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Effect of Indomethacin on Thrombin-Induced Pulmonary Edema in the Rat

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ABSTRACT

The preventive effect of indomethacin on thrombin-induced pulmonary edema was studied in rats. Administration of thrombin caused a significant increase in lung weight, wet weight to dry weight ratio (WW/DW), and relative lung water content. During infusion of thrombin, mean pulmonary artery pressure rose and mean systemic artery pressure fell, PaO_2 decreased progressively and there was a continuous rise in pH and $PaCO_2$.

An inhibitor of cyclooxygenase, indomethacin, at a dose of 1 mg/kg body weight, induced a significant further increase in lung weight ($p<0.05$), and a tendency towards an increase in WW/DW and water content compared with animals given thrombin alone. Treatment with indomethacin, however, counteracted the elevated pulmonary artery pressure occurring in the early phase after thrombin infusion, but not that in the late phase. Systemic artery pressure was not affected by indomethacin. The increases in pH and $PaCO_2$ after thrombin infusion were attenuated and remained stable almost at baseline level after indomethacin administration. Indomethacin did not prevent the hypoxemia induced by thrombin infusion.

In conclusion, although indomethacin prevented the early increase in pulmonary artery pressure due to thrombin and the decrease in pH and the increase in $PaCO_2$, it caused lung vascular permeability to protein to increase more than with thrombin alone.

INTRODUCTION

In both the dog (2) and the rat (6), pulmonary microembolism induced by infusion of thrombin during inhibition of fibrinolysis has been found to induce pulmonary insufficiency with similarities to the clinical adult respiratory distress syndrome. Infusion of thrombin results in systemic hypotension, acute pulmonary hypertension, and increased pulmonary vascular permeability to protein with subsequent pulmonary edema (12). The pathophysiology of thrombin-induced microembolism seems to involve the activity of cyclooxygenase-derived arachidonate metabolites (3). Evidence for this assumption is mostly based on studies using various cyclooxygenase inhibitors. Among these arachidonate metabo-

lites, thromboxane is known to be associated with sequences of events that follow infusion of thrombin in the rat (20), and with the development of thrombin-induced pulmonary edema in sheep (8, 9). Increased permeability to protein may also result from entrapment of fibrin (6) and leukocytes (21, 5) in the lung. The cyclooxygenase inhibitor indomethacin is known to inhibit prostaglandin synthesis (24) and the release of thromboxane A_2 in guinea pig lung parenchymal strips (26). It also inhibits several leukocyte activities in vitro such as neutrophil migration (4), aggregation (13), and adherence (25), and also the myeloperoxidase-H_2O_2-Cl system of neutrophils (22).

The aim of the present study was to test the effects of indomethacin on thrombin-induced changes in pulmonary artery and systemic artery pressure and in arterial blood gas exchange variables, and on pulmonary edema induced by thrombin, in rats.

MATERIALS AND METHODS

Animals

Fifty-two male Sprague-Dawley rats (Alab, Stockholm, Sweden) were used. They were divided into three treatment groups: saline controls (n=8), thrombin plus AMCA (n=21), and thrombin plus AMCA plus indomethacin (n=23). All rats weighed between 200 and 250 g and had free access to food (Ewos rat pellet) and tap water.

Materials

Bovine thrombin (Topostasine^R) was kindly supplied by Hoffman La Roche, Switzerland. It was dissolved in physiological saline to a concentration of 100 IU/ml, and kept at - 20° C until used in the experiment. The fibrinolytic inhibitor tranexamic acid (trans-4-aminomethyl-cyclohexane-carboxylic acid, AMCA) was purchased from Kabi, Stockholm, Sweden. Indomethacin (Confortid^R) was supplied by Dumex A/S, Copenhagen, Denmark. Pentobarbital (Inactin^R) was obtained from Byk-Gulden, FRG. S-2238, a chromogenic substrate for detection of antithrombin activity, was obtained from Kabi, Stockholm,
Sweden.

Methods

The rats were anesthetized intraperitoneally with 125 mg/kg of pentobarbital. They were placed in a supine position and tracheostomized.

