The Role of Fibrin in the Genesis of Pulmonary Edema after Embolization in Dogs

ASRAR B. MALIK, BING C. LEE, HOYTE VAN DER ZEE, AND ARNOLD JOHNSON

SUMMARY We determined the degree of pulmonary edema at various times postembolization in mechanically ventilated dogs in which pulmonary emboli were induced with 0.7 g/kg glass beads (100 μm in diameter). All dogs received 141I-fibrinogen prior to embolization. The extravascular lung water contents were determined at 1 or 2 hours postembolization. The increases in pulmonary arterial pressure (Ppa) and vascular resistance (PVR) were similar in both groups. Lung 141I activity per g bloodless dry lung increased (P < 0.01) at 1 hour postembolization and decreased (P < 0.05) at 2 hours postembolization which suggested a time-dependent decrease in fibrin localization. Extravascular lung water/bloodless dry lung weight ratio of 3.10 ± 0.30 in control dogs was increased (P < 0.05) to 6.05 ± 0.82 1 hour postembolization and was decreased (P < 0.05) to 4.17 ± 0.38 at 2 hours postembolization. The possibility that the decrease in lung water at 2 hours postembolization was due to decreased fibrin in the lung at this time was studied in another group of dogs by infusing tranexamic acid to inhibit fibrinolysis after embolization. At 2 hours postembolization, a greater fibrin accumulation (P < 0.05) in the tranexamic acid-treated group was associated with an extravascular lung water/bloodless dry lung weight ratio of 6.33 ± 1.02, which was greater (P < 0.05) than the value in the untreated group at 2 hours postembolization. Therefore, the reduction in lung water at 2 hours postembolization was not due to decreases in Ppa or PVR. Because inhibition of fibrinolysis prevented the time-dependent decrease in pulmonary edema following microembolization, the decrease in extravascular lung water content may be due to clearance of fibrin from the lungs and subsequent dissipation of humoral factors which promote fluid accumulation.

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THE INCREASE in extravascular lung water following pulmonary microembolization is due to increased lung vascular permeability (Saldeen, 1976; Vaage et al., 1976; Okhuda et al., 1978; Malik and van der Zee, 1978b). The evidence for increased permeability comes from studies in intact sheep (Okhuda et al., 1978; Malik and van der Zee, 1978b), dogs (Saldeen, 1976), and isolated rabbit lungs (Vaage et al., 1976). Recent studies have suggested that humoral factors partly mediate the increase in extravascular lung water after pulmonary thromboembolization (Saldeen, 1976; Vaage, 1976; Malik and van der Zee, 1978a; Malik and van der Zee, 1978b). These factors may be released secondary to intravascular coagulation and platelet aggregation induced by pulmonary microembolization (Saldeen, 1976; Vaage, 1976; Malik and van der Zee, 1978a). The development of pulmonary edema and progressive hypoxia in dogs after microembolization was related to fibrin accumulation in the lung rather than to platelet aggregation (Saldeen, 1976). In contrast, other studies in sheep have suggested that the release of humoral factors subsequent to fibrin and platelet entrapment in the lung may not be important since it was not possible to prevent the microembolization-induced increases in lung vascular permeability after defibrinogenation (Binder and Staub, 1978), thrombocytopenia (Binder et al., 1978), or pretreatment with heparin (Staub and Binder, 1978).

Studies have also indicated that fibrin deposition in the lung following microembolization occurred rapidly, reaching a maximum within 10 minutes, and was cleared within 1 hour after microembolization (Saldeen, 1969a; Saldeen, 1976). The rapid fibrinolysis was due to the activation of plasminogen since its inhibition with epsilon aminocaproic acid or tranexamic acid prevented clearance of fibrin from the lungs (Saldeen, 1976; Malik and van der Zee, 1977). Because the time-dependent alterations in fibrinolysis and fibrin entrapment in the lung may alter the degree of fluid accumulation, in the present study we examined the time course of changes in lung water following microembolization in the dog.

Methods

We studied 31 supine mongrel dogs anesthetized with sodium pentobarbital, 25 mg/kg, iv. Catheters (6F) were positioned in the main pulmonary artery, left atrium or pulmonary arterial wedge, aortic arch, and right atrium. The left atrial catheter was passed...
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retrogradely via a femoral artery in random experiments to ensure the validity of the pulmonary arterial wedge pressure. Vascular pressures were recorded using Statham P23Db pressure transducers referred to the level of the left atrial appendage. The reference level was verified at autopsy. Pulmonary blood flow was determined in triplicate using the indicator-dilution technique. Cardiogreen dye was injected into the right atrium and dilution curves were recorded from the aorta using a Gilford Instruments 1031R cuvette-densitometer and Harvard infusion-withdrawal pump. The pulmonary vascular resistance was calculated as the pulmonary arterial-pulmonary arterial wedge pressure gradient/pulmonary blood flow ratio.

