0014-4754/95/100956-05$1.50 + 0.20

Pentoxifylline attenuates acute lung injury induced by microemboli

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Received 20 May 1994; received after revision 25 October 1994; accepted 13 March 1995

Abstract. Pentoxifylline (PTX), a methylxanthine derivative, effectively prevents acute lung injury in different animal models. To investigate whether PTX would attenuate acute lung injury induced by microemboli resulting from treatment with calcium chloride ($CaCl_2$) suspension, an isolated blood-perfused rat lung model was used. Pretreatment with PTX prevented the increase in pulmonary arterial pressure (PAP), lung weight gain and protein concentration in the lavage fluid after $CaCl_2$ treatment.
Key words. Pentoxifylline; microemboli; lung injury.

Pentoxifylline (PTX), a methylxanthine derivative, has been demonstrated to improve peripheral vascular circulation[1,2]. Recent work also demonstrates that this agent reduces phagocytic activity and superoxide anion formation by human neutrophils and monocytes in vitro, and increases cyclic-AMP level in endothelial cells[3–5]. PTX has also been shown to attenuate acute lung injury due to *E. coli* infection, tumor necrosis factor, phorbol myristate acetate (PMA) and protamine[6–9]. However, its working mechanisms are not fully understood. PTX might attenuate lung injury either through the suppression of neutrophil activation, which is a major source of mediators and proteases, or by directly protecting the endothelial cells, the main target of mediators, which characteristically show pathology in pulmonary edema.

Because most of the protective effects of PTX on acute lung injury are neutrophil-dependent, the important effect of this agent on neutrophils has been emphasized in the literature[3,6,10–12]. On the other hand, the roles of platelets in acute lung injury and the protective effects of PTX on platelet-dependent lung injury are rarely mentioned. In a previous report, we showed that platelets were actively involved in the mechanism of acute lung injury induced by microemboli after treatment with $CaCl_2$ suspension[13]. The purpose of this study was to determine whether PTX has a protective action against this platelet-dependent acute lung injury in the isolated rat lung model.

As parameters of lung injury, we determined lung weight gain and pulmonary arterial pressure (PAP) during experiments. The protein concentration of the bronchoalveolar lavage fluid (BAL) was also measured.

Materials and methods

Preparation of isolated lungs. Male Sprague-Dawley rats weighting 275–300 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (20–30 mg). After a tracheostomy has been performed, each rat was artificially ventilated with a small animal ventilator with room air. The chest was opened at the midline. An intracardiac injection of heparin (1000 units/kg b. wt.) was given, and 10 ml of blood was collected from the right ventricle, mixed with 12 ml of Krebs Henseleit buffer (KHB) solution, and used as a perfusing fluid. The chemical composition of the KHB solution was (in mg/100 ml): NaCl, 484; KCl, 35.4; $MgSO_4$, 14.35; KH_2PO_4, 35.4; $NaHCO_3$, 210; dextrose, 200; and $CaCl_2 \cdot 2H_2O$, 37.3. The main pulmonary artery was cannulated through a right ventricular puncture. To divert the pulmonary venous outflow into a reservoir, a wide-bore cannula was inserted into the left atrium via the left ventricular inflow tract. The cannula was then fixed with a ligature at the apex of the heart. The ligature around the pulmonary artery was also placed around the aorta, preventing the loss of perfusate into the systemic circulation. The recirculating perfusion circuit consisted of a roller pump with a perfusion flow rate of 8 ± 0.5 ml/min, a heat exchanger for temperature maintenance of the perfusate at 37 ± 0.5 °C, a tubing system, and a perfusate reservoir. Weight changes in the perfuse reservoir and pulmonary arterial pressure (PAP) were continuously measured by a force transducer and a pressure transducer, respectively, and recorded on a polygraph. Lung weight changes were recorded as the inverse of the weight change of the perfusate reservoir. After perfusion began, the lungs were ventilated with a mixture of 95% room air and 5% CO_2 gas at a rate of 65–70 breaths/minute and a tidal volume of 2 ml.

The animal's condition was allowed to stabilize for 5–10 min before the experiment began. At the end of the perfusion, the lungs were lavaged with 3 ml of normal saline. The fluid was centrifuged at $1500 \times g$ for

10 min, and the concentration of protein in the supernatant was determined by the dye binding method[14].

Preparation of calcium chloride suspension. When calcium chloride ($CaCl_2 4 \cdot 2H_2O$) was added to the KHB solution, a suspension formed if the concentration was high enough. In this study, the concentration of calcium chloride was 25 mg/ml.

Microscopically, the suspension consists of a uniformly formed granular precipitate of particles with estimated size of one-tenth of that of red blood cell (RBC) in a hemocytometer. Most of the granules form clumps with a diameter ranging from 3 to 10 times that of a RBC. This suspension was used to induce acute lung injury when added to the perfusate, which consisted of blood with platelets in KHB solution.