A tracheal cannula (PE 240, Clay Adams, Becton Dickson & Co., USA) was inserted for airway support and an abdominal cannula (Portex; outer diameter 0.80 mm, HYTHE, Kent, England) was inserted into the peritoneal cavity for AMCA injection and maintenance of anesthesia. All rats breathed air spontaneously through a tracheostomy, and the body temperature was kept constant at 38° C with an electric pad throughout the experimental period.

Intravenous injection sites were prepared in one of the saphenous veins, using polyethylene catheters (Portex; outer diameter 0.80 mm, HYTHE, Kent, England) and 26 gauge needles for injection of indomethacin and thrombin. Indomethacin was dissolved in sterile distilled water to a final concentra-

tion of 2 mg/mL. The solution was slowly administered through a saphenous vein 30 min prior to the infusion of thrombin.

Pulmonary microembolization was induced essentially as described previously (16) and modified as described below. In brief, 15 min prior to thrombin administration rats were injected through the abdominal catheter with 200 mg/kg of AMCA to prolong fibrin entrapment in the lungs and increase pulmonary damage (2).

Three hundred sixty IU/kg of bovine thrombin was injected manually by the intravenous route over a period of 10 min, using a stopwatch and a 1.0 ml disposable syringe. The doses of indomethacin and thrombin used in this study was based on the findings in a preliminary study, in which animals treated with indomethacin did not tolerate the thrombin dose used in previous studies (500 IU/kg). The thrombin dose was therefore reduced to 360 IU/kg. The rats were killed 90 min after termination of thrombin infusion and the lungs were excised, gently cleaned with gauze, weighed, and dried at 37° C in a warm incubator for approximately 72 h. The lungs were reweighed until their weight was constant.

Calculation of wet weight to dry weight ratio (WW/DW) and water content

The relative lung water content was calculated in the left lung as described previously (17). Water content (%) = WW - DW / WW x 100.

Pressure measurements

Pulmonary artery pressure (PAP) was recorded via a Silastic^{R} catheter (Dow Corning Co., USA; outer diameter 0.025 inch) inserted into the right jugular vein and then gently advanced to the pulmonary artery. Systemic artery pressure (SAP) was recorded via a polyethylene catheter (PE 50) inserted into the femoral artery. The catheters were connected to Statham transducers and the pressure tracing was performed throughout the experimental period. The position of the catheters was checked from the pressure tracings and confirmed when the rats were killed. Rats in which catheters were not properly positioned were excluded from further analysis.

Arterial blood gas studies

Blood samples of 100 µL were drawn into heparinized microcapillary tubes from the femoral artery and analyzed immediately with a blood gas analyzer (Instrumental Laboratory System 1302, Italy). The analyses were carried out at 37° C.

Antithrombin activity

Antithrombin activity was measured in vitro by means of a chromogenic substrate, S-2238. One vial of S-2238 was dissolved in 15 mL sterile water to a concentration of 0.75 nmol/L and 50 µL of test plasma or standard was diluted with 3.0 ml working buffer solution. Bovine thrombin 53(nkat) was reconstituted with 1.5 mL sterile water. At assay, 400 µL of diluted test plasma or standard, and indomethacin in different concentrations were incubated at 37° C for 3-6 min. Then 100 µL of thrombin was added and incubated at 37° C for 30 min. S-2238 was thereafter added, mixed well, and incubated at 37° C for exactly 30 s. The reaction was stopped by addition of 300 µL of 50 % acetic acid, which was mixed

immediately. The absorbance of the sample was read against distilled water in a photometer at 405 nm.

