JCI The Journal of Clinical Investigation

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J Clin Invest. 1983;71(2):351-357. https://doi.org/10.1172/JCI110776.

Research Article

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Acetyl Glyceryl Ether Phosphorylcholine-stimulated Human Platelets Cause Pulmonary Hypertension and Edema in Isolated Rabbit Lungs

ROLE OF THROMBOXANE A_2

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ABSTRACT Macrophages, neutrophils, and platelets may play a role in acute edematous lung injury, such as that seen in the adult respiratory distress syndrome (ARDS), but their potential actions and interactions are unclear. Because stimulated human macrophages and neutrophils can release acetyl glyceryl ether phosphorylcholine (AGEPC), a potent platelet activator, we hypothesized that in ARDS, leukocyte release of AGEPC might stimulate platelets to release thromboxane A_2 (TXA_2), which then produces pulmonary hypertension and lung edema. In support of this premise, we found that pulmonary hypertension and edema occurred in isolated rabbit lungs perfused with human platelets and AGEPC, but not with platelets or AGEPC alone. Infusion of a vasodilator (nitroglycerin) to maintain base-line pulmonary artery pressures in lungs perfused with platelets and AGEPC prevented the development of lung edema suggesting that platelet and AGEPC-induced edema was hydrostatic in nature. Additional experiments suggested that the increased pressure was a result of TXA_2 release from platelets stimulated by AGEPC. Specifically, preincubation of platelets with imidazole, a thromboxane synthetase blocker, prior to infusion with AGEPC significantly diminished pulmonary hypertension and prevented lung edema. Furthermore, pretreating lung preparations with 13-azaprostanoic acid, a TXA_2 antagonist, before infusion of AGEPC and untreated platelets also reduced the pulmonary hypertension and blocked the lung edema. The role of TXA_2 was further suggested when perfusates from lungs infused with platelets and AGEPC developed high levels of TXA_2, whereas perfusates from controls did not. These results suggest that platelet aggregation induced by AGEPC may contribute to ARDS by releasing TXA_2, which raises microvascular pressure and increases edema formation, especially when an underlying permeability defect is present.

INTRODUCTION

The occurrence of thrombocytopenia (1), pulmonary platelet microthrombi (2), and intrapulmonary platelet sequestration (3) in the adult respiratory distress syndrome (ARDS)[1] suggests that platelets may contribute to this form of acute edematous lung injury. Moreover, previous investigations in whole animals (4–9) and isolated lungs perfused with whole blood (10) have indicated that activated platelets may release vasoactive substances that generate pulmonary hyper-

Preliminary reports of this work were presented to the Western Section of the American Federation of Clinical Research, February 1982, American Thoracic Society, May 1982, and the American Federation of Clinical Research, May 1982.

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Received for publication 20 May 1982 and in revised form 19 October 1982.

[1] *Abbreviations used in this paper*: AGEPC, acetyl glyceryl ether phosphorylcholine; ARDS, adult respiratory distress syndrome; HBSS, Hanks' balanced salt solution; 6-k-PGF_{1a}, 6-keto prostaglandin F_{1a}; PRP, platelet-rich plasma; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2.

tension and increased hydraulic conductivity. The complicated nature of these studies with their multiple organ and cell interactions, however, has not allowed definition of the specific contribution of platelets to these results or the exact mechanisms involved. Recently, the elucidation of platelet activating factor (acetyl glyceryl ether phosphorylcholine, AGEPC) has created further interest in the potential role of platelets in the pathogenesis of ARDS. This naturally occurring phospholipid is released by human alveolar macrophages (11) and neutrophils (12) and is a potent stimulant of neutrophil degranulation (13) and platelet aggregation (14). AGEPC also causes platelets to release cyclooxygenase-dependent arachidonate metabolites such as thromboxane A_2 (TXA_2) (15). Furthermore, infusion of AGEPC into whole animals decreases the number of circulating neutrophils and platelets and generates respiratory distress and acute pulmonary hypertension (16–18). Although these observations suggest a role for AGEPC in acute lung injury, it is unclear whether these pulmonary effects are dependent on platelets, neutrophils, other cellular agents, or direct vascular effects of AGEPC (19).