Experimental protocol. Animals were randomly assigned to one of three groups. Group I ($n = 8$) was a control group, receiving neither calcium chloride suspension nor pentoxifylline. Group I ($n = 9$) was the 'microemboli group', and received calcium chloride suspension (3 ml) but no PTX. Group III ($n = 8$) was the PTX group, which received calcium chloride suspension (3 ml) and pretreatment with PTX (10 mg/300 g Trental, Hoechst-Roussel, Somerville, NJ, USA).

Statistical analysis. The values are expressed as means $\pm$SE. Comparisons within each group were performed using Student's paired t-test. Comparisons among groups were using a one-way analysis of variance (ANOVA) and Scheffe's comparison a posteriori; $p < 0.05$ was considered to be statistically significant.

Results

In order to evaluate the protective effect of PTX on acute lung injury, induced by calcium chloride, the severity of lung injury was determined by measuring lung weight gain, elevation of pulmonary arterial pressure, and the protein concentration in the BAL fluid. All of these three parameters remained essentially constant during the experimental period in the control group. The calcium suspension caused discernible increase in lung weight gain, pulmonary arterial pressure and protein concentration in the BAL fluid. Pretreatment with PTX significantly attenuated the severity of lung injury; all three parameters were significantly reduced when compared with the microemboli group.

Effect of PTX on lung weight gain. In the control group, lung weight was maintained at a stable level, and there was no significant lung weight gain during the 60 min course of the experiments. In the microemboli group, lung weight significantly increased after administration of calcium chloride suspension, and lung weight gain attained 11.2 ± 1.1 gm after 60 min of perfusion. In the PTX group, lung weight gain was significantly reduced when compared with the microemboli group throughout the 60 min period (fig. 1); it attained 2.0 ± 0.6 gm at 60 min ($p < 0.001$).

Effect of PTX on pulmonary arterial pressure. The changes in PAP among the different groups throughout the experimental procedure are shown in figure 2. As with lung weight gain, there were almost no changes in PAP throughout the 60 min perfusion period in the control group. PAP dramatically increased 10 min after calcium chloride suspension had been added in group

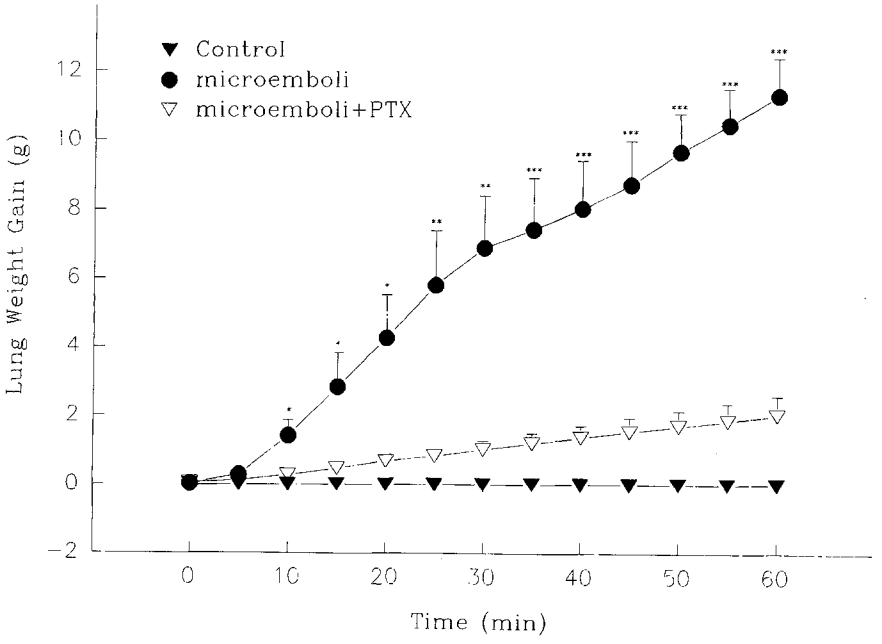

Figure 1. Lung weight gain in various study groups during a 60 min period of time. PTX = Pentoxifylline. Bar represents SE. Asterisks indicate significant differences between the microemboli and PTX protective groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