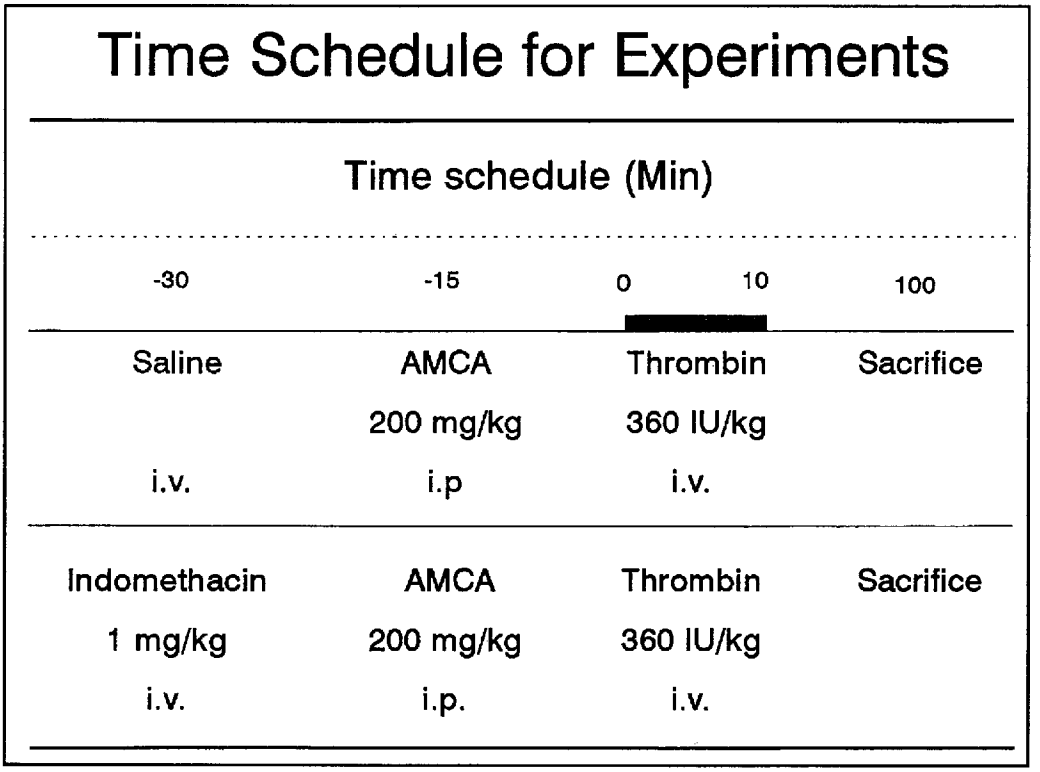
Statistical analyses

Statistical analyses of differences between groups were performed with the Wilcoxon-White two-sample ranks test.

Two-way analysis of variance was used to determine the significance of changes in mean PAP (MPAP), mean SAP (MSAP), and arterial blood gas exchange from the baseline value after infusion of thrombin. The baseline value was the value just prior to thrombin infusion. Student's unpaired t test was used for comparisons between groups at equivalent time periods. A p value of < 0.05 was considered significant in all cases.

RESULTS

Changes in lung weight, WW/DW, and water content



The experiment was performed on 23 rats according to the protocol shown in Figure 1. The results are presented in Figure 2. Rats injected with thrombin + AMCA showed a significant increase in lung weight, WW/DW, and water content. Rats pretreated with indomethacin had a higher lung weight than those not treated with indomethacin ($p<0.05$). WW/DW and the water content in the indomethacin-treated rats were slightly higher but not significantly different from those in rats given thrombin alone.

