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Methylprednisolone Attenuates Airway and Vascular Responses Induced by Reactive Oxygen Species in Isolated, Plasma-Perfused Rat Lungs

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The effects of methylprednisolone (MP) on the acute airway and pulmonary vascular responses induced by reactive oxygen species (ROS) were investigated in isolated, plasma-perfused rat lungs. ROS were generated by adding xanthine oxidase and hypoxanthine to the perfusate. MP was administered in 3 different ways: 1. Added to the perfusate (1 $mg*ml^{-1}$) 5 min prior to xanthine oxidase and hypoxanthine, 2. Given as intraperitoneal injections (40 $mg*kg^{-1}$) to lung donor rats 12 and 2 hours prior to the experiments, or 3. Combining 1 and 2. The lungs were perfused at constant volume inflow (15 $ml*min^{-1}$). Pulmonary arterial pressure and transpulmonary pressure were followed for 30 min after addition of xanthine oxidase and hypoxanthine. ROS induced a powerful, acute broncho- and vasoconstriction, which was inhibited by addition of MP to the perfusate. Pretreatment with MP also inhibited the vascular and airway responses. Adding MP to the perfusate of pretreated lungs further reduced the ROS-induced smooth muscle constriction. In conclusion, MP inhibits vasoconstriction and bronchoconstriction induced by ROS in isolated rat lungs.

Keywords: Adult respiratory distress syndrome, bronchoconstriction, corticosteroids, free radicals, lung injury, pulmonary vasoconstriction.

INTRODUCTION

Reactive oxygen species (ROS) participate in the pathogenesis and pathophysiology of lung injuries induced by a variety of causes such as air pollutants, cigarette smoke, inhalation of mineral dust, bleomycin, paraquat, ischaemia-reperfusion injury, oxygen toxicity, and in lung diseases such as asthma, fibrotic lung disorders, and the adult respiratory distress syndrome (ARDS).[1,2]

ARDS is characterized by increased microvascular permeability, lung oedema, bronchoconstriction and pulmonary vasoconstriction, causing ventilation-perfusion mismatching, intrapulmonary shunting, and hypoxaemia.[3] Activation of inflammatory mediators such as granulocytes and platelets, the complement system, proteases, the coagulation cascade, and arachidonic acid metabolism appears to be involved in the pathogenesis of ARDS.[3] There is substantial evidence that ROS are important

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mediators of ARDS.[1] Pharmacological doses of corticosteroids, in particular methylprednisolone (MP), have been used both as prophylaxis and treatment of ARDS[4,5] and blunt chest trauma.[6] This use of MP is controversial, and the mechanisms of the possible beneficial action of steroids are largely unknown.

Experimentally generated ROS cause vasoconstriction, lung oedema, and airway constriction, increase microvascular permeability, and impair endothelial function of isolated lung preparations.[7–14] These effects of ROS are to some extent mediated by lipid peroxidation and release of eicosanoids.[1,2,8] Corticosteroids are known inhibitors of eicosanoid generation through inhibition of phospholipase A_2,[15] and corticosteroids may modify lipid peroxidation.[16,17] We have previously shown that MP inhibits the ROS-induced increase of microvascular permeability in isolated rat lungs, both when given directly into the perfusate, or when given as pretreatment to the lung donor.[18]

The aim of this investigation was to study whether MP attenuates the acute vasoconstriction and bronchoconstriction caused by experimentally generated ROS in isolated, plasma-perfused rat lungs. The effects of MP were studied by adding MP to the perfusate shortly before the exposure to ROS, or pretreating the lung donor rats with MP. The effect of combining these 2 ways of MP administration was studied as well.

MATERIALS AND METHODS

Lung Model

Male Wistar rats weighing 200–300 g (Møllegaard Breeding-Anter LTD, Skensved, Denmark) were used. The lungs were prepared and perfused as previously described.[9,12] The lung preparation was suspended in a humidified perspex chamber at 37°C, and perfused at constant volume inflow (15 ml*min^{-1}) in a recirculating system with 20 ml of horse plasma. Plasma was obtained from whole blood mixed with heparin (20 U*ml^{-1}), centrifuged for 15 minutes at 5000 rpm, and stored at –70°C. The outlet pressure was just below 0 mmHg. The lungs were ventilated with 5% CO_2 in air (70 strokes*min^{-1}, tidal volume 2–3 ml and end-expiratory pressure 0.15 kPa).

