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Protein kinase C inhibition attenuates hypochlorite-induced acute lung injury ☆

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Summary
Neutrophil-derived oxidative stress plays a crucial role in acute lung injury. Hypochlorite/hypochlorous acid (HOCl) is a major oxidant of neutrophils. Protein kinase C (PKC) may be an appropriate target for HOCl due to its functionally important thiols. This study investigates the role of PKC in HOCl-induced acute lung injury.
Isolated lung preparations were from 30 rabbits. HOCl (1000 nmol min^{-1}) or buffer (control) were infused into isolated rabbit lungs. Pulmonary artery pressure (PAP [Torr]) and lung weight were continuously measured. Capillary filtration coefficient ($K_{f,c}$), was measured at baseline and at 30, 60, 90 min. Experiments were terminated at 105 min or when fluid retention exceeded 50 g. The non-selective protein kinase inhibitor staurosporin (100 nM) or the selective PKC inhibitor bisindolylmaleimide I (GF109203X, 10 nM) were added to the perfusate 5 min prior to the start of the experiments.
Staurosporin completely prevented the HOCl-induced increase in PAP (no change versus ΔPAP_{max} 5.2 ± 0.78) but did not influence the increase in vascular permeability. GF109203X delayed the HOCl-induced increase in PAP and vascular permeability. PAP_{max} was observed significantly later in the HOCl-GF109203X group (84.4 ± 4.0 min) in comparison with the HOCl group (52.1 ± 3.5 min). Termination of the experiments due to edema formation occurred significantly later in experiments with GF109203X (91.8 ± 1.9 versus 79.2 ± 4.1 min).
Protein kinases are involved in HOCl-induced acute lung injury. Specifically PKC inhibition delayed HOCl-induced increases in PAP and vascular permeability.

Introduction

Neutrophils play a crucial role in the pathogenesis of acute lung injury.[1] The sequestration of neutrophils in

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the pulmonary microvasculature due to chemotactic stimuli is regarded as an initiating event of acute respiratory distress syndrome.[2] It is accompanied by a large increase in neutrophils and myeloperoxidase activity in bronchoalveolar lavage fluid.[2] Stimulated neutrophils may affect lung tissue through the release of proteolytic enzymes,[1] the production of prostanoids[3] or the generation of highly reactive oxygen species.[1] Oxidative stress is considered as a major pathway in acute lung injury. Various experimental models and clinical studies demonstrate the involvement of oxidative stress in acute lung injury.[4,5]

Hypochlorous acid/hypochlorite (HOCl) is the predominant oxidant of the stimulated neutrophil. HOCl is synthesized by neutrophil-derived myeloperoxidase. This enzyme targets free functional groups of proteins and amino acids, predominantly sulfhydryl groups.[6] The effects of HOCl on isolated rabbit lungs are comparable to the effects of stimulated neutrophils.[7] The increase in pulmonary artery pressure (PAP) and vascular permeability is accompanied by accumulation of lipid peroxidation products.[8] The mechanisms mediating HOCl-induced effects during acute lung injury are not entirely elucidated. However, the following considerations suggest an involvement of protein kinase C (PKC).