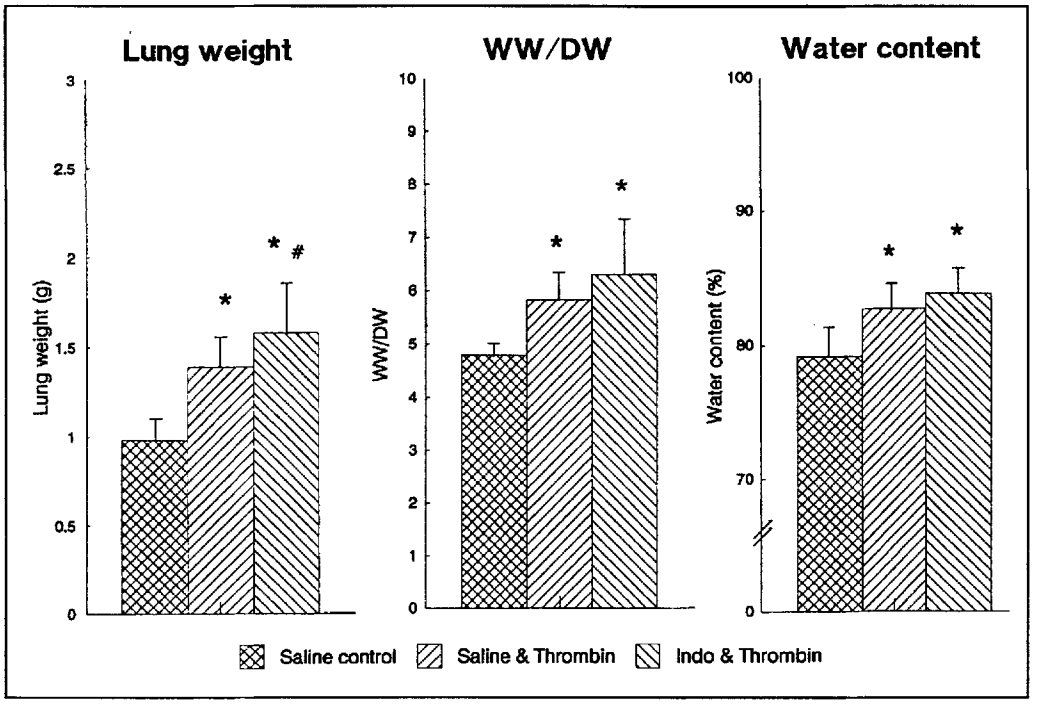


Fig 2. Preventive effect of indomethacin, 1 mg/kg, on changes in lung weight, WW/DW ratio, and relative lung water content induced by thrombin infusion in rats. * indicates $p<0.05$ vs saline control group. # denotes $p<0.05$ compared to animals given thrombin & AMCA alone. Mean ± S.D.

Hemodynamic changes

The percent changes in MPAP and MSAP are illustrated in Figure 3. The infusion of thrombin resulted in a large increase in PAP (by 87±23 %). The values then fell progressively during the remainder of the experiment. The increase in PAP after thrombin infusion was diminished by pretreatment with indomethacin ($p<0.05$). Five and 15 min. after completion of thrombin infusion these differences were statistically significant.

Thrombin produced an immediate fall in MSAP. The decreased arterial pressure after thrombin infusion had slightly recovered 5, 15, and 30 min after completion of thrombin infusion, but began to decrease again after 30 min and had fallen by about 30 % from the baseline at 90 min.

Treatment with indomethacin showed a tendency to counteract the thrombin-induced fall in arterial pressure, but the difference was not statistically significant. Changes in arterial pressure after thrombin infusion, considered for the whole experimental period, were not affected by treatment of indomethacin.

