmonary fluid and protein exchange following microembolization with glass beads

<table>
<thead>
<tr>
<th>Exptl Groups</th>
<th>Bead Dosage, g/kg body wt</th>
<th>Qlym, ml/h</th>
<th>L/P Ratio</th>
<th>Ct, ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PE</td>
<td>BL</td>
<td>PE</td>
</tr>
<tr>
<td>I. Normal Pla</td>
<td>5.92 ± 0.87 14.56 ± 2.36</td>
<td>0.76 ± 0.04 0.49 ± 0.05</td>
<td>4.40 ± 0.59 7.01 ± 1.12</td>
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<tr>
<td>PE/BL</td>
<td>2.54 ± 0.24 12.41 ± 1.88</td>
<td>0.64 ± 0.05 0.74 ± 0.03</td>
<td>4.40 ± 0.52 9.30 ± 1.67</td>
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</tr>
<tr>
<td>II. Control-embolized Pla</td>
<td>0.46 ± 0.04 7.08 ± 2.16*</td>
<td>0.71 ± 0.05 0.81 ± 0.06</td>
<td>2.63 ± 0.04 5.74 ± 1.90*</td>
<td></td>
</tr>
<tr>
<td>PE/BL</td>
<td>1.97 ± 0.22 2.49 ± 0.70</td>
<td>1.05 ± 0.066</td>
<td>5.91 ± 2.22 11.64 ± 4.14*</td>
<td></td>
</tr>
<tr>
<td>III. Defibrinogenated-embolized Pla</td>
<td>0.50 ± 0.00 3.77 ± 0.70</td>
<td>0.82 ± 0.10 0.77 ± 0.05</td>
<td>2.20 ± 0.66*</td>
<td></td>
</tr>
<tr>
<td>PE/BL</td>
<td>1.92 ± 0.53 2.31 ± 0.21</td>
<td>0.99 ± 0.12†</td>
<td>3.11 ± 0.19†</td>
<td></td>
</tr>
<tr>
<td>IV. Defibrinogenated-embolized Pla</td>
<td>0.78 ± 0.07 6.49 ± 2.31</td>
<td>0.50 ± 0.07 0.27 ± 0.15</td>
<td>3.11 ± 0.19†</td>
<td></td>
</tr>
</tbody>
</table>

* Different from base line (P < 0.05). † Different from group I (P < 0.05).

Values are means ± SE. Qlym, pulmonary lymph flow; L/P ratio, lymph-to-plasma protein concentration ratio; Ct, transvascular protein clearance; BL, base line; PE, postembolization; Pla, left atrial hypertension.

Figure 3. A: pulmonary lymph flow (Qlym), lymph-to-plasma protein ratio (L/P), and lymph protein clearance during base line and following embolization in control (group II) and in fibrinogen-depleted (group III) groups receiving 0.5 g/kg glass beads. Values are expressed as means ± SE. * Significantly different from base line at P < 0.05. B: pulmonary lymph flow (Qlym), lymph-to-plasma protein ratio (L/P), and lymph protein clearance during base line and following embolization in control (group II) and in fibrinogen-depleted groups (group IV) receiving 0.78 g/kg glass beads. Values are expressed as means ± SE. * Significantly different from base line at P < 0.05.

Discussion

In our previous study, we demonstrated that defibrinogenation prevented the increase in extravascular lung water content after glass bead embolization in dogs (7). However, these studies were based solely on the measurement of the lung wet-dry weight ratios, which did not provide an assessment of vascular permeability. The finding in the present study, that pulmonary lymph flow (Qlym) and transvascular protein clearance (Ct) increased after glass bead microembolization in defibrinogenated sheep to the same extent as in control sheep, indicates that defibrinogenation does not prevent the increase in vascular permeability to proteins following glass bead embolization. The changes in Qlym and Ct in
the defibrinogenated groups cannot be explained by recruitment of additional vessels, since well over 50% of the pulmonary vascular bed was embolized as evident by the 250–450% increase in PVR immediately after infusion of the microemboli (Fig. 1); thus the increases in Qlym and C_{L} despite the massive reduction in vascular surface can only be explained by increased pulmonary endothelial permeability. These results agree with the study of Binder et al. (1) in which anecrod pretreatment also did not modify the increase in transvascular protein flow following embolization with siliconized glass beads. Therefore, the protective effect of defibrinogenation on extravascular lung water content cannot be explained by the inhibition of the increase in pulmonary vascular permeability.