All PKC isoforms consist of a N-terminal regulatory domain and a C-terminal catalytic domain. These domains are connected by flexible hinge regions.[9,10] In the resting state, the enzyme is kept inactive by intramolecular interaction of an auto-inhibitory sequence (pseudosubstrate) of the regulatory domain and the substrate-binding site of the catalytic domain.[10] Activation of the enzyme requires the binding of diacylglycerol (DAG) to the regulatory domain thus enhancing the affinity of PKC to the cell membrane.[9] The binding of phospholipids, such as phosphatidylserine, and DAG causes conformational changes rendering the enzyme active.[9,10] The DAG binding sites exhibit two pairs of zinc fingers located at the regulatory site. Each zinc finger consists of six cysteine residues that coordinate two zinc atoms.[11] This positively charged zinc thiolate structure is highly susceptible to negatively charged oxidants, such as the HOCl ion (OCl^-).[12] Oxidative modification of these cysteine residues destroys the zinc fingers. The autoinhibition is relieved and co-factor independent activity is commenced.[13] In addition oxidative stress-induced activation of phospholipase A2 (PLA2),[14] phospholipase C (PLC)[15] and phospholipase D (PLD)[16] results in the release of DAG and phospholipids from the cell membrane and subsequently in co-factor dependent PKC activation. Hydrogen peroxide has been shown to activate PKC in bovine tracheal smooth muscle cells.[17] PKC activation has been shown to induce pulmonary vasoconstriction,[18] to be involved in pulmonary hypoxic vasoconstriction[19] and to increase endothelial cell permeability.[20]

We hypothesized that the acute increase in PAP and vascular permeability in response to HOCl is mediated by PKC activation. This study therefore investigates the influence of PKC inhibition with a non-specific serine-/threonin kinases inhibitor, Staurosporin, and a specific inhibitor of PKC (bisindolylmaleimide I; GF 109203X [BIM]) on HOCl-induced increase in PAP and vascular permeability.

Methods

Isolated rabbit lung model

General procedure

Rabbits of either sex between 2.5 and 3.0 kg were used. Isolated lungs were prepared according to the method described in detail by Seeger et al.[21] and as published previously.[7,22] Lungs were ventilated with a mixture of 4% CO_2, 17% O_2 and 79% N_2 to maintain perfusate pH between 7.37 and 7.40. A rodent animal respirator was used. The ventilatory frequency was set to 30 min^{-1}. Positive end-expiratory pressure was set to 2 cmH_2O and tidal volume was set to 30 ml. Perfusion flow was gradually increased to 100 ml min^{-1}. Krebs–Henseleit buffer (NaCl 125 mM, KCl 4.3 mM, glucose 13.32 mM, KH_2PO_4 1.1 mM, $MgCl_2$ 1.3 mM, $CaCl_2$ 2.4 mM, $NaHCO_3$ 24 mM) was used for perfusion. The total volume of the re-circulating perfusate was 300 ml and perfusate temperature was kept at 37 °C. PAP, pulmonary venous pressure, ventilation pressure and weight gain of the isolated organ were continuously recorded. Following a steady-state period only those lungs were selected for the study that fulfilled entry conditions: no signs of leakage, a homogeneous white appearance, lack of macroscopically visible edema, no increase in lung weight. Pulmonary venous pressure was adjusted to 2 mmHg.

Hydrostatic challenge

Capillary filtration coefficient ($K_{f,c}$) was determined gravimetrically from the slope of lung weight gain after a sudden venous pressure elevation of 10 cmH_2O for 8 min.[21] It was expressed as 10^{-4} ml s^{-1} cmH_2O^{-1} g^{-1}. The total rapid change in weight over the first 2 min following the onset of the venous pressure rise was designated as pure vascular filling and used for the calculation of vascular compliance (C, [g cmH_2O^{-1}]).[21] Retention (ΔW, [g]) was determined as the remaining difference in weight before and after a hydrostatic challenge.

Substances

BIM was purchased from Calbiochem (Darmstadt, Germany). All other reagents were obtained from Sigma (Munich, Germany). The concentration of the HOCl stock solution was determined spectrophotometrically ($\varepsilon_{290\,nm} = 350$ mol^{-1} cm^{-1}) immediately before use.

Perfusate concentrations of potassium and lactate dehydrogenase activity

Potassium concentration and lactate dehydrogenase were used to exclude severe tissue damage. The measurements were performed by standard methods.

Experimental protocol

Experimental groups are summarized in Table 1. The continuous application of 1000 nmol min^{-1} HOCl (HOCl group) or of buffer for control (C group) into the arterial line of the system was started at $t = 0$ min following a