In the present investigation, we used the isolated perfused lung model to address these issues. This approach eliminates extrapulmonary influences and allows isolation of platelets or neutrophils as the sole perfusing cellular blood product. The results of this investigation show that AGEPC-activated human platelets, but not neutrophils, cause acute pulmonary hypertension and hydrostatic edema in isolated rabbit lungs, and that (TXA_2) released from platelets is the major mediator of these changes.

METHODS

Source of reagents. Imidazole and bovine serum albumin (BSA, Cohn fraction V) were obtained from Sigma Chemical Co., St. Louis, MO. Imidazole was dissolved in 1% BSA (250 μg/ml). Dr. R. Broersma (Dow Chemical Corp., Indianapolis, IN) provided 13-azaprostanoic acid that was dissolved in absolute ethanol before dilution in 1% BSA. The majority of the studies were performed using highly purified AGEPC obtained from Sedary Research Laboratory (London, Ontario). AGEPC obtained from Bachem (Budendorf, Switzerland) produced similar results. After evaporation of the chloroform diluent, AGEPC was dissolved in absolute ethanol and then diluted with 1% BSA (10 μg/ml). Ficoll-Hypaque and 6% Dextran were supplied by Pharmacia Fine Chemicals, Piscataway, NJ. Heparin (1,000 U/ml) was supplied by Abbott Laboratories, No. Chicago, IL. Aqueous nitroglycerin lactose (Eli Lilly & Co., Indianapolis, IN) was used as the vasodilator. Acetylsalicylic acid (Sigma Chemical Co.) 0.4% stock solutions were prepared in normal saline and titrated to a pH of 7.4.

Preparation of platelets. Venous blood was obtained with a two-syringe technique from normal volunteers who had not taken any medications for 7 d. Each 45 ml of blood was anticoagulated with 5 ml acid citrate dextrose and centrifuged at 100 *g* for 10 min at 24°C for production of platelet-rich plasma. Platelet-poor plasma was made by centrifuging citrated blood at 1,000 *g* for 20 min after removal of the platelet-rich plasma fraction as prepared above.

Preparation of neutrophils. Neutrophils were purified from human blood as described (20). Briefly, 100 ml of venous blood was drawn into a syringe that contained heparin (100 U). Dextran 6% (8 ml) was then added and the erythrocytes were allowed to sediment for 90 min. Subsequently, the leukocyte-rich serum was aspirated, underlain with Ficoll-Hypaque and centrifuged at 800 rpm for 10 min and then at 1,500 rpm for 10 min. Sedimented leukocytes were washed and resuspended in Hanks' balanced salt solution (HBSS) for addition to the lung perfusate. These preparations contained 94–96% neutrophils and 4–6% monocytes.

Preparation of isolated perfused lungs. Male and female New Zealand White rabbits, weighing 2–2.5 kg, were anesthetized with intramuscular xylazine (10 mg/kg; Cutter Laboratory, Shawnee, KS) and intravenous ketamine (25–50 mg/kg; Parke-Davis & Co., Detroit, MI). Tracheostomies allowed ventilation with room air by a respirator (model 6700; Harvard Apparatus Co., Millis, MA). After a midsternal thoracotomy, heparin (500 U) was injected into the right ventricle. Plastic cannulae were then inserted into the pulmonary artery and left ventricle. Lung preparations were subsequently flushed with 500 ml of a mixture of 3% BSA and Greenberg-Bohr physiologic salt solution (21) (38°C, pH 7.4) using a peristaltic pump (model 1203; Harvard Apparatus Co.) at a flow rate of 40 ml/kg body wt per min. After isolating the lungs and suspending them in a humid chamber of 38°C, an extracorporeal lung perfusion circuit was instituted with 300 ml of the 3% BSA-physiologic salt solution at the previous flow rate. Pulmonary artery pressures were measured continuously with a pressure transducer (Bell and Howell Co., Pasadena, CA, type 4-327-0010) and recorded with a multichannel recorder (VR-6 Electronics for Medicine, White Plains, NY). Perfusate samples drawn from the left ventricular cannula were analyzed for pH, P_{CO_2}, and P_{O_2} with Radiometer microelectrodes (Radiometer Co., Copenhagen, Denmark). After the start of perfusion, the lungs were ventilated with a 95% room air and 5% CO_2 gas mixture that was warmed and humdified. Tidal volume was 10–15 ml/kg body wt, the respiratory rate was 24 breaths/min, and the end-expiratory pressure was 2 cm H_2O. Perfusate gas tensions and pH were maintained at P_{CO_2} = 20–28 mmHg, P_{O_2} = 100–130 mmHg and pH = 7.38–7.46.