The endotracheal tube was connected to a Harvard respirator that was adjusted to provide blood gases and pH in the normal range at the start of the experiment. Tidal volume and respiratory rate were determined prior to the experiment using a Collins spirometer. The baseline arterial P02 ranged from 72 to 95 mm Hg, arterial Pco2 values ranged from 33 to 38 mm Hg, and arterial pH values ranged from 7.34 to 7.39. The dogs were paralyzed with pancuronium bromide (0.1 mg/kg, iv), and the lungs were hyperinflated periodically to prevent atelectasis.

Glass beads 100 μm in diameter (3-M Co.) were used to induce pulmonary microembolization. Earlier experiments indicated that glass beads (0.7 g/kg body weight) infused into the jugular vein over a 5-minute period provided a sufficient degree of embolization to induce hypoxemia and prevent homogenization of the lung was assessed by subtracting the 125I activity predicted from the lung blood weight measured from the 125I activity. This value is referred to as the excess 125I activity and is expressed as counts per minute (cpm) per gram bloodless dry lung weight to correct for any changes in the lung water and amount of blood trapped in the lungs during the experiment.

Studies were made in four groups: group I (n = 6), control dogs; group II (n = 8), dogs injected with 0.7 g/kg glass beads and killed 1 hour postembolization; group III (n = 11), dogs injected with 0.7 g/kg glass beads and killed 2 hours postembolization; and group IV (n = 6), dogs injected with 0.7 g/kg glass beads and killed 2 hours postembolization. In addition, these dogs received an inhibitor of plasminogen activation, tranexamic acid, 50 mg/kg (transaminoethyl cyclohexane carboxylic acid), 15 minutes prior to embolization and received 100 mg/kg as an infusion during the 2-hour postembolization period. The significance of differences between groups was tested by an overall one-way analysis of variance combined with a multiple comparison method (Eilers, 1967). In the dogs receiving glass bead emboli, the wet and dry lung weights were corrected for beads injected by subtracting the weight of the beads from the wet and dry lung weights, thereby enabling comparison of lung water between the nonembolized and embolized groups.

We determined the fraction of the injected beads that were lodged in the lungs in three dogs. The lung containing emboli was homogenized and the homogenate was dissolved with 18 M H2SO4. The mixture then was passed through a fine wire-mesh filter (USA Standard Testing Sieve, 180 mesh) to recover the beads. In the three dogs, the percents of injected beads recovered were 89%, 92%, and 95% of the total injected. Because none was found at autopsy in the right heart, jugular vein, and inferior and superior vena cavae, the incomplete recovery may be due to the insensitivity of the extraction procedure and the loss occurring from the pulmonary artery during removal of the lung.

Pulmonary intravascular coagulation following glass bead microembolization was assessed by injection of 100 μCi of 125I-labeled fibrinogen (Abbott Laboratories) 3 hours prior to the experiment as described previously (Malik and van der Zee, 1978a). Arterial and mixed venous blood samples were taken at the end of the experiment. Prior to homogenization, the dried inflated lung was cut into small pieces (approximately 2 cm long and 1 cm wide) and inserted into counting tubes. The lung and blood were counted with similar geometry in a Nuclear-Chicago gamma counter. The occurrence of intravascular coagulation in the lung was assessed by subtracting the 125I activity predicted from the lung blood weight measured from the 125I activity. This value is referred to as the excess 125I activity and is expressed as counts per minute (cpm) per gram bloodless dry lung weight to correct for any changes in the lung water and amount of blood trapped in the lungs during the experiment.

Results

Figure 1 indicates the changes in pulmonary hemodynamics in the 1- and 2-hour untreated groups.
with pulmonary emboli. The left atrial pressure, pulmonary arterial wedge pressure, and pulmonary blood flow did not change significantly following embolization in either group. The increases in pulmonary arterial pressure \( (P_{pa}) \), pulmonary perfusion pressure \( (\Delta P_{p}) \), and pulmonary vascular resistance \( (PVR) \) in dogs that had emboli 1 or 2 hours after embolization were not significantly different (Fig. 1). \( P_{pa}, \Delta P_{p}, \) and \( PVR \) remained elevated in both groups for the duration of the study.