We noted that there was a gradual rise in PVR in both defibrinogenated groups after glass bead microembolization in contrast to rapid increases in maximum levels occurring in control animals. This is in accord with our previous data in defibrinogenated dogs (7). The steady increases in PVR after embolization in defibrinogenated animals may be due to several mechanisms. One possibility is that there is an inhibition of the release of a vasodilator substance, such as prostacyclin, which is normally released by the endothelium after intravascular thrombosis (4) but cannot be released in defibrinogenated groups because of the absence of intravascular thrombosis. Thus a decrease in production of a pulmonary vasodilator in the defibrinogenated animals may cause the steady increase in resistance. The release of such a vasodilator substance after pulmonary microembolism would be similar to the release of vasodilator prostaglandins that occurs after endotoxin (4) and hypoxia (5) and that modulates pulmonary vasomotor tone after these insults. The second possibility is that there is a steady release of a pulmonary vasoconstrictor substance, such as thromboxane A_{2}, after microembolization in the defibrinogenated animals that does not occur in untreated animals. Because platelet aggregation may have occurred, as evident from the decrease in platelet counts after embolization (Fig. 2), thromboxane A_{2} may be released after platelet activation (4). The final possibility is that the increase in PVR after embolization is the result of a reduction in vascular surface area due to a decrease in pulmonary blood flow in the defibrinogenated groups. This is suggested by the decreasing trend in pulmonary blood flow in these groups and by the significant decreases in mean aortic pressure.

In the present study, we observed less edema in defibrinogenated sheep than in control sheep when lungs were embolized by the same number of glass beads; furthermore we noted a relationship between the steady-state pulmonary arterial pressure and the degree of extravascular water accumulation after microembolization (Fig. 4). Although this study agrees with our previous data in dogs insofar as fibrinogen depletion reduced the degree of pulmonary edema after microembolization (7), the results indicate the protective effect is not due to an inhibition of the increase in permeability. Because there was a smaller increase in pulmonary arterial pressure, the protective effect of defibrinogenation after injection of the same number of beads as in the control group may have been due to a smaller increase in pulmonary microvascular hydrostatic pressure (P_{mv}). A lung in which endothelial permeability to proteins is increased is especially sensitive to a change in P_{mv} (18), which depends on the magnitude of pre- and postcapillary resistances and pulmonary blood flow. We can only speculate about the basis of the smaller increase in P_{mv} in the defibrinogenated groups. It may be due to an increase in the precapillary-to-postcapillary resistance ratio and to a decrease in pulmonary blood flow; both of these ideas are tenable because PVR increased steadily only in the defibrinogenated groups and there was an associated decrease in pulmonary blood flow.

In our previous study (7), we noted that defibrinogenation prevented pulmonary edema induced by embolization with nonsiliconized beads in dogs. However, Binder et al. (1) did not observe such an effect after pulmonary microembolization of sheep lungs with siliconized beads. In the present study, there was a protective effect of defibrinogenation when the same number of glass beads were injected in the defibrinogenated group as in the control group. The differences with Binder’s study (1) may be related to the different types of glass bead microemboli and the fact that the increase in pulmonary arterial pressure in the defibrinogenated group (group III) was much less after embolization in the control group. Binder et al. (1), on the other hand, matched the pulmonary arterial pressures in control and defibrinogenated groups as in our group IV, which could account for their lack of protective effect.
The increase in permeability after glass bead microembolization does not require fibrinogen, but the involvement of other factors, in particular granulocytes, appears to be important. Flick et al. (3) and Johnson and Malik (6, 8) found that granulocytopenia prevented the increase in CI, (3, 8) and pulmonary edema (6) following glass bead embolization in sheep and dogs. The present study does not rule out clotting factors, in addition to granulocytes, as contributing to the increase in permeability. The fibrinolytic pathway, which has been implicated in the pathogenesis of lung vascular injury after microembolization (12), can be activated directly by glass beads via the activation of Hageman factor (9). Leukotactic complement peptides in defibrinogenated animals can be generated independently of intravascular coagulation (9). Therefore, the fibrinolytic system and leukocytes may still mediate lung vascular injury after glass bead embolization even in the defibrinogenated animal.

There was no relationship between increase in Qlym after embolization and the increase in extravascular lung water content. The lungs were the most edematous in the pathogenesis of lung vascular injury after microembolization even in the defibrinogenated animal. This may be related to fluid accumulation in alveoli and in spaces around extra-alveolar airways and vessels after pulmonary embolization in the control group. Because this fluid would not be available to the terminal lymphatics it would not be possible for Qlym to increase to a greater extent in the control group after embolization, despite greater degree of extravascular fluid accumulation.

In conclusion, defibrinogenation reduces the degree of pulmonary edema after the same degree of glass bead embolization as in control sheep. This effect was not due to an inhibition of the microembolization-induced increase in lung vascular permeability but rather to a smaller increase in Pmv that may be the result of the steady increases in PVR in the defibrinogenated sheep after glass bead microembolization. The results suggest that increases in both Pmv and lung vascular permeability are required to produce pulmonary edema after microembolization.

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