Measurements of lung weights and lung lavage albumin concentrations. The isolated lungs were suspended from a force-displacement transducer (Grass Instrument Co., Quincy, MA, model FT10 G) for continuous weight monitoring. All experiments except for pressure controls were stopped 55 min after the start of perfusion. The left lung was then isolated and lavaged via the left mainstem bronchus with 20 ml isotonic saline. Albumin concentrations on samples of these lung lavages were determined by the bromocresol green method (Sigma Technical Bulletin no. 630, revised October 1976).

Measurement of perfusate platelet counts. Perfusate samples (3 ml) were drawn from the left ventricular cannula 25 min after the start of perfusion (5 min after infusion of platelet-rich plasma). Samples were placed in tubes containing EDTA. Platelet counts were then determined by a Coulter counter (ZBI, Coulter Electronics, Inc., Hialeah, FL).

Protocol for infusion of platelets and AGEPC. After a 20-min stabilization period, platlet-rich plasma (10.1±1.9 ml) containing 4.3±0.3 × 10^9 platelets was infused into the reservoir resulting in a peak perfusate platelet count of

16.0±3.0. 5 min later, AGEPC (20 μg) was infused into the pulmonary arterial cannula. Platelet control studies were done by infusing platelet-rich plasma (10.0±2.0 ml) (4.3±0.3 × 10^9 platelets) at 20 min (peak platelet count of 16.0±2.5 × $10^3/mm^3$) without subsequent infusion of AGEPC. Platelet-poor plasma control studies involved infusion of platelet-poor plasma (5.0±0.3 × 10^7 platelets in 10 ml of plasma) at 20 min with subsequent infusion of AGEPC 5 min later. AGEPC and saline controls were also performed with these agents infused at 25 min in the absence of platelet infusion (peak platelet count 3.0±0.1 × $10^3/mm^3$).

Protocol for infusion of neutrophils and AGEPC. After a 20-min stabilization period, purified neutrophils (2.4±0.3 × 10^8 neutrophils in 10 ml of HBSS) were infused into the reservoir. In previous isolated lung studies, lung injury was produced when this quantity of neutrophils was infused with phorbol myristate acetate (22). 5 min after infusion of neutrophils, AGEPC (20 μg) was instilled into the PA cannula.

Sample collection and measurement methods of TXA_2 and 6-k-$PGF_{1\alpha}$ by radioimmunoassay. Perfusate samples (3 ml) were collected at 20, 25, 30, 40, and 55 min after start of lung perfusion. Acetylsalicylic acid (0.4%, 20 μl) was then added to the samples, which were immediately placed on ice. Samples were subsequently spun at 2,000 *g* for 15 min at 4°C and the supernates were stored at −70°C. Assays for thromboxane B_2 (TXB_2, the stable metabolite of TXA_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-k-$PGF_{1\alpha}$, the measurable metabolite of prostacyclin) were done on samples by radioimmunoassay methods previously described. Prostaglandins for standard curves were gifts from Dr. John Pike of the Upjohn Co. (Kalamazoo, MI). The collection techniques and radioimmunoassay methods have been shown to give prostaglandin and thromboxane measurements comparable to those obtained with gas chromatography-mass spectrometry (23).