Table 1 shows the body weights and weights of wet lung, dry lung, residual blood, extravascular lung water, and bloodless dry lung in the control, 1- and 2-hour untreated postembolization groups, and 2-hour postembolization group treated with tranexamic acid. According to the analysis of variance, the weights of residual blood content and bloodless dry lung in the four groups were not significantly different. The wet lung weights in the three postembolization groups were greater \( (P < 0.05) \) than control, and there were no significant differences between postembolization groups. The dry lung weight was increased above control \( (P < 0.05) \) only in the tranexamic acid-treated group, but this value was not different from the values in the other postembolization groups. The extravascular lung water contents were significantly greater \( (P < 0.05) \) in all postembolization groups than in the controls; however, the increase at 1 hour was greater than at 2 hours postembolization, since the 1- and 2-hour values were significantly different from each other \( (P < 0.05) \). The mean extravascular lung water/bloodless dry lung weight ratio at 1 hour postembolization of 6.05 ± 0.82 was greater \( (P < 0.05) \) than the control value of 3.10 ± 0.30 and decreased \( (P < 0.05) \) to 4.17 ± 0.38 at 2 hours postembolization; the value at 2 hours postembolization was not significantly different from the control.

Tracheal fluid indicative of gross alveolar edema was present in two of the eight 1-hour postembolization dogs with extravascular lung water/bloodless dry lung weight ratios of 9.30 and 8.62, but tracheal edema fluid was not present in any of the 10 2-hour postembolization dogs in which the extravascular lung water content was determined. The excess \(^{125}\)I activity per g bloodless dry lung of 9898.2 ± 1070.7 cpm/g in the 1-hour postembolization groups has been corrected for the weight of glass beads injected. The values are indicated as mean ± 1 SEM.

In the postembolization groups, the lung weights have been corrected for the weight of glass beads injected. The values are indicated as mean ± 1 SEM.

\* Different from control \( (P < 0.05) \).

† Different from 120-minute embolization \( (P < 0.05) \).
The excess $^{125}$I activity per g bloodless dry lung of $4957.5 \pm 1193.1$ cpm/g at 2 hours postembolization in the tranexamic acid-treated group was greater ($P < 0.05$) than the value of $1321.6 \pm 341.8$ cpm/g in the untreated group in which fibrinolysis was not inhibited. There was a greater degree of pulmonary edema at 2 hours postembolization when fibrinolysis was inhibited ($P < 0.05$) than in the untreated group (Table 1). The extravascular lung water content/bloodless dry lung weight ratio in the tranexamic acid-treated group was greater ($P < 0.05$) than the values in the 2-hour untreated postembolization group (Table 1). The extravascular lung water content in the treated group was not statistically different from the untreated postembolization groups (Table 1), possibly due to the lower body weights of the tranexamic acid-treated dogs. The greater extravascular lung content/bloodless dry lung weight ratio in the postembolization dogs that received tranexamic acid was not due to differences in the pulmonary hemodynamic response to microembolization. In tranexamic acid-treated dogs, $P_{w}$ increased from a baseline value of $12.5 \pm 1.6$ mm Hg to $33.1 \pm 2.7$ mm Hg at 5 minutes postembolization, and the value at 2 hours postembolization of $35.2 \pm 4.1$ mm Hg was not significantly different from the 5-minute postembolization value. PVR increased from a baseline value of $5.7 \pm 1.1$ mm Hg/liter per min to $12.8 \pm 2.8$ mm Hg/liter per min, 5 minutes postembolization, and the value at 2 hours postembolization of $15.3 \pm 3.1$ mm Hg/liter per min also was not significantly different from the 5-minute postembolization value. The pulmonary hemodynamic changes in tranexamic acid-treated group following embolization were not significantly different from changes in the 2-hour untreated embolized group (Fig. 1).