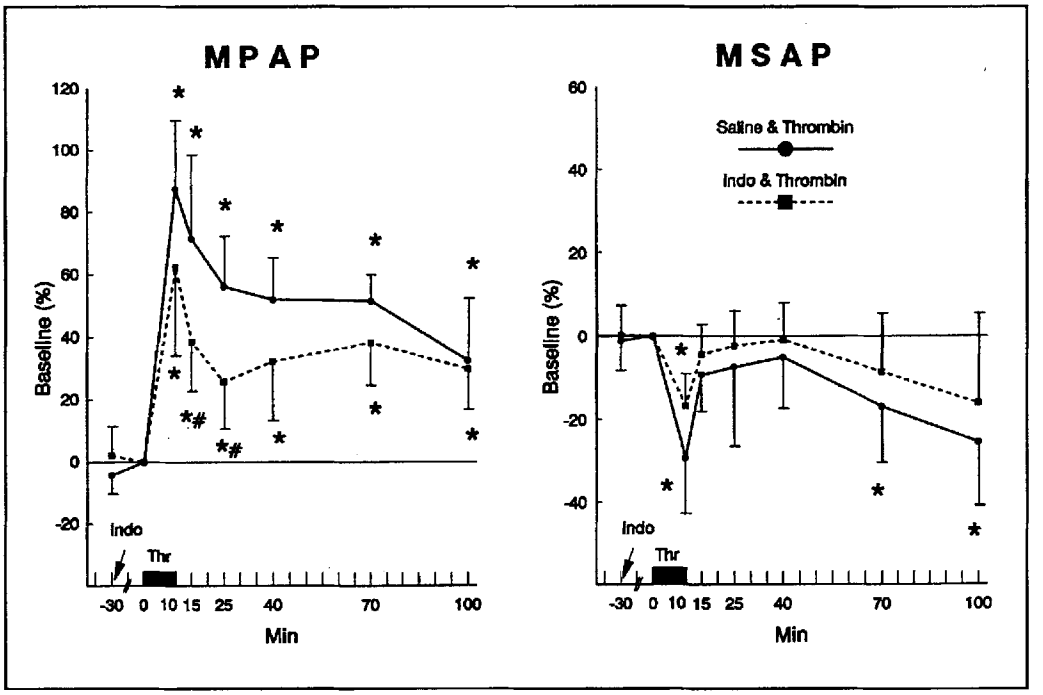


Fig 3. Preventive effect of indomethacin, 1 mg/kg, on changes in mean pulmonary artery pressure (MPAP) and mean systemic artery pressure (MSAP) induced by thrombin infusion in rats. Baseline represents steady-state values before thrombin infusion. * indicates $p<0.05$ compared with animals given thrombin and AMCA alone. Mean ±S.E.

Arterial Blood Gas Studies

The changes in arterial blood gas variables are illustrated in Figure 4. Thrombin caused a progressive decline in pH and PaO_2, while $PaCO_2$ increased continuously. The fall in pH and the rise in $PaCO_2$ after the infusion of thrombin were ameliorated by indomethacin. The pH in the indomethacin group was significantly higher 15 min after completion of thrombin infusion and subsequently.

$PaCO_2$ was significantly lower 60 and 90 min after thrombin infusion in the indomethacin group. However, the thrombin-induced decrease in PaO_2 was not affected by indomethacin.

Antithrombin activity in vitro

To elucidate the question as to whether indomethacin possessed intrinsic antithrombin activity, this drug was incubated at various concentrations with thrombin in a chromogenic substrate system. As seen in Figure 5, indomethacin had no inhibitory effect at reasonable doses.

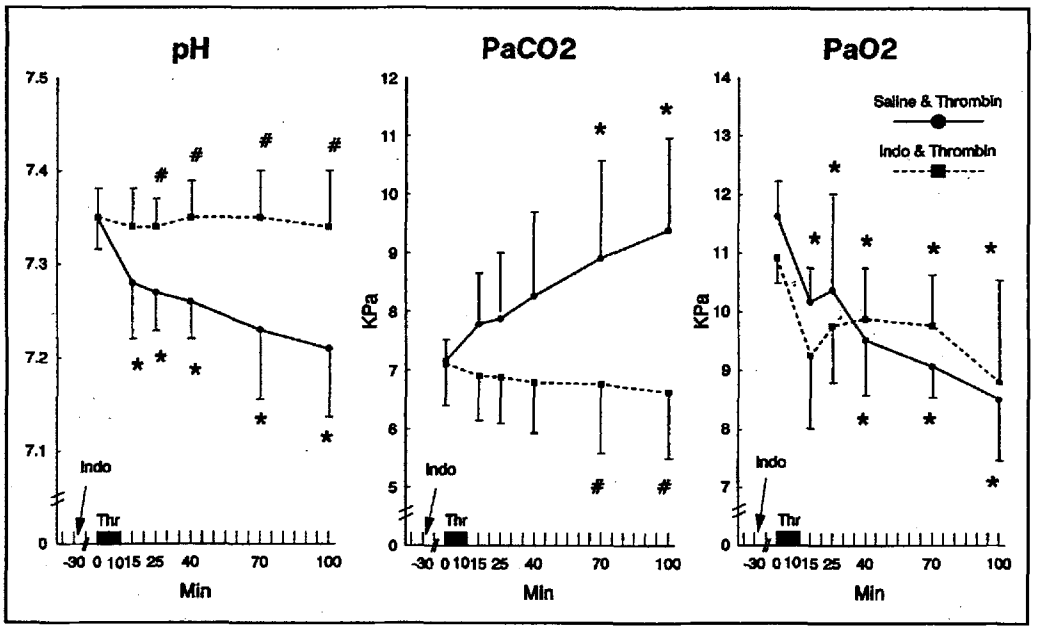


Fig 4. Changes in pH, $PaCO_2$ and PaO_2 after thrombin infusion in rats treated and not treated with indomethacin (Indo), 1 mg/kg body weight. Baseline represents steady-state values before thrombin infusion. * indicates $p<0.05$ compared with animals given thrombin and AMCA alone. Mean ± S.E.