Perfusion pressure control experiments. Pressure control studies were conducted by infusing the vasodilator nitroglycerin (2.0±1.0 mg) to maintain base-line perfusion pressures (9.0±2.0 mmHg) after activating platelets (10.1±1.9 ml platelet-rich plasma (PRP), 4.3±0.3 × 10^9 platelets, peak platelet count 15.0±1.0 × $10^3/mm^3$) with AGEPC (20 μg). In most experiments, nitroglycerin was not added until several seconds after perfusion pressures had initially doubled to ensure an active system. The infused nitroglycerin returned the perfusion pressures to base-line values within several seconds. Left atrial pressures were increased after the usual 55-min protocol to stress the alveolar-capillary membrane and reveal a subtle permeability defect if one was present. This pressure challenge was applied by placing a partially occlusive clamp on the left ventricular cannula to generate a 10-mmHg pressure load on the left atrium. After 10 min the clamp was released, and the lung was perfused for an additional 5 min (total perfusion time, 70 min). The lungs were then lavaged as previously described.

Preincubation of platelets with imidazole. Protocols using imidazole-treated platelets were identical to those in AGEPC-platelet studies except that platelets (10.1±0.9 ml PRP, peak platelet count of 14.0±0.5 × $10^3/mm^3$) were preincubated for 30 min with 50 μg/ml imidazole before infusion. This concentration of imidazole predominantly inhibits thromboxane synthesis without major effects on the synthesis of other arachidonate metabolites (24).

Preincubation of the lung with 13-azaprostanoic acid. Protocols using the thromboxane receptor site blocker were the same as those used in the AGEPC-platelet studies except that 0.93 mg of 13-azaprostanoic acid (0.3 μM) was infused into the pulmonary artery cannula 10 min after the start of perfusion. Platelets (10.0±0.9 ml PRP, peak platelet count of 15.0±2.0 × $10^3/mm^3$) were subsequently infused followed by AGEPC as in previous studies.

Statistical analysis. All data was analyzed using one-way analysis of variance except for the lung weight data, which were compared using Kruskal-Wallis one-way analysis by ranks.

RESULTS

Effect of platelets and AGEPC or neutrophils and AGEPC on the development of hypertension and edema formation in isolated perfused lungs. Addition of platelet-rich plasma and AGEPC caused increases in perfusion pressures (Fig. 1), lung weights, and lung lavage albumin concentrations (Fig. 2) in isolated lungs that were significantly greater than control lungs infused with platelets alone, AGEPC alone, saline, or AGEPC plus platelet-poor plasma. The platelet-poor plasma control studies excluded the possibility that AGEPC interacted with a plasma factor producing the observed lung pressure and weight changes. Lungs infused with neutrophils and AGEPC showed no changes in perfusion pressures (12.3±1.8 mmHg), lung weights (0 wt gain), or increases in lung lavage albumin concentrations (8.0±3.2 mg/dl). There were no significant differences in peak perfusate platelet counts between experiments where AGEPC and platelets or platelets alone were infused.

Effect of perfusion pressures on edema formation in isolated lungs perfused with platelets and AGEPC. Edema formation in isolated lungs observed after infusion of AGEPC and platelets could have been caused

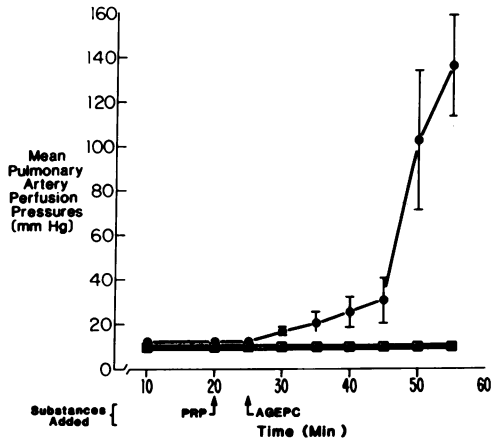

FIGURE 1 Addition of platelets (PRP) plus AGEPC (●) caused significant ($P < 0.005$) increases in mean perfusion pressures in isolated perfused lungs compared to lungs infused with platelets (▲) or AGEPC (□) alone. Each point is the mean±SEM. Infusion of platelet-poor plasma (PPP) plus AGEPC caused no increase in mean perfusion pressures (data not shown).

