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Cyclosporine, an Immunosuppressant, Attenuates Phorbol-Induced Lung Injury in Rats

C.L. Su, C.F. Chen, L.L. Chiang, C.N. Lee, D. Wang, S.L. Lo, and S.J. Kao

ABSTRACT

Objective. White cell activation in the lung plays a critical role to induce lung injury and lymphocytes in the thoracic duct system may also participate. We evaluated the effect of cyclosporine on phorbol myristate acetate (PMA)-induced lung injury.

Materials and Methods. We used an in situ isolated, blood perfused rat lung model to measure pulmonary arterial pressure (PAP) and lung weight gain (LWG; g) for 50 minutes after a bolus injection of PMA (0.05 μg/mL). Oxygen radical release was estimated by an LKB 1251 luminometer and by nitric oxide (NO) release as measured by an ENO-20 NO analyzer.

Results. In the group exposed to PMA alone, the mean PAP increased from 16.53 $\pm$ 1.28 to 43.33 $\pm$ 3.40 mm Hg ($P < .001$), and lung weight increased by 4.35 $\pm$ 0.67 g during the 50-minute perfusion after PMA challenge ($P < .001$). In vitro measurement showed that PMA induced a significant increase in oxygen radical release ($P < .001$). PMA attenuated NO release ($P < .001$) into the perfusion system. Pretreatment with cyclosporine (3 mg/kg) for 3 days prevented the increases in both PAP ($P < .01$) and LWG ($P < .001$). NO release was maintained in cyclosporine-pretreated rats. Cyclosporine also showed dose-dependent attenuation of oxygen radical release by PMA-activated white blood cells.

Conclusion. The mechanisms responsible for the protective effect of cyclosporine on the lung injury induced by phorbol may be related to an attenuation of oxygen radical production with maintenance of NO release.

ACUTE LUNG INJURY induced by phorbol myristate acetate (PMA) has been commonly used as an experimental model to study the adult respiratory distress syndrome.[1] Although the etiology and pathogenesis of the condition are uncertain and therapy is problematic, a number of studies have suggested that activated white cells

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0041-1345/08/$–see front matter
doi:10.1016/j.transproceed.2008.07.116

play an important part in its development.[2,3] PMA, an ester derived from croton oil, causes activation and aggregation of white cells. This compound causes white cells to accumulate in the lungs, where they release oxygen radicals as well as secretory granular enzymes that induce pulmonary edema due to endothelial damage both in the whole animal and in isolated lungs.[4,5] Cyclosporine is a potent immunosuppressive agent causing inhibition of lymphocytes,[6] of respiratory burst,[7,8] and of calcineurin.[9] It seems to have antioxidant properties[10] and to inhibit cytokine transcription.[11] In this study, we sought to study the antioxidant effects of cyclosporine on the phorbol-induced respiratory burst and the protective effect of cyclosporine on the phorbol-induced lung injury.

MATERIALS AND METHODS

Preparation of the Isolated Lungs

Sprague-Dawley rats weighing 300 to 350 g were deeply anesthetized with intraperitoneal sodium pentobarbital. The animals were artificially ventilated with room air following tracheostomy, and heparin (1 U/kg, IV) was administered to prevent coagulation. Blood (10–11 mL) was taken from the right ventricle for the preparation of perfusate. A cannula was placed in the pulmonary artery through a puncture into the right ventricle and mitral valve. The catheter fixed by ligature at the apex was used to divert the pulmonary venous outflow into a blood reservoir. A roller pump was used to perfuse the pulmonary artery with constant flow from the blood reservoir. The effluent from the left atrium was freely diverted into the reservoir. The rats were laid on an electrical balance to measure lung weight gain (LWG). The pulmonary arterial perfusion pressure (PAP) was continuously monitored. The pulmonary perfusion flow (8–10 mL/min) was initially adjusted to achieve a PAP of about 15 cm H_2O.[12]

Preparation of Experimental Drugs

PMA (Sigma Chemical Co, St Louis, Mo, United States) dissolved in dimethyl sulfoxide (DMSO; Sigma Chemical Co) at 100 μg/mL was frozen at −70°C. Thawed aliquots were diluted with 0.9% NaCl solution to yield a final perfusate concentration of 0.05 μg/mL immediately before use.