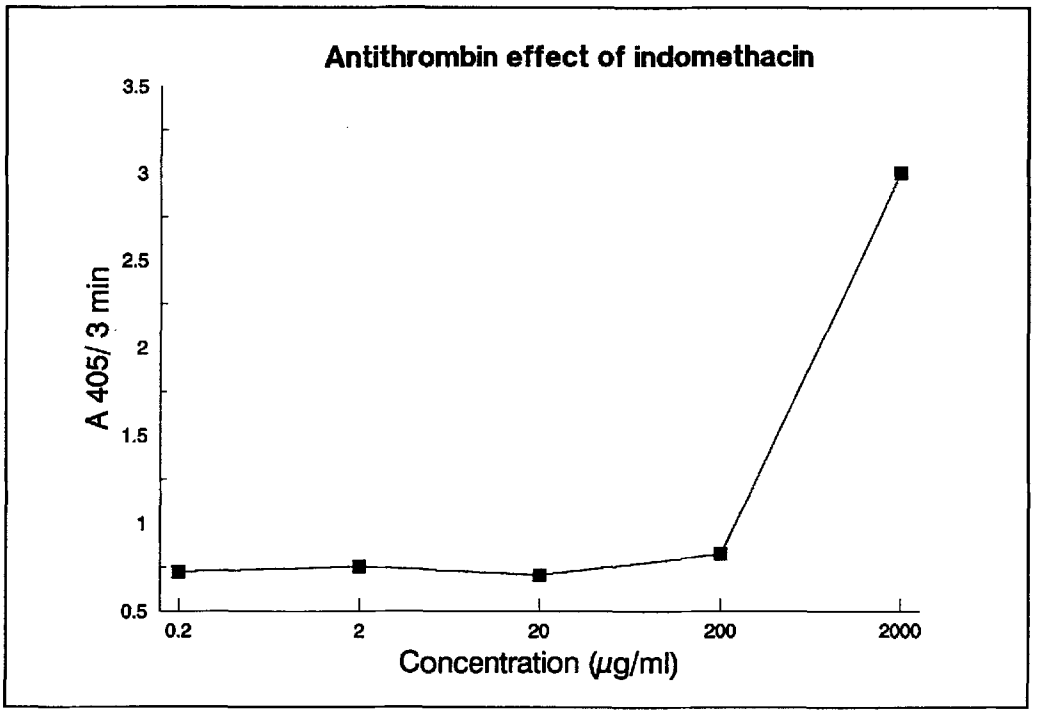


Fig 5. Antithrombin activity measured in a chromogenic substrate assay (S-2238) at various indomethacin concentrations, expressed as the absorbance change at 405 nm after 3 min of incubation.

DISCUSSION

The initial pressure response to the infusion of thrombin in the present study was analogous to that seen in previous experiments (20, 21), i.e. a rapid increase in mean pulmonary artery pressure and a concomitant decrease in mean systemic artery pressure. Pretreatment with indomethacin attenuated the increase in pulmonary artery pressure in the early phase after thrombin infusion.

The rise in mean pulmonary artery pressure after infusion of thrombin may probably be due to a profound increase in pulmonary vascular resistance resulting from thrombin-induced release of thromboxane A_2 (20, 8). It is therefore conceivable that the improvement of the mean pulmonary artery pressure after thrombin infusion in the indomethacin group might be due mainly to inhibition of thromboxane synthesis resulting from cyclooxygenase inhibition, and consequently to a decrease in pulmonary vasoconstriction.

The reduced mean systemic artery pressure during the infusion of thrombin was slightly improved in the early phase after thrombin infusion by pretreatment with indomethacin. However, indomethacin did not influence the progressive fall in mean systemic artery pressure caused by thrombin. In this phase the lung weight started to increase, as seen in previous studies (6, 10).

The progressive decrease in mean systemic artery pressure probably reflects a progressive disturbance of the systemic circulation secondary to the pulmonary damage.

The infusion of thrombin produced a progressive decline in pH and PaO_2 and a continuous rise in $PaCO_2$. As indomethacin attenuated the changes in pH and $PaCO_2$ but not the pulmonary edema, the changes in pH and $PaCO_2$ should not be secondary to edema and are probably due to bronchoconstriction and vasoconstriction induced by cyclooxygenase derivatives. The decline in PaO_2 after thrombin infusion was not affected by pretreatment with indomethacin and might partly be secondary to the pulmonary edema. Indomethacin exaggerated the hypotension caused by N-formyl methyl-leucyl-phenyl-alanine in pentobarbital anesthetized rats (18) and has also been known to enhance bronchoconstriction (23). It is therefore conceivable that hypotension and bronchoconstriction may also lead to increased hypoxemia in the indomethacin-treated group.