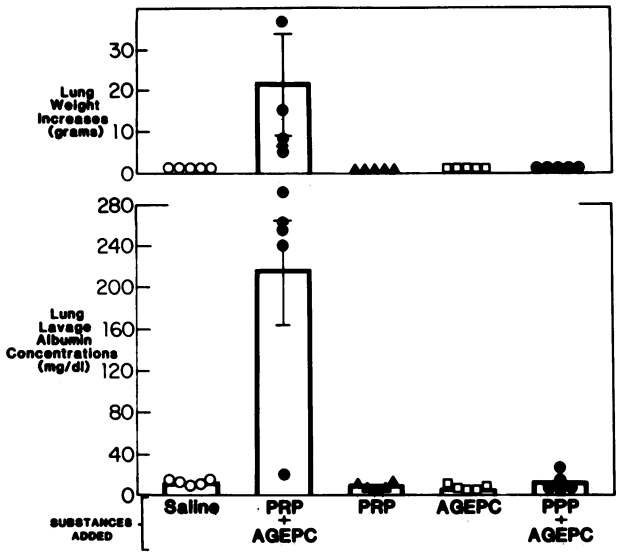

FIGURE 2 Platelets plus AGEPC (●) caused significant increases in lung weights ($P < 0.05$) (top panel) and lung lavage albumin concentrations ($P < 0.005$) (bottom panel) compared to control experiments. Each point represents data from one experiment.

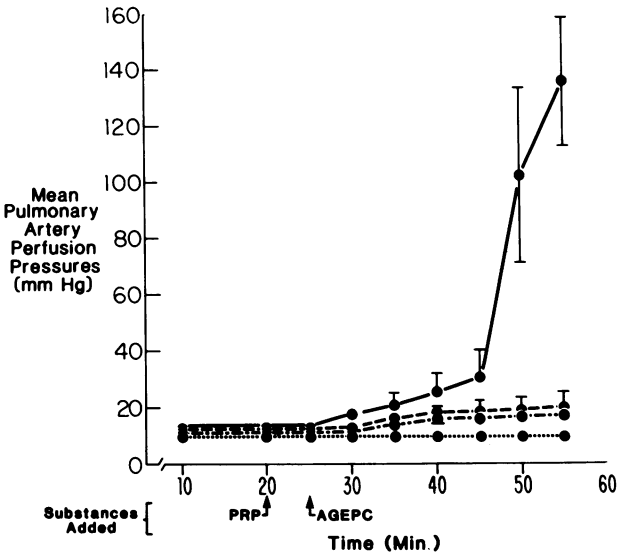

FIGURE 3 Incubation of platelets and AGEPC with imidazole (● -·-· ●) or infusion of 13-APA (● - - - ●) into lungs perfused with platelets and AGEPC prevented ($P < 0.005$) increased perfusion pressures. Infusion of nitroglycerin (● · · · ●) completely blocked the pressure rise in lungs perfused with platelets and AGEPC. (● —— ●).

by a membrane permeability defect or by hydrostatic forces. To determine which form of lung edema developed, we infused AGEPC and platelets into lungs in which base-line perfusion pressures were maintained by parallel infusion of nitroglycerin (Fig. 3). In contrast to the previous findings, during the usual 55-min perfusion period and a subsequent 10-min pressure challenge period (10 mmHg left atrial pressure load), there were no increases in lung weights or lung lavage albumin concentrations in nitroglycerin-treated lungs perfused with AGEPC and platelets (Fig. 4). These observations suggested that the edema was caused by the increased perfusion pressure and resultant hydrostatic forces rather than by direct alveolar-capillary membrane injury.