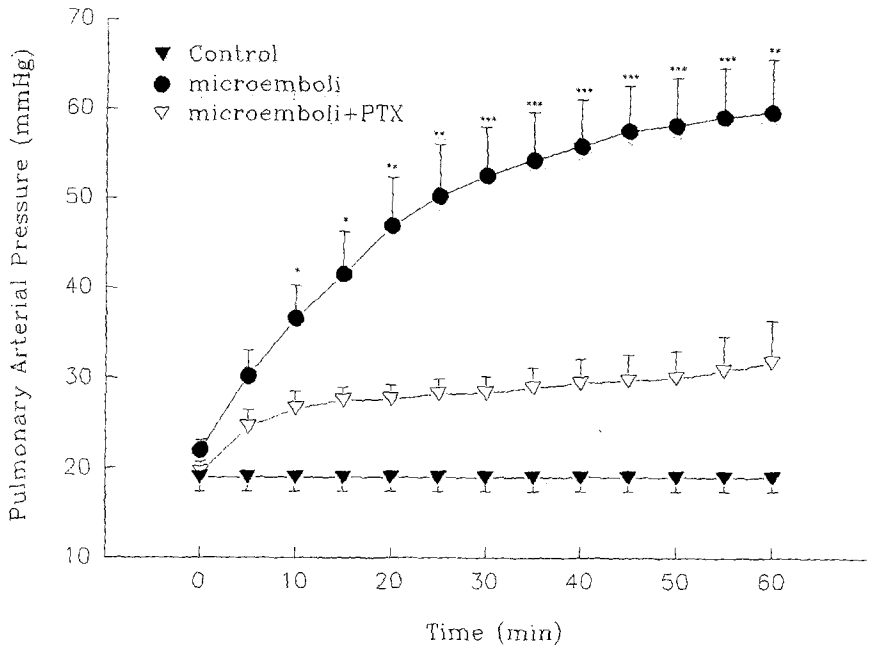

Figure 2. Changes in pulmonary arterial pressure (PAP) during a 60 min period of time. PTX = Pentoxifylline. Bar represents SE. Asterisks indicate significant differences between the microemboli and PTX protective groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

II, and reached 59.5 ± 6.1 mm Hg at 60 min ($p < 0.001$ compared with the control group). There was a marked protective effect against pulmonary hypertension in group III, in which the animals were pretreated with PTX (fig. 2). The peak PAP in the PTX-pretreated group was only 31.7 ± 4.5 mm Hg, which was significantly lower than in the microemboli group ($p < 0.01$).

Effect of PTX on protein concentration of BAL fluid. The protective effect of PTX in acute lung injury was further confirmed by the measurement of the protein concentration of the BAL fluid (fig. 3). The mean portein concentration of the BAL fluid in the microemboli group was 217 ± 20 mg/dl, significantly higher than in the control group (23.0 ± 6.4 mg/dl, $p < 0.001$). PTX pretreatment of the animals significantly reduced the protein leakage into the air space of the lungs (65 ± 10 mg/dl), with a p value smaller than 0.001 compared with the microemboli group.

Discussion

The adult respiratory distress syndorme (ARDS) is associated with a variety of clinical conditions. The mechanism of this syndrome is still not very clear and the

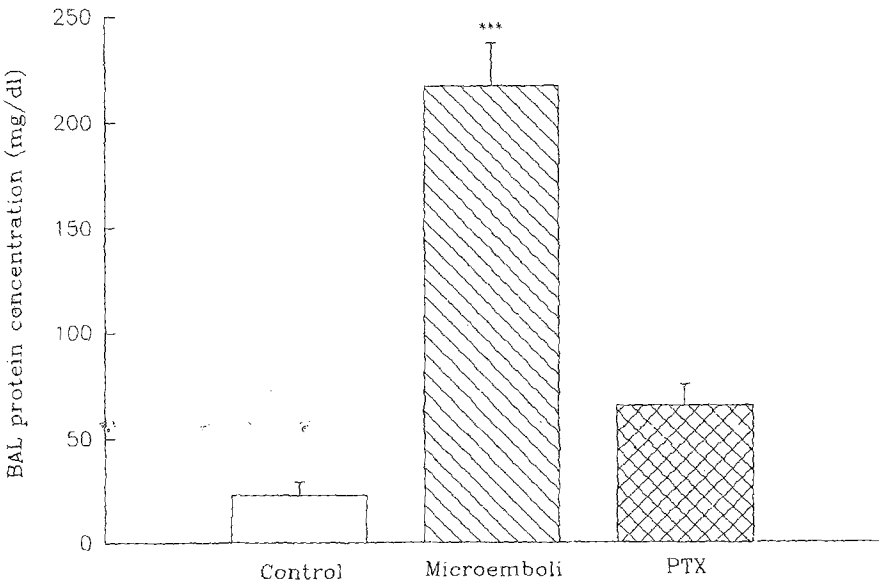

Figure 3. Protein concentration of lavage fluid. Bar represents SE. Asterisks indicate significant differences ($p < 0.001$) when the microemboli group was compared with the PTX protective group.

only treatment available is supportive. Despite improvement in critical and ventilatory supportive care during the past two decades, the morbidity and mortality of ARDS remains high. Many circulatory inflammatory cells and cytokines have been implicated in the pathogenesis of acute lung injury. Although the role of platelets in acute lung injury has been mentioned in the literature[13,15–23], the possible effect of PTX on platelet-dependent acute lung injury has never been explored.