Thrombin infusion following administration of AMCA caused an increase in lung vascular permeability to protein, manifested as an increase in lung weight, WW/DW and the lung water content. Pretreatment with indomethacin aggravated thrombin-induced increase in lung weight. The same effect was observed in sheep with lung injury induced by endotoxin (17). In contrast, indomethacin prevented an increase in lung vascular permeability to protein caused by thrombin in sheep in another study (3). This discrepancy in the effects may be explained by species differences and different experimental conditions.

Why did indomethacin aggravate lung vascular permeability to protein in this study?

Lipoxygenase metabolites are known to be potent increasers of lung vascular permeability to protein, and they seemed to be involved in the late phase of thrombin-induced microembolism in sheep (3, 19). Indomethacin may shunt arachidonic acid metabolites into the lipoxygenase pathway, leading to

increased synthesis of chemotactic leukotrienes (7, 15). It has also been found to increase leukocyte chemotaxis in carrageenin-induced inflammation (11).

This effect may explain the increased lung weight after administration of indomethacin. Ibuprofen, on the other hand, another cyclooxygenase inhibitor, does not seem to induce a shift to the lipoxygenase pathway and inhibited the release of leukotriene B_4 from rat neutrophils in vitro (14). Also, we found that ibuprofen decreased, and did not increase pulmonary edema in the same model as was used in the present experiment (1).

In summary, although indomethacin prevented the early increase in mean pulmonary artery pressure due to thrombin and had favourable effects on pH and $PaCO_2$, it caused the lung vascular permeability to protein to increase more than did thrombin alone.

These findings suggest that indomethacin may not be beneficial in the adult respiratory distress syndrome, which is characterized by an increased permeability to protein and pulmonary edema.

REFERENCES

1. Ahn, C.M., Sandler, H., Wegner, T.& Saldeen, T.: Effect of ibuprofen on thrombin-induced pulmonary edema in the rat. (Manuscript)
2. Arfors, K-E., Busch, C., Jacobson, S., Lindquist, O., Malmberg, P., Rammer, L.& Saldeen, T.: Pulmonary insufficiency following intravenous infusion of thrombin and AMCA (Tranexamic acid) in the dog. Acta Chir Scand *138:* 445-452, 1972.
3. Bizios, R., Minnear, F.L, van der Zee, H.& Malik, A.B.: Effects of cyclooxygenase and lipoxygenase inhibition on lung fluid balance after thrombin. J Appl Physiol *55*: 462-471, 1983.
4. Brown, K.A.& Collins, A.J.: Action of nonsteroidal, anti-inflammatory drugs on human and rat peripheral leukocyte migration in vitro. Ann Rheum Dis *36*: 239-243, 1977.
5. Cooper, J.A., Solano, S.J., Bizios, R., Kaplan, J.E.& Malik, A.B.: Pulmonary neutrophil kinetics after thrombin-induced intravascular coagulation. J Appl Physiol *57*: 826-832, 1984.
6. Diffang, C.& Saldeen, T.: Effect of brinase (Protease I from Aspergillus oryzae) on the elimination of fibrin from the lungs and pulmonary damage in rats with induced intravascular coagulation and inhibited fibrinolysis. Thrombos Res *9*: 611-622, 1976.
7. Docherty, J.C.& Wilson, T.W.: Indomethacin increases the formation of lipoxygenase products in calcium ionophore stimulated human neutrophils. Bichem Biophys Res Commun *148*: 534-538, 1987.
8. Garcia-Szabo, R.R., Peterson, M.B., Watkins, W.D., Bizios, R., Kong, D.L.& Malik, A.B.: Thromboxane generation after thrombin. Protective effect of thromboxane synthetase inhibition on lung fluid balance. Circ Res *53*: 214-222, 1983.
9. Garcia-Szabo, R.R., Johnson, A.& Malik, A.B.: Thromboxane increases pulmonary vascular resistance and transvascular fluid and protein exchange after pulmonary microembolism. Prostaglandins *35*: 707-721, 1988.
10. Gerdin, B., Diffang, C.& Saldeen, T.: Pulmonary insufficiency in the rat after intra vascular coagulation and inhibition of fibrinolysis. I. Studies on some pathogenetic mechanisms. Eur Surg Res *13*: 402-413, 1981.