Effect of imidazole, a thromboxane synthetase blocker, on the development of hypertension and edema formation in isolated lungs perfused with platelets and AGEPC. Infusion of AGEPC and imidazole-treated platelets did not produce significant increases in perfusion pressures (Fig. 3), lung weights, or lung lavage albumin concentrations (Fig. 4) in comparison to infusion of AGEPC with untreated platelets. Peak platelet counts in perfusates in these experiments were similar to counts in experiments where untreated platelets and AGEPC were infused.

Effect of 13-azaprostanoic acid, a TXA_2 antagonist, on the development of hypertension and edema formation in isolated lungs perfused with platelets and AGEPC. The previous studies suggested that platelet TXA_2 might contribute to production of hypertension

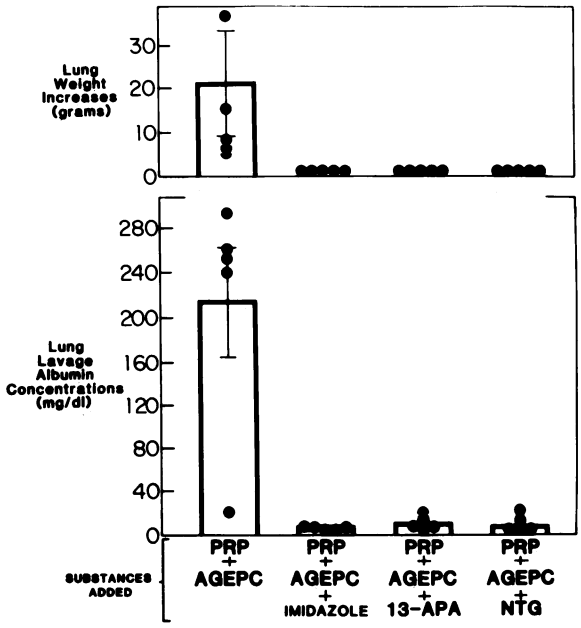

FIGURE 4 Infusion of imidazole-treated platelets and AGEPC did not cause increases in lung weights ($P < 0.05$) or lung lavage albumin concentrations ($P < 0.005$). Pretreatment of lungs with 13-azaprostanoic acid prior to infusion of platelets and AGEPC also prevented increases in lung weights ($P < 0.05$) and lung lavage albumin concentrations ($P < 0.005$). Maintenance of base-line perfusion pressures additionally prevented AGEPC-stimulated platelet increases in lung weights ($P < 0.05$) and lung lavage albumin concentrations ($P < 0.005$).

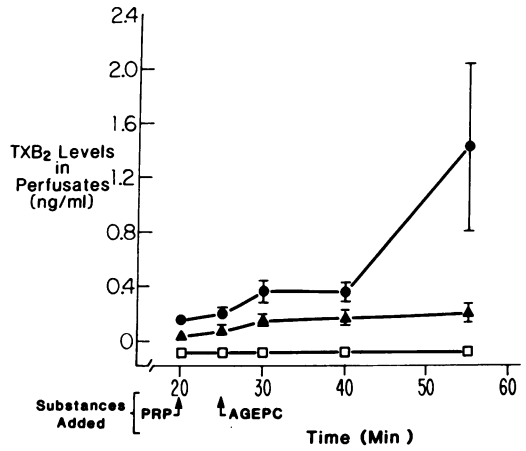

FIGURE 5 TXB_2 increased in perfusates of lungs infused with platelets and AGEPC (●) (n = 5), but not in lungs perfused with platelets (▲) (n = 4) or AGEPC (□) (n = 2) alone.

and edema in isolated lungs perfused with platelets and AGEPC. To further identify a role for TXA_2, lung preparations were infused with 13-azaprostanoic acid (a TXA_2 antagonist) prior to instillation of platelets and AGEPC. Instillation of 13-azaprostanoic acid inhibited increases in perfusion pressures (Fig. 3) and prevented increases in lung weights and lung lavage albumin concentrations (Fig. 4) in isolated lungs perfused with platelets and AGEPC. There were no differences in peak perfusate counts measured at 25 min in these experiments compared to experiments where only platelets and AGEPC were infused.