Reactive Oxygen Species

ROS were generated by adding xanthine oxidase and hypoxanthine to the perfusate. Xanthine oxidase was locally extracted from cows' milk, purified and tested as previously described.[19] It was added to the perfusate to obtain concentrations of either 0.1 or 0.25 U*ml^{-1}. The same batch of xanthine oxidase was used in all experiments, and its activity was checked before each set up by measuring the conversion of hypoxanthine to uric acid. Hypoxanthine (Sigma Chemical Co, St. Louis, Mo, USA) was dissolved in 5% glucose, and added to the perfusate along with xanthine oxidase in a final concentration of 1 mM.

Measurements

Transpulmonary pressure (Ptp) and pulmonary artery pressure (Ppa) were recorded by pressure transducers (AE 840 AME, Horten, Norway). The transducers were connected to a Beckman polygraph (R 500 A) with a preamplifier (9853 A) and an amplifier (411) (Beckman Instruments Inc, Schiller Park, Ill. USA).

Experimental Protocol

Methylprednisolone succinate (MP) (Solu-Medrol, Upjohn International, Kalamazoo, USA) was administered in two different ways: 1) In acute studies MP was added as a bolus dose to the perfusate 5 min prior to xanthine oxidase and hypoxanthine to obtain a MP concentration of 1 mg*ml^{-1} in the perfusate; 2) In pretreatment studies the rats were given MP (40 mg*kg^{-1}) intraperitoneally 12 and 2 hours prior to the experiments.

After start of perfusion the preparation was stabilized for 30 min. Then xanthine oxidase and hypoxanthine were given, and the lungs were observed for 30 minutes.

Acute Studies (Series 1)

The following groups were included: Group 1.1.: Xanthine oxidase (0.25 $U*ml^{-1}$) and hypoxanthine (1 mM) added to the perfusate (n = 8). Group 1.2.: Similar to group 1.1., but MP ($1mg*ml^{-1}$) was given 5 min prior to xanthine oxidase and hypoxanthine (n = 6).

Pretreatment Studies (Series 2)

Group 2.1.: Xanthine oxidase ($0.1\ U*ml^{-1}$) and hypoxanthine (1 mM) were added to the perfusate (n = 10). Group 2.2.: Like group 2.1., but the rats were pretreated with MP ($40\ mg*kg^{-1}$) (n = 6). Group 2.3.: Like group 2.2., but MP was also given to the perfusate as in group 1.1. (n = 7).

Series 1 and series 2 were performed with some months in between. We have previously experienced considerable variations in susceptibility to oxidant injury both in rat lungs and heart.[9,12,20] The concentration of xanthine oxidase to be used was decided empirically before each series, and a lower concentration of xanthine oxidase was required in series 2 to achieve similar lung reactions as in series 1. We have no valid explanation for this phenomenon of "seasonal variations" in the sensitivity to ROS.[21] Lungs with greater macroscopic areas of atelectasis before addition of xanthine oxidase and hypoxanthine were excluded.

Statistics

Results are given as mean ± SEM. The increase in Ppa and Ptp is presented as per cent increase compared to baseline values (ΔPpa and ΔPtp). An ANOVA test was first performed to detect differences between groups during the whole observation period. Apparently significant differences were verified or falsified with a Tukey's multiple range test. Differences at the actual time points were evaluated by a one-way analysis of variance.

