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Prevention of Ischemia/Reperfusion-Induced Accumulation of Matrix Metalloproteinases in Rat Lung by Preconditioning With Nitric Oxide

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Background. **Pulmonary ischemia/reperfusion (I/R) injury is associated with degradation of structural proteins. Preconditioning by short-term inhalation of nitric oxide (NO) ameliorates some of the severe consequences of an I/R cycle. The aim of this study was to evaluate the effects of NO preconditioning on I/R-induced changes of matrix metalloproteinase (MMP) activity.**

Materials and methods. **Left lung *in situ* ischemia in rats was maintained for 1 h, followed by reperfusion for 30 min or 4 h. In the NO group, animals inhaled NO (15 ppm) for 10 min directly before ischemia. Changes of expression or activity of MMPs (MMP-2, MMP-7, MMP-9, MMP-14) and of neutrophil elastase (NE) in bronchoalveolar lavage fluid (BALF), lung tissue, and arterial plasma were analyzed by zymography and Western blotting. Western blotting was also used to detect tissue inhibitors of matrix proteases, the extracellular metalloproteinase inducer (EMMPRIN or CD147), and endostatin, a proteolytic collagen fragment.**

Results. **Ischemia resulted in an increase of lavagable MMP activity (12.3-fold MMP-2, 8.1-fold MMP-7) at 30 min reperfusion. The activity of MMP-9 and NE in lung tissue progressively increased with time, whereas MMP-14 and MMP-2 were constant. Inhalation of NO prevented the early increase of MMP-2 and MMP-7 in BALF, but the level of MMP-9 and NE in tissue was not affected. The expression of tissue inhibitors of matrix proteases and EMMPRIN did not respond to any treatment. The release of endostatin proceeded in parallel to the level of MMPs in BALF. Significant correlations between MMP-9 and myeloperoxidase in lung tissue and between MMP-2/MMP-7 and plasma protein extravasation were found.**

Conclusions. **The early rise of MMP-2 and MMP-7 in BALF resulted from plasma protein extravasation, whereas MMP-9 and NE were imported into lung tissue via leukocyte invasion. The effect of NO inhalation on lavagable MMPs was secondary to the sealing of the permeability barrier.**

Key Words: **nitric oxide; ischemia/reperfusion; lung; matrix metalloproteinase; endostatin; EMMPRIN; TIMP.**

INTRODUCTION

Organ transplantation is inevitably associated with tissue ischemia/reperfusion (I/R). Despite major improvements in perioperative and surgical techniques, I/R injury still constitutes a major cause of graft failure after lung transplantation. Pulmonary I/R injury is defined by a complex cascade of systemic and cellular events resulting in inflammation, edema, impairment of gas exchange, microvascular injury, and pulmonary hypertension [1–3].

Evidence has accumulated that the underlying mechanisms of some of these sequelae of an I/R cycle are related to proteolytic degradation processes. The integrity of the lung permeability barrier relies on intact endothelial cell–cell interactions and the adhesion to the extracellular matrix (ECM). Barrier function can be compromised by enzymatic degradation of essential constituents in the cell adhesive structures—tight junctions, adherens junctions, and desmosomes—by enzymes originating from lung tissue itself, like several matrix metalloproteinases (MMPs), or from invading leukocytes like neutrophil elastase [4, 5]. Furthermore, MMPs are involved in the release and activation of a vast array of ECM-bound cytokines, chemokines,

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and growth factors [6, 7]. In this way, they contribute to the development of inflammatory processes but also to repair mechanisms like angiogenesis [8].

The MMPs are a group of zinc-containing hydrolases with the ability to attack all major constituents of the ECM, including native collagens [9]. They are tightly regulated on different levels [10, 11], expression, hydrolytic activation of zymogens, and binding to their endogenous inhibitors, the tissue inhibitors of matrix proteases (TIMPs). Dysregulation is related to chronic obstructive or fibrotic diseases of the lung, like emphysema or chronic obstructive pulmonary disease [9], but the role of MMPs in the dynamics of ECM is not restricted to degradation and remodeling. Hydrolysis of several ECM constituents and also of other substrates leads to exposure of cryptic sites or the release of soluble fragments, both with bioactivities that the parent molecules do not have [12, 13]. Among many others, endostatin and angiostatin, fragments from collagen XVIII and plasminogen function as inhibitors of angiogenesis and regulators of viability and motility of endothelial cells [14].