11. Higgs, G.A., Eakins, K.E., Mugridge, K.G., Moncada, S.& Vane, J.R.: The effects of nonsteroidal anti-inflammatory drugs on leukocyte migration in carrageenin-induced inflammation. Eur J Pharmacol *66*: 81-86 1980.

12. Johnson, A., Tahamont, M.V., Kaplan, J.E.& Malik, A.B.: Lung fluid balance after pulmonary microembolization. Effects of thrombin vs. fibrin aggregate. J Appl Physiol *52*: 1565-1570, 1982.

13. Kaplan, H.B., Edelson, H.S., Korchak, H.M., Given, W.P., Abraham, S.& Weissman, G.: Effects of nonsteroidal anti-inflammatory agents on human neutrophil functions in vitro and in vivo. Biochem Pharmacol *33*: 371-378, 1984.

14. Konstan, M.W., Vargo, K.M.& David, P.B.: Ibuprofen attenuates the inflammatory response to Pseudomonas aeruginosa in a rat model of chronic pulmonary infection. Am Rev Respir Dis *141*: 186-192, 1990.

15. Lonigro, A.J., Stephenson, A.H.& Sprague, R.S.: Role of arachidonic acid metabolites in the pathogenesis of acute lung injury. Adv Prostaglandin Thromboxane Leukotriene Res *21*: 421-428, 1991.

16. Nettelbladt, O., Tengblad, A.& Hällgren, R.: Accumulation of hyaluronan (hyaluronic acid) in lung tissue during experimental alveolitis parallels development of interstitial edema. Am J Physiol *257*: 379-84, 1989,.

17. Ogletree, M.L.& Brigham, K.L.: Indomethacin augments endotoxin induced increased lung vascular permeability in sheep. (Abstract). Am Rev Respir Dis *119*: A303, 1979.

18. Olsen, U.B.& Bille-Hansen, V.: Exaggerated hypotension by N-formyl-methionylphenylalanine in indomethacin pretreated rats. Role of toxic oxygen. Agents Actions *17*: 489-94, 1986.

19. Perlman, M.B., Johnson, A., Jubiz, W.& Malik, A.B.: Lipoxygenase products induce neutrophil activation and increase endothelial permeability after thrombin-induced pulmonary microembolism. Circ Res *64*: 62-73, 1989.

20. Sandler, H., Gerdin, B.& Saldeen, T.: Studies on the role of thromboxane in thrombin-induced pulmonary insuffiency in the rat Thrombos Res *42*: 165-175,1986.

21. Sandler, H.& Gerdin, B.: Pulmonary insufficiency in the rat after intravascular coagulation and inhibition of fibrinolysis. III. Studies on the role of polymorphonuclear leukocytes (PMNLs). Acta Universatis Upsaliensis *17*: VII.1-VII.12 1985.

22. Shacter, E., Lopez, R.L.& Pati, S.: Inhibition of the myeloperoxidase-H_2O_2-Cl-system of neutrophils by indomethacin and other nonsteroidal anti-inflammatory drugs. Biochem Pharmacol *41*: 975-84, 1991.

23. Undem, B.J., Pickett, W.C., Lichtenstein, L.M.& Adams, G.K.: The effect of indomethacin on immunologic release of histamine and sulfidopeptide leukotrienes from human bronchus & lung parenchyma. Am Rev Respir Dis *136*: 1183-1187, 1987.

24. Vane, J.R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology *231*: 232-235, 1971.

25. Venezio, F.R., Divincenzo, C., Pearlman, F.& Phair, J.P.: Effects of the newer nonsteroidal anti-inflammatory agents, ibuprofen, fenoprofen, and sulindac on neutrophiladherence. J Infect Dis *152*: 690-694, 1985.

26. Vincent, J.E., Zijlstra, F.J.& Bonta, I.L.: The effect of inhibitors on the LTD_4-induced contractions and release of thromboxane A_2 in the guinea pig lung parenchymal strip. Agents Actions Suppl *14:* 69-81, 1984.

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