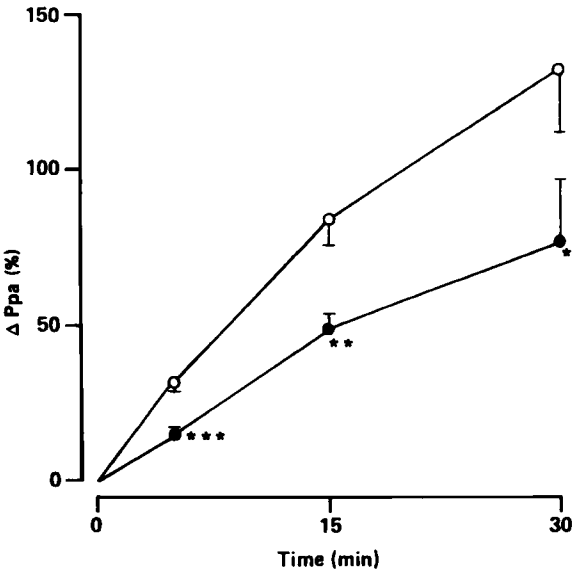

FIGURE 1 The increase in pulmonary arterial pressure (ΔPpa) induced by reactive oxygen species in isolated, plasma-perfused rat lungs (addition of xanthine oxidase and hypoxanthine to the perfusate at time 0) (controls, n = 8, ○—○). The inhibitory effect of methylprednisolone ($1\ mg*ml^{-1}$) in the perfusate is shown (n = 6, ●—●). ΔPpa is in per cent of baseline value, and presented as mean ± SEM. *, **, and *** denote $p < 0.03$, $p < 0.001$, and $p < 0.0001$ compared to the control group.

RESULTS

Pulmonary Arterial Pressure

Acute effects of MP Baseline perfusion pressure was 0.9 ± 0.1 kPa and 1.1 ± 0.1 kPa (groups 1.1 and 1.2). Xanthine oxidase and hypoxanthine increased Ppa as previously described[12] (Fig. 1). This vasoconstriction was significantly inhibited by MP ($p < 0.01$, ANOVA). At 30 min ΔPpa was 132 ± 20% in control lungs, and 77 ± 5% in lungs where MP had been added to the perfusate (Fig. 1).

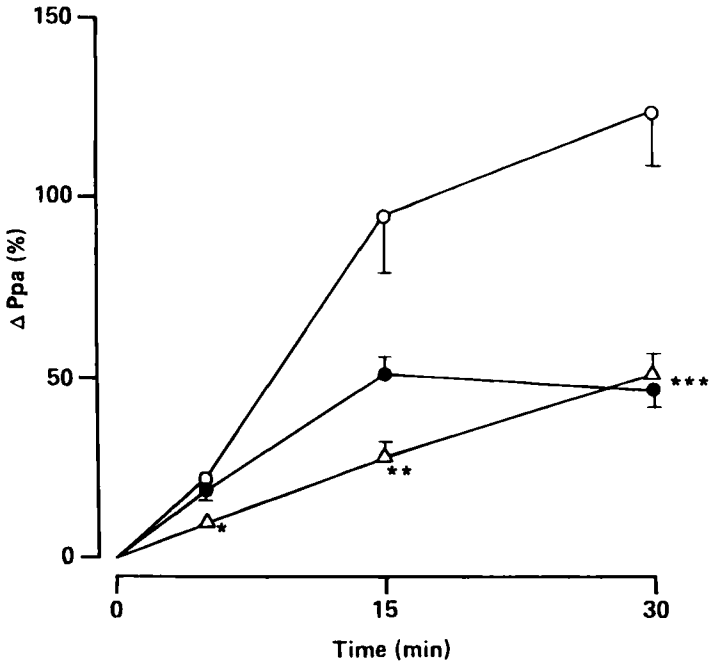

FIGURE 2 The increase in pulmonary arterial pressure (ΔPpa) induced by reactive oxygen species in isolated, plasma-perfused rat lungs (addition of xanthine oxidase and hypoxanthine to the perfusate at time 0) (controls, n = 8, ○—○). The inhibitory effect of pretreatment of the lung donor by methylprednisolone is shown (40 $mg*kg^{-1}$ given 12 and 2 hours before the experiment) (n = 6, ●—●). In another group of pretreated rats, methylprednisolone (1 $mg*ml^{-1}$) was also added to the perfusate (n = 7, Δ—Δ). ΔPpa is in per cent of baseline value, and presented as mean ± SEM. *, **, and *** denote $p < 0.04$, $p < 0.003$, and $p < 0.0002$ compared to the control group.