TXB_2 and 6-k-PGF_{1a} concentrations in lung perfusates. TXB_2 levels, which are used as an index of TXA_2 production, were higher in perfusates from lungs infused with AGEPC and platelets (1,421±624 pg/ml) than in controls (platelet controls, 208±76 pg/ml, AGEPC controls, 12±12 pg/ml) (Fig. 5). There were no significant differences in perfusate 6-k-PGF_{1a} levels in these experimental groups. The addition of nitroglycerin did not significantly affect peak TXB_2 concentrations (1,105±802 pg/ml). The time course of TXB_2 generation after infusion of platelets and AGEPC paralleled the pressure rise in these experiments. Finally, 6-k-PGF_{1a} (the measurable metabolic product of prostacyclin) concentrations were measured in the pressure control experiments to exclude the possibility that nitroglycerin stimulated vascular production of prostacyclin that could inhibit platelet activation. 6-k-PFG_{1a} concentrations, however, did not increase after nitroglycerin infusion.

DISCUSSION

Our data show that AGEPC stimulates human platelets to release TXA_2 and cause acute pulmonary hypertension and hydrostatic edema in isolated rabbit lungs. An interaction between AGEPC and platelets was required as shown by the absence of response when either AGEPC or platelets alone, or AGEPC plus platelet-poor plasma were infused into the isolated lung. Since AGEPC also stimulates neutrophils to degranulate (13) and since our platelet preparations may have contained a small number of neutrophils, additional control studies were performed to determine if neutrophils participated in the development of hypertension and edema. The results demonstrated that purified neutrophils and AGEPC did not cause pulmonary hypertension or lung edema.

The observed pulmonary edema in isolated lungs perfused with platelets and AGEPC could have developed from direct alveolar-capillary membrane injury (permeability edema) or from vascular barotrauma resulting from high pulmonary artery pressures (hydrostatic edema). To determine if the pulmonary edema was hydrostatic or due to increased membrane permeability, we infused platelets and AGEPC into isolated lungs in which low perfusion pressures were maintained by addition of nitroglycerin. When pulmonary hypertension was blocked by the vasodilator, no edema occurred. Furthermore, when a left atrial pressure challenge was applied at the end of the usual 55-min protocol to stress the alveolar-capillary membrane and reveal a subtle permeability defect if one was present, no increases in lung weights or albumin concentrations were detected. This finding indicated that the AGEPC-platelet induced edema was purely hydrostatic in origin. These results are in contrast to previous work from our laboratory showing that phorbol myristate acetate stimulated neutrophils or oxygen radicals generated by purine and xanthine oxidase do cause a permeability edema in isolated lungs that occurs in the absence of a pressure rise and is enhanced by a left atrial pressure challenge (25).

Although these findings suggest that platelets do not alter capillary permeability, it is still possible that a coexisting permeability defect could have been obscured by effects of vasodilators on platelet aggregation and endothelial cell prostaglandin synthesis. High levels of nitroglycerin directly limit platelet aggregation and stimulate endothelial cells to release prostacyclin, which prevents AGEPC-induced platelet aggregation (26). These possibilities are unlikely, however, since no increased perfusate levels of 6-k-PGF_{1a} (the measurable prostacyclin metabolite) were detected in the experiments where nitroglycerin was infused compared to isolated lungs perfused with platelets and AGEPC without a vasodilator. Also, the small amount of nitroglycerin infused indicates that its perfusate concentration was below that reported to block platelet aggregation (26).