Measurement of Nitric Oxide

High-performance liquid chromatography (HPLC) was used to measure nitrite and nitrate anions derived from nitric oxide (NO) in plasma.

Measurement of Chemiluminescence In Vitro

Chemiluminescence was determined with a luminometer (Model 1251, LKB-Wallac, Sweden). Human whole blood (200 μL) in Dulbecco's buffer (300 μL) was incubated with 10 μL lucigenin (10^{-4} mol/L) and 0.5 μL PMA. We administered various doses of cyclosporine (5, 25, and 50 μg/mL).

Measurement of Protein Concentration in Lung Lavage Fluid

All experiments were terminated after 50 minutes of perfusion, at which time the lungs were removed and lavaged twice with saline (2.5 mL/lavage). Lavaged samples were centrifuged at 1500*g* at room temperature for 10 minutes. The concentration of albumin in the supernate was determined spectrophotometrically by measuring the change in absorbance at 630 nm after addition of bromocresol green.

Experimental Protocol

The 3 groups were: The control group (n = 8) of isolated rat lungs with PAP and LWG continuously monitored over a 50-minute period. At the end of the experiment, lung lavage was performed as described above. The PMA group had the agent added to the perfusate after a 20-minute baseline period. PAP, LWG, and lung lavage protein were obtained as described for the control group. The PMA and cyclosporine group rats were pretreated with cyclosporine (3 mg/kg) for 3 days. PMA was added to the perfusate. PAP, LWG, and lavage protein were obtained as described for the control group.

Data Analysis

Data were expressed as mean values ± SEM. Comparisons within each group for a given parameter were made using paired and unpaired Student *t* tests. Values of $P < .05$ were considered statistically significant.

RESULTS

Phorbol-Induced Increase in PAP and LWG

Figure 1 shows the PAP and LWG tracings during the 50 minutes of perfusion. Phorbol induced a significant increase

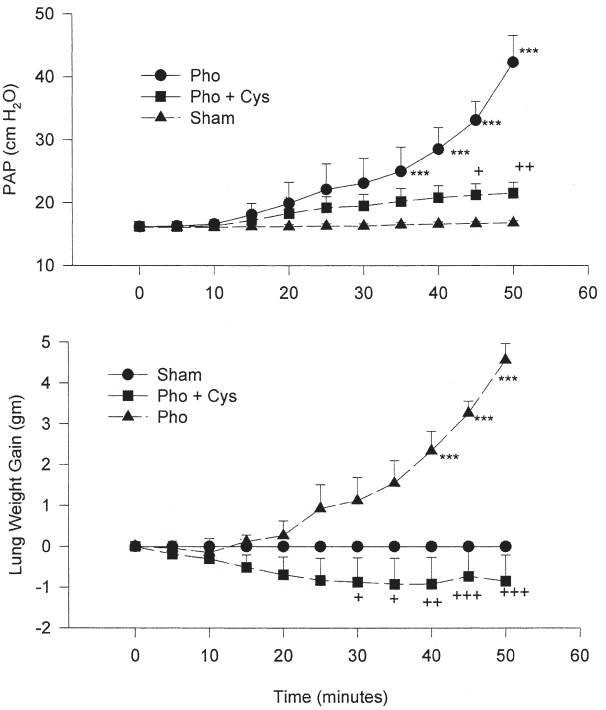

Fig 1. PAP and LWG tracings during the 50 minutes of perfusion after phorbol administration and the interventions from cyclosporine. $^{***}P < .001$, significant differences between phorbol (Pho) and sham group $^{+}P < .05$, $^{++}P < .01$, and $^{+++}P < .001$, significant differences between Pho and cyclosporine (Cys) group.