Pretreatment with MP Baseline perfusion pressure was 1.3 ± 0.1, 1.2 ± 0.1 and 1.3 ± 0.1 kPa for groups 2.1, 2.2., and 2.3., respectively. ΔPpa was attenuated both by pretreatment with MP, as well as pretreatment plus MP added to the perfusate ($p < 0.01$, ANOVA) (Fig. 2). Pretreatment with MP attenuated the late pulmonary vasoconstriction (at 30 min), but had no significant effect on the early vasoconstriction after addition of xanthine oxidase and hypoxanthine (Fig. 2). Combining pretreatment with addition of MP to the perfusate (group 2.3.) reduced the early vasoconstriction significantly (Fig. 2).

Transpulmonary Pressure

Acute effects of MP Baseline Ptp was 0.57 ± 0.02 kPa for both groups (1.1. and 1.2.). Xanthine oxidase and hypoxanthine immediately increased Ptp as previously described[12] (Fig. 3). ΔPtp was 57 ± 15% and 86 ± 15% after 5 and 15 min, respectively, in group 1.1., versus 14 ± 2 and 18 ± 6% in group 1.2. (Fig. 3). MP significantly attenuated ΔPtp when comparing the whole observation period ($p < 0.01$, ANOVA) (Fig. 3).

Pretreatment with MP Baseline Ptp was 0.74 ± 0.01, 0.78 ± 0.01 and 0.76 ± 0.02 kPa in groups 2.1., 2.2., and 2.3., respectively. The ROS-induced ΔPtp was attenuated in both groups given MP ($p < 0.01$, ANOVA) (Fig. 4). The immediate increase of Ptp was only reduced in group 2.3. Adding MP to the perfusate had no advantage over pretreatment alone in the late ΔPtp (Fig. 4).

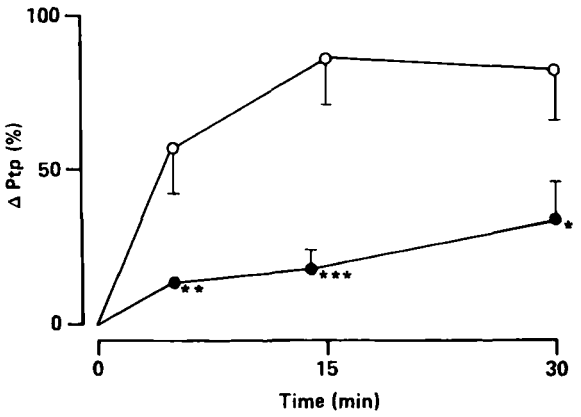

FIGURE 3 The increase in transpulmonary arterial pressure (ΔPtp) induced by reactive oxygen species in isolated, plasma-perfused rat lungs (addition of xanthine oxidase and hypoxanthine to the perfusate at time 0) (controls, n = 8, ○—○). The inhibitory effect of methylprednisolone (1 mg*ml^{-1}) in the perfusate is shown (n = 6, ●—●). ΔPtp is in per cent of baseline value, and presented as mean ± SEM. *, **, *** denote $p < 0.05$, $p < 0.04$, and $p < 0.002$ compared to the control group.