In a previous study we showed that platelets are actively involved in pathogenesis in a model in which acute lung injury is induced by a calcium chloride suspension lung[13]. As the results for the 'microemboli group' in this study illustrated, a significant increase in pulmonary arterial pressure (PAP), lung weight and the protein concentration in bronchoalveolar lavage fluid occurs when 3 ml of $CaCl_2$ suspension is added to a perfusate solution containing KHB and blood. It was shown in a previous study that such an increase was not observed when the perfusate contained only the $CaCl_2$ suspension and the buffer solution[13], but that when platelets were added instead of blood, PAP, lung weight gain and the protein concentration of the lavage fluid increased significantly[13].

Several possible mechanisms may contribute to platelet-dependent acute lung injury after treatment with calcium chloride suspension. First, since the precipitate forms clumps several times larger than RBCs these may cause microembolization in the pulmonary microcirculation and induce acute lung injury. Second, platelets may be perferentially more sensitive to this chemical suspension than other circulating cells, which would result in the release of platelet-associated cytokines, aggregation and the formation of microemboli. Third, plasma membrane injury of the endothelial cells, induced by calcium phosphate precipitates which in turn attract, accumulate and activate circulating platelets, may also cause acute lung injury. It is possible that calcium phosphate precipitates when $CaCl_2$ is added to KHB buffer. Calcium phosphate precipitation has long been used to induce cell membrane injury and facilitate gene transfer in the transfection of eukaryotic cells[24].

PTX, a drug previously used as a peripheral vasodilator for intermittent claudication, is known to increase intracellular cAMP levels in neutrophils, monocytes, endothelial cells and platelets[25–26], and has important anti-inflammatory actions that make it helpful in mitigating lung injury[3,10]. The ability of PTX to attenuate neutrophil activation, and neutrophil-dependent and independent endothelial cell damage in acute lung injury, has been documented in both animal models and isolated cells[3,6,8–12,25,27–29]. The results of these studies indicate that the protective effect of PTX on acute lung injury may act both on inhibit neutrophil activation and to maintain endothelial cell integrity. However, little is known about the role of PTX in platelet-dependent acute lung injury.

The purpose of this investigation was to evaluate the protective effect of PTX on a platelet-dependent acute lung injury model in rats. As presented in the results section, pretreatment of animals with PTX in the experimental group clearly attenuated the degree of acute lung injury, as judged by the significant inhibition of the increase in PAP, lung weight and protein concentration of BAL fluid.

The significant protective effect of PTX on this platelet-dependent microemboli-induced acute lung injury suggests that the mechanism of the protective effect of PTX on acute lung injury might be through the inhibition of the activation of platelets as well as neutrophils, both of which disrupt the integrity of endothelial cells. We have recently found that infusion of PMA or platelets alone into isolated rat lung perfused with a bloodless perfusate did not cause lung injury, but that platelets and PMA together caused pulmonary hypertension and edema[23]. This result indirectly indicates that the activation of platelets rather than the platelets themselves causes acute lung injury. We have also confirmed that the cyclooxygenase pathway is more important than the lipoxygenase pathway in this PMA-mediated platelet dependent lung injury[23]. However, this requires further confirmation in the present model, as the pulmonary response to foreign body microemboli (intravenous injection of crushed pentazocin suspension of talc, the filler in these tablets) in dogs is prevented by diethylcarbamazine, a lipoxygenase inhibitor, and by FPL 55712, a receptor antagonist for the peptido-leukotrienes. However, it is not prevented by indomethacin, a cyclooxygenase inhibitor[30].

In clinical practice, intravenous administration of multiple drugs may cause precipitates to form if the buffer solution is not carefully chosen and handled. Occasionally, respiratory distress can be found in patients receiving infusions of these drug suspensions. In addition, some intravenous drug addicts, such as a pentazocin abusers, may suffer from talc-induced acute lung injury if the crushed pentazocin suspension is injected intravenously[30]. Pentoxifylline treatment has recently been shown to protect effectively against talc-induced acute lung injury in rat (Kang Hsu et al., unpubl. observ.). The results of this study not only expand our understanding of the pathogenesis of acute lung injury, but may have significant clincial applications and provide a good animal model for the study of particle-induced acute lung injury. Further extension of this model in the future may result in the exploration of the pathophysiology of particle-induced acute lung injury in clinical practice, and also provide a strategy for the development and testing of effective therapeutic modalities.

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