**Discussion**

The release of humoral factors subsequent to pulmonary thromboembolization may mediate the increase in extravascular lung water content (Saldeen, 1976; Malik and van der Zee, 1978a). Pulmonary edema did not develop in dogs given heparin prior to embolization, which suggested that the release of humoral factors was related to intravascular coagulation (Malik and van der Zee, 1978a). Saldeen (1976) suggested that microembolization-induced pulmonary edema occurs in association with fibrin in the lung since the decrease in arterial $P_{O_2}$ following pulmonary thromboembolization was associated with fibrin accumulation rather than platelet aggregation. Depletion of platelets and leukocytes in dogs did not appear to prevent the pulmonary lesions resulting from microembolization induced by thrombin infusion, which suggested that pulmonary edema was not due to platelet aggregation or leukocyte trapping in the lung (Busch et al., 1974). These findings have suggested indirectly that pulmonary edema after embolism is the consequence of fibrin accumulation in the lung. Evidence indicates that, after vascular injury, fibrin may be present not only in the lung vessels but also in the extravascular spaces (Meyer and Ottaviano, 1973). Bachofen and Weibel (1977) observed that intravascular fibrin was rare in septic lung disease, but was found in the interstitial space and alveoli. The lack of intravascular fibrin may be due to an increase in pulmonary endothelial fibrinolytic activity following microembolization, resulting in dissolution of fibrin "plugs" (Risberg, 1975). In contrast to studies in dogs in which intravascular coagulation may play a role in mediating pulmonary edema after microembolization (Saldeen, 1976), other studies in sheep have shown that the microemboliza-
tion-induced increases in lung vascular permeability could not be prevented by pretreatment with heparin (Staub and Binder, 1978), defibrinogenation (Binder and Staub, 1978), or platelet depletion (Binder et al., 1978).

Because the total cardiac output passes through the pulmonary vascular bed, the lung endothelial cells play a crucial role in fibrinolysis (Ryan and Ryan, 1977). In addition, the tissue factors responsible for fibrinolysis, the plasminogen activators, occur most abundantly in association with vessels of the brain, heart, and lungs (Meyer and Ottaviano, 1973; Risberg, 1975; Ryan and Ryan, 1977). Deposition of fibrin occurred rapidly within 10 minutes after microembolization with homogenized adipose tissue in rabbits and rats; by 60 minutes most of the fibrin had disappeared from the lungs (Saldeen, 1969a; Saldeen, 1969b). The inhibition of fibrinolysis with inhibitors of plasminogen activation such as epsilon amino caproic acid or tranexamic acid prevented the clearance of fibrin after microembolization (Saldeen, 1969a; Saldeen, 1969b; Saldeen, 1976). Because the clearance of fibrin from the lung after thromboembolization normally occurs rapidly and because intravascular coagulation and fibrin accumulation have been implicated in the development of microembolus-induced pulmonary edema, in the present study we examined the time course of changes in extravascular lung water after microembolization.

The occurrence of intravascular coagulation and secondary thrombosis in the lungs postembolization was assessed by determining the accumulation of $^{125}$I activity in the lung following the injection of $^{125}$I-fibrinogen (Malik and van der Zee, 1978a). Previous studies indicated that thrombi were formed in dog lungs after glass bead embolization, as assessed by an increase in the $^{125}$I activity, and that this could be prevented by prior treatment with heparin (Malik and van der Zee, 1978a). In the present study, the lung $^{125}$I activity increased at 1 hour postembolization and decreased toward control levels at 2 hours postembolization. These changes were associated with an increase in the extravascular lung water content at 1 hour and return toward control levels at 2 hours. The possibility exists that there may be increased leakage of $^{125}$I-fibrinogen, particularly if the pulmonary vascular permeability is increased, as in the case after pulmonary microembolization (Vaage et al., 1976; Ohkuda et al, 1978; Malik and van der Zee, 1978b), thereby overestimating the intravascular $^{125}$I activity and fibrin accumulation. However, it was shown that the extravascular $^{125}$I-fibrinogen activity remained constant after the induction of intravascular coagulation and pulmonary microembolization with thrombin (Busch and Rammer, 1973; Busch et al., 1973). Although the amount of fibrin in the lungs postembolization may have been overestimated due to extravascular fibrinogen leakage, this does not preclude the marked differences in the excess $^{125}$I activity per g bloodless dry lung in the two embolization groups ($9898.2 \pm 1070.7$ cpm/g/1 hour postembolization vs. $1321.6 \pm 341.8$ cpm/g/2 hours postembolization ($P < 0.01$)). The leakage of fibrin degradation products into the extravascular space may, however, account for approximately 50% of the excess $^{125}$I activity observed at 1 hour postembolization because, when fibrinolysis was inhibited with tranexamic acid, the excess $^{125}$I activity averaged $4967.5 \pm 1193.1$ cpm/g/2 hours postembolization.