DISCUSSION

Adding xanthine oxidase and hypoxanthine to the plasma perfusate of isolated rat lung induced ROS-mediated responses in the vasculature and the airways as previously described.[12] Since perfusion was with constant flow, changes in perfusion pressure (Ppa) reflect changes in pulmonary vascular resistance. In this model dynamic insufflation pressure and Ptp are the same. From previous studies we know that this also represents end tidal pressure at zero flow in the present model (Vaage, unpublished observations). Consequently, increases in Ptp most likely indicate a reduction in dynamic lung compliance. No detailed evaluation of lung mechanics was performed in the present study, and the increase in Ptp might theoretically be caused by increased airway resistance, a decrease in dynamic lung compliance, a decrease in lung volume, or by a combination of all these factors. Dynamic lung compliance is not good for detection of sudden interstitial fluid accumulation, but a sudden decrease may indicate peripheral airway obstruction by fulminant oedema and alveolar flooding.[22] The "late" increase (at 30 min) in Ptp coincided with development of fulminant oedema,[12] whereas the early, much smaller increase in Ptp most probably was caused by airway constriction and/or airway obstruction, in particular constriction of the small, peripheral airways.[12]

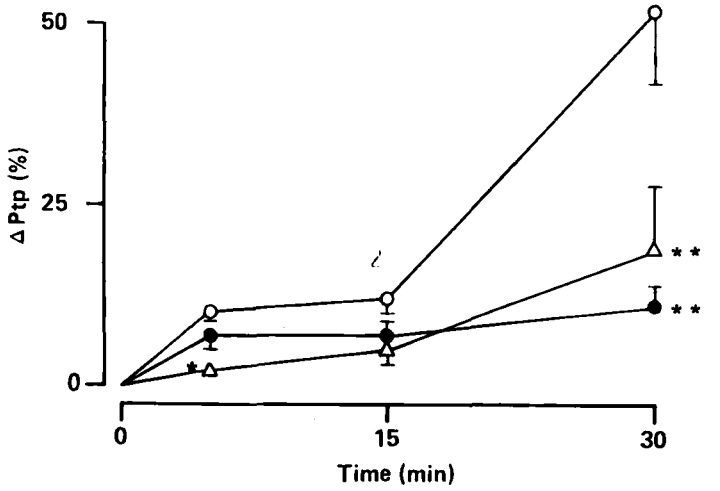

FIGURE 4 The increase in transpulmonary pressure (ΔPtp) induced by reactive oxygen species in isolated, plasma-perfused rat lungs (addition of xanthine oxidase and hypoxanthine to the perfusate at time 0) (controls, n = 8, ○—○). The inhibitory effect of pretreatment of the lung donor by methylprednisolone is shown (40 mg*kg^{-1} given 12 and 2 hours before the experiment) (n = 6, ●—●). In another group of pretreated rats, methylprednisolone (1 mg*ml^{-1}) was also added to the perfusate (n = 7, Δ—Δ). ΔPtp is in per cent of baseline value, and presented as mean ± SEM. *, and ** denote $p < 0.02$ and $p < 0.01$ compared to the control group.

Adding xanthine oxidase and hypoxanthine to the perfusate is a useful model to study the pulmonary effects of ROS, the mechanisms of and the conditions governing these responses, and how ROS-induced lung reactions and injury may be inhibited. The constriction of pulmonary smooth muscle and associated lung injury in the present model is mainly due to generation of H_2O_2 and/or the hydroxyl radical derived from H_2O_2.[12] An isolated organ model has clear limitations in itself, but the present model allows detailed studies of ROS-induced injury without the interaction of blood cells, and without the whole body neuroendocrine modifications.

In the present study MP attenuated the acute airway and vascular constriction induced by ROS, both when MP was given as pretreatment, or when given directly into the lung perfusate. Both ways of MP administration was previously shown to inhibit ROS-induced increase of microvascular permeability in the same model.[18] Although both acute administration and pretreatment with MP had protective effects, addition of MP to the perfusate prior to xanthine oxidase and hypoxanthine was superior to pretreatment alone in inhibiting the early vasoconstriction and bronchoconstriction, possibly because a higher concentration was obtained in the lungs. With pretreatment of the lung donor, only the MP which is incorporated into lung tissue is present during the experiment. With pretreatmant the pattern of protection was also different from protection by administration of MP given directly into the perfusate, since the effect on the late effects of ROS were relatively more pronounced. This observation may suggest that pretreatment protected by mechanisms totally different from those of acute administration. The pretreatment doses of MP correspond to pharmacological doses given to patients with ARDS.[4–6] The concentration of MP when given into the perfusate probably exceeds therapeutic concentrations in patients, but corresponds to the administration of MP in some experimental studies.[16,23,24] MP is almost evenly distributed throughout total body water, reflecting its lipophilicity.[16] MP rapidly penetrates lung tissue, and within 1 hour high concentrations are obtained in bronchial lavage fluid after intravenous administration.[25] Some data suggest that MP is more rapidly incorporated into lung cells than other corticosteroids.[26]