AGEPC causes platelets to synthesize and release TXA_2 in vitro (15). Since TXA_2 is a potent vasoconstrictor, we considered that the pulmonary hypertension and resultant hydrostatic edema were mediated by TXA_2 released from platelets stimulated by AGEPC. The measurements of increased TXB_2 levels (the stable metabolite of TXA_2) in perfusates of isolated lungs infused with AGEPC and platelets supported this idea. Furthermore, the time course of TXB_2 production closely paralleled the increase in pulmonary artery pressure.

Additional evidence for the role of TXA_2 was derived from experiments showing that AGEPC stimulated platelets made deficient in TXA_2 synthetic capabilities by pretreatment with imidazole did not generate significant pulmonary hypertension or edema. Moreover, the protective effects on the lung of 13-azaprostanoic acid, a TXA_2 receptor-site antagonist (27), further supported a role for TXA_2. These findings are consistent with previous observations suggesting a pathogenetic role for TXA_2 in models of acute edematous lung injury. Specifically, thromboxane synthetase inhibition by imidazole limits pulmonary hypertension and membrane permeability in sheep (28) and 13-azaprostanoic pretreatment improves the survival of rats (29) infused with endotoxin.

Our observations in the isolated lung complement and help interpret previous investigations in whole animals that indicate that platelet activation promotes increased pulmonary vascular resistance in acute edematous lung injury. Infusion of endotoxin increases pulmonary artery pressure and induces edema in several animal models of ARDS. The pulmonary hypertension is inhibited in thrombocytopenic animals (9, 30, 31) and in animals pretreated with cyclooxygenase inhibitors (32, 33), which would prevent platelet release of prostaglandins and thromboxanes. Our study extends these observations by clearly implicating platelet release of TXA_2 as a major mechanism involved in experimental platelet-induced acute pulmonary hypertension. These findings have potential clinical importance since pulmonary hypertension is a common accompaniment of ARDS in humans (34). Furthermore, studies have demonstrated that circulating platelets sequester in the pulmonary circulation in patients in the early phase of respiratory failure (3) suggesting that platelets mediate the pulmonary pressor response through the release of vasoactive substances. Our data would indicate that platelets do have the capability of generating acute pulmonary hypertension by releasing thromboxane A_2, which causes pulmonary vascular constriction.

Extending these observations further, our study suggests that platelets may contribute to ARDS by increasing the hydrostatic pressure in the pulmonary microvascular bed, thereby promoting edema formation. This mechanism may be most important when a coexisting alveolar-capillary membrane defect is present. To affect the capillary bed, a portion of the increased pulmonary vascular resistance would have to develop in postcapillary vessels. Whereas we did not selectively measure pre- and postcapillary pressures in our system, the development of hydrostatic edema after platelet activation suggests that a portion of the vasoconstriction occurred at postcapillary sites. This conclusion is supported by observations that arachidonic acid infusion in sheep pretreated with endotoxin aggravates lung edema formation in a similar pattern (35). Since arachidonic acid is metabolized to thromboxanes and endoperoxides in the lung, these agents appear to increase microvascular pressures thereby worsening edema formation in the setting of an underlying membrane permeability defect.

These results suggest a potential role for AGEPC and platelets in the pathogenesis of acute edematous lung injury. Additionally, they indicate that a potential therapeutic rationale may exist for considering the use of agents that limit TXA_2 synthesis or antagonize TXA_2 expression in the care of patients predisposed to ARDS.

ACKNOWLEDGMENTS

Dr. Heffner is an American Lung Association Fellow. Dr. Repine is an Established Investigator of the American Heart Association. We are indebted to Elaine Berger, Gail Nakata, and Nancy Sherman for technical assistance.

This work was supported in part by grants from the American Heart Association of Colorado, National Institutes of Health (HL 24248 and HL 21248), American Lung Association of Colorado, Council for Tobacco research, and the Kroc, Hill, Swan, and Kleberg Foundations.

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