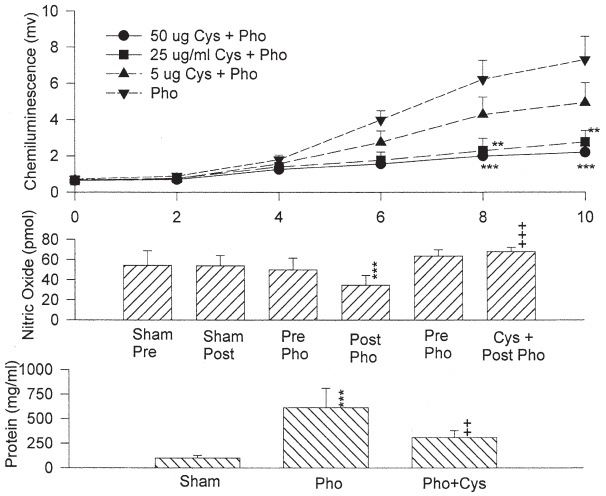

Fig 2. In vitro luminescence response, NO release in the perfused lung, and the lavage protein changes in the various groups. **(Upper)** $^{**}P < .01$, $^{***}P < .001$, significant differences between phorbol (Pho) and cyclosporine (Cys) intervention group. **(Middle)** Significant difference in NO release between pre- and post-phorbol challenge ($^{***}P < .001$). Cyclosporine pretreatment reversed the NO decrease ($^{+++}P < .001$). **(Lower)** Significant increase in the lavage protein concentration after Pho challenge ($^{***}P < .001$). Pretreatment with Cys attenuated the lavage protein increase ($^{++}P < .01$).

in PAP and LWG ($P < .001$). Pretreatment with cyclosporine significantly attenuated the increase in PAP and LWG (see Fig 1 for P values).

In Vitro Luminescence Response, NO Release in the Perfused Lung, and Lavage Protein Concentration

Figure 2 (upper portion) shows the dose-dependent attenuating effects of cyclosporine on phorbol-induced oxygen radical production ($P < 0.01$, $P < .001$). The middle portion shows that phorbol significantly attenuated NO release ($P < .001$) and cyclosporine reversed it ($P < .001$). The lower portion shows that phorbol induced lung injury by increasing the lavage protein content ($P < .001$). Pretreatment with cyclosporine attenuated the lavage protein ($P < .01$).

DISCUSSION

The results of the present study showed that cyclosporine attenuated both the PAP increase and the LWG induced by PMA in a rat model (Fig 1). Phorbol administration increased lung weight slowly over 50 minutes, reflecting edema development. Under control conditions (sham group), lung weight remained unchanged during the entire perfusion period. Since the accumulation of fluid in the lung was associated with increased membrane permeability to protein, phorbol significantly increased the lavage protein concentration ($P < .001$) (Fig 2). The increased lavage potein concentration was attenuated by cyclosporine ($P < .01$). In vitro results showed that chemiluminescence significantly increased when whole blood was activated by PMA. This increase in chemiluminescence was dose-dependently attenuated by cyclosporine (Fig 2, upper portion). The in vivo and in vitro results showed that the protective effect of cyclosporine was due a decreased oxygen radical production by leukocytes and maintenance of NO release so that there was less oxidative stress. Related studies have suggested that cyclosporine, a potent immunosuppressive drug, may have effects of oxygen radical scavenge[7,8] on attenuated neutrophil and alveolar macrophage chemotaxis,[13,14] and on decreased lymphocyte sequestration in the lung.[6] The effects of cyclosporine to attenuate oxygen radical generation and to maintain NO production suggest that the drug mitigates phorbol-induced lung injury by attenuation of the oxidative stress induced by PMA.

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