The generation of ROS in the present model mainly takes place during the first 15 minutes after addition of xanthine oxidase and hypoxanthine,[12] and the peak concentration of ROS is probably reached after a few minutes.[12] Pretreatment may have failed to give a sufficient concentration of MP in the vessel wall to deal with the initial burst of ROS. Consequently, in a model of less intense, but longer lasting ROS production, the difference between the modes of administration and different concentrations may theoretically be reduced.

The present study does not elucidate why MP protects against injury by ROS, which can only be the subject of speculations. MP may have direct effects on vascular and bronchial smooth muscles. With their diversity of actions corticosteroids are also circulatory hormones modulating normal and pathologic vasomotion.[27] This is also the case in the pulmonary vascular bed, where MP in some situations may act as a vasodilator.[28] Corticosteroids are not able to directly inhibit xanthine oxidase or to scavenge ROS.[21] However, there are strong indications that MP might be incorporated into cell membranes and inhibit lipid peroxidation.[16,17] This effect is concentration-dependent with a biphasic dose-response curve, and the antioxidant effect follows the tissue concentration of methylprednisolone quite closely, indicative of a nonclassic mechanism of steroid action.[29] A concentration-dependent inhibition of lipid peroxidation by MP might explain why addition of MP to the perfusate, and not pretreatment, had so powerful inhibitory effects during the first minutes of ROS generation. Pretreatment with MP has recently been reported in rats to increase the activity of antioxidant

enzymes: superoxide dismutase, glutathione peroxidase, and catalase in rats.[30] Subsequent studies have demonstrated that MP is able to induce transcriptional activation of the manganese superoxide dismutase gene both in human granulocytes[31] and in rat glomerular cells.[32] An increased activity of antioxidants in lung tissue may be a likely explanation of the protective effects of MP pretreatment.

One possible mode of MP action is blockade of phospholipase A_2,[15] and thus inhibition of ROS-induced arachidonic acid release from membrane phospholipids. Inhibition of membrane lipid hydrolysis and arachidonic acid release is, in addition to inhibiting lipid peroxidation, an important part of the neuroprotective effect of MP.[16] However, in the lungs phospholipase A_2 inhibition occurs only after more than 30 min treatment with corticosteroids.[15] Thus, in the present experiments phospholipase A_2 in lungs was not inhibited by acute administration of MP, but this mechanism might have been important in pretreated lungs, since arachidonic acid metabolites partly mediate ROS-induced lung injury.[1,2,10]

The present study indicates that MP may have a therapeutic role in lung disease and injury where generation of ROS is an important part of the pathogenesis. The pretreatment experiments suggest that MP therapy should be instituted as soon as possible, in some situations prophylaxis might be beneficial. But caution must be exerted, and the effect of MP has to be test in each situation. For instance, from the present findings MP would be expected to attenuate oxygen toxicity. However, MP actually enhanced oxygen toxicity in a sheep model,[33] and in an in vivo rat model of oxygen toxicity MP had no effect on either mortality or histological damage.[34] Obviously in vivo situations with blood cell activation and involvement of a variety of mediators is quite different from our isolated organ model with a more "pure" ROS-induced injury.

In conclusion, MP inhibits the acute effects of ROS on airways and the pulmonary vasculature. The exact mechanisms of these actions of MP are not clarified. Several possible mechanisms exist, and they may differ, dependent on whether MP is given as pretreatment or as an acute treatment. The present study provides a rationale for treatment with MP, and possibly corticosteroids in general, in lung diseases and injuries where ROS is a part of the pathology.

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