Despite the overestimation in the extent of fibrin entrapment, the results suggest a time-dependent decrease in fibrin accumulation after embolization which was associated with a decrease in the extravascular lung water content. These data are consistent with observations that fibrin is cleared rapidly from the lung vessels in dogs due to the activation of fibrinolysis (Saldeen, 1976; Risberg, 1975).

The increases in pulmonary arterial pressure and pulmonary vascular resistance were not significantly different in the 1- or 2-hour postembolization groups. The similar increases in pulmonary vascular resistance suggest comparable degrees of vascular obstruction. Because the time-dependent decrease in lung water following microembolization cannot be explained on the basis of differences in the pulmonary hemodynamic response, the decrease in the extent of pulmonary edema 2 hours following microembolization may be due to clearance of fibrin from the lungs.

To determine whether the initial increase and then the decrease in extravascular lung water content 2 hours after microembolization were due to varying levels of fibrin in the lung, we determined the severity of pulmonary edema in dogs given tranexamic acid, which inhibits plasminogen activation and thereby prevents fibrinolysis (Risberg, 1975). As expected, the tranexamic acid-treated dogs had greater fibrin accumulation 2 hours postembolization, but also had a greater degree of pulmonary edema than the untreated 2-hour postembolization group. Therefore, the time-dependent decrease in lung water toward control values after microembolization may be due to clearance of fibrin from lung vessels as the result of enhanced fibrinolytic activity (Risberg, 1975; Saldeen, 1976) and the subsequent "washout" of humoral factors that produce edema (Saldeen, 1976; Malik and van der Zee, 1978a).

Studies in sheep and dogs indicated increases in protein clearance from lung vessels (Ohkuda et al., 1978; Costabella et al., 1978; Malik and van der Zee, 1978b) and in the lymph/plasma protein concentration ratio following microembolization (Costabella et al., 1978; Malik and van der Zee, 1978b), suggesting an increase in lung vascular permeability. Vaage et al. (1976) demonstrated that the increase in
vascular permeability in the isolated perfused rabbit lungs was transient; a decrease to baseline permeability levels was apparent 30 minutes after embolization. However a reversible increase in lung vascular permeability was not observed in the intact sheep (Okhuda et al., 1978; Malik and van der Zee, 1978b). The mechanism by which humoral factors lead to transiently increased permeability is not clear, but humoral factors may "round up" junctions of endothelial cells, leaving larger interendothelial junctions, or promote increased vesicular transport (Effros, 1978). Substances such as histamine open interendothelial junctions in systemic microvessels by contracting fibrils in endothelial cells (Majno et al., 1969), although there are no data to support this mechanism in lung vessels. The time-dependent decrease in permeability following microembolization in isolated lungs may be due to a decrease in the levels of circulating humoral factors (Vaage et al., 1976). Our observation that extravascular lung water content initially increased and then decreased following embolization is consistent with the data indicating reversible increases in lung vascular permeability following embolization (Vaage et al., 1976).

Another mechanism which may partly explain the time-dependent decrease in the extravascular lung water content following microembolization is the intrinsic ability of the lung to clear excess extravascular fluid by increasing pulmonary lymph flow (Staub, 1974). The results from studies on sheep in which pulmonary lymph was obtained indicated that lymph flow increased 2- to 3-fold after microembolization (Okhuda et al., 1978; Malik and van der Zee, 1978b). A reasonable estimate of the resting total lung lymph flow in a dog is 10 ml/hr (Staub, 1974). To assume that the increase is 3-fold would explain only a fraction of the decrease in the extravascular lung water content occurring between the first and second hours after embolization (Table 1), which suggests that the main factor is clearance of fibrin from lung vessels.

In summary, the data indicate a time-dependent decrease in the degree of pulmonary edema which was related to a decrease in fibrin accumulation in the lung following microembolization in dogs. The decrease in lung water following embolization was prevented by inhibiting fibrinolysis. The time-dependent changes in lung water were not due to differences in the pulmonary hemodynamic response, since pulmonary arterial pressure and pulmonary vascular resistance remained elevated after embolization. Therefore, the reversibility of pulmonary edema following microembolization may be due to clearance of fibrin. This may be a reparative mechanism in the lung for reducing the degree of fluid accumulation after microembolization.

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References

Busch C, Rammer L (1973) Quantitation of fibrin deposition and elimination in organs of rats injected with labelled fibrinogen and by isolation of the labelled fibrin from water soluble tracer. Thromb Diath Haemorrh 29: 108-114
The role of fibrin in the genesis of pulmonary edema after embolization in dogs.
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