In contrast to the contribution of proteases to chronic lung diseases and the associated remodeling of lung structure, the I/R-induced consequences that occur within minutes or hours have rarely been investigated. In different animal models and experimental settings, I/R of the lung resulted in the increase of expression or activation of the gelatinases, MMP-2 and MMP-9 [15–17]. These effects were associated with degradation of collagen IV [17] and alveolar-capillary permeability alterations [15]. Conclusive evidence of a role of MMPs in the induction of I/R injury was obtained by prophylactic application of broad band inhibitors of MMPs and neutrophil elastase (NE) in different models of lung I/R and adult respiratory distress syndrome. The treatment ameliorated symptoms of I/R injury like inflammation, neutrophil invasion, interstitial edema, and impaired gas exchange [17–20].

A frequently investigated approach to prevent I/R injury, at least in animal models, is ischemic preconditioning, meaning that short episodes of ischemia can suppress the effects of an extended period of ischemia later on. Studies on the mechanism have shown that the release of nitric oxide (NO) is an essential step in the complex signaling chain of preconditioning [21]. Consequently, application of NO by inhalation for a short period before onset of ischemia has been demonstrated to prevent the detrimental effects of an I/R cycle on lung function [22].

Several lines of evidence have shown that NO suppresses expression or secretion and activation of MMPs, at least of gelatinases, in lung tissue or in isolated lung epithelial cells [23–26], but contradictory results have also been reported [27]. In the present communication, the hypothesis is tested that NO inhalation in the preconditioning mode suppresses I/R-induced effects on MMPs and NE. A previously described [28] rat *in situ* model of left lung ischemia is used for this purpose. These recent studies have shown that NO inhalation for 10 min at a moderate concentration of 15 ppm is sufficient to prevent the increase of permeability and to ameliorate the deterioration of gas exchange capacity [28]. Here we present evidence that accumulation of MMP activity without compensation by endogenous inhibitors proceeds by extravasation from plasma, secondary to the impairment of the permeability barrier, and by invasion of leukocytes. The extravasation of MMPs and the release of endostatin, a collagen fragment, could be partially blocked by NO inhalation.

MATERIALS AND METHODS

Surgical Protocol

The study was approved by the Animal Care Section of the Saxonian Government, Dresden, Germany, and the Animal Care Committee of the University of Dresden. Male rats (Sprague Dawley, 300–350 g) were obtained from Charles River Laboratories (Sulzfeld, Germany).

The surgical protocol was described in detail in a recent report [28]. Briefly, the animals were initially anesthetized by intraperitoneal injection of 100-150 mg/kg sodium pentobarbital (Merial GmbH, Hallbergmoos, Germany). Tracheostomy was carried out, and animals were ventilated using a pressure-controlled RUS-1300 ventilator (FMI, Seeheim, Germany), at a rate of 60/min, an inspired oxygen content of 99%, a positive end-expiratory pressure of 2 cm H_2O, and a maximal peak pressure of 10 cm H_2O. Atropine sulfate (B. Braun AG, Melsungen, Germany) was injected (intramuscular, 0.1 mg) to stabilize heart rates and blood pressure. The animals were positioned on the right side before left lateral thoracotomy in the fifth intercostal space was carried out, and the pulmonary hilum was dissected. Animals were heparinized with 500 IU sodium heparin (Ratiopharm GmbH, Ulm, Germany), and isotonic NaCl (0.5 mL, subcutaneous) was injected. At the end of the experiment, the animals were placed in supine position to create a midline incision. Blood samples were taken from the aorta ascendens for blood gas analysis and to obtain ethylenediaminetetraacetic acid plasma. The animals were then sacrificed by severing the aorta abdominalis. The heart–lung block was excised after flushing the pulmonary circulation with 20 mL isotonic saline, and bronchoalveolar lavage was carried out as outlined elsewhere [28]. The 4 lobes of the right lungs were separated, and the left lungs were dissected into 3 equal parts. The lung samples were snap-frozen in liquid nitrogen and stored in a nitrogen tank.

Treatment Groups

In the I/R groups, the left pulmonary hilum was closed with a noncrushing clamp for 1 h with subsequent reperfusion for 30 min (I/R30) or 4 h (I/R4). Anesthesia was maintained, as required, by a further injection of 16 mg pentobarbital. Animals in the NO groups (NO30 and NO4) received the same treatment as in the I/R groups, except that NO to a concentration of 15 ppm was blended into the inspiratory gas flow from a reservoir of 1000 ppm NO, balance N_2 (Westfalen AG, Münster, Germany) for 10 min, directly before ischemia was initiated by occluding the left hilum. The concentrations of NO and O_2 were measured with electrochemical sensors in a flow cell of a Polytron 7000 transmitter (Dräger Safety, Lübeck, Germany). An-