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Selective phosphodiesterase 3 inhibitor olprinone attenuates meconium-induced oxidative lung injury

Daniela Mokra [a,*], Anna Drgova [b], Rudolf Pullmann Sr. [c], Andrea Calkovska [a]

[a] Department of Physiology, Jessenius Faculty of Medicine, Comenius University and Martin University Hospital, Mala Hora 4, SK-03601 Martin, Slovakia
[b] Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University and Martin University Hospital, Martin, Slovakia
[c] Department of Clinical Biochemistry, Jessenius Faculty of Medicine, Comenius University and Martin University Hospital, Martin, Slovakia

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ABSTRACT

Since inflammation and oxidation play a key role in the pathophysiology of neonatal meconium aspiration syndrome, various anti-inflammatory drugs have been tested in the treatment. This study evaluated whether the phosphodiesterase (PDE) 3 inhibitor olprinone can alleviate meconium-induced inflammation and oxidative lung injury. Oxygen-ventilated rabbits intratracheally received 4 ml/kg of meconium (25 mg/ml) or saline. Thirty minutes after meconium/saline instillation, meconium-instilled animals were treated by intravenous olprinone (0.2 mg/kg) or were left without treatment. All animals were oxygen-ventilated for an additional 5 h. A bronchoalveolar lavage (BAL) of the left lungs was performed and differential leukocyte count in the sediment was estimated. The right lungs were used to determine lung edema by wet/dry weight ratio, as well as to detect oxidative damage to the lungs. In the lung tissue homogenate, total antioxidant status (TAS) was determined. In isolated lung mitochondria, the thiol group content, conjugated dienes, thiobarbituric acid-reactive substances (TBARS), dityrosine, lysine-lipid peroxidation products, and activity of cytochrome c oxidase (COX) were estimated. To evaluate the effects of meconium instillation and olprinone treatment on the systemic level, TBARS and TAS were determined in the blood plasma, as well. Meconium instillation increased the relative numbers of neutrophils and eosinophils in the BAL fluid, increased edema formation and concentrations of oxidation markers, and decreased TAS. Treatment with olprinone reduced the numbers of polymorphonuclears in the BAL fluid, decreased the formation of most oxidation markers in the lungs, reduced lung edema and prevented a decrease in TAS in the lung homogenate compared to non-treated animals. In the blood plasma, olprinone decreased TBARS and increased TAS compared to the non-treated group. Conclusion, the selective PDE3 inhibitor olprinone has shown potent antioxidative and anti-inflammatory effects in the meconium-induced oxidative lung injury.

1. Introduction

Meconium aspiration syndrome (MAS) is a serious respiratory disorder in the term and post-term neonates. When aspirated, the first faeces of the newborn - meconium - moves distally and obstructs the peripheral airways. In the alveoli, meconium components deteriorate the surfactant function and induce inflammation. All together they lead to oxidative lung injury, lung edema, pulmonary vasoconstriction, and airway hyperreactivity [1]. Inflammation and subsequent damage of the lung tissue may be caused by both hydrophilic and hydrophobic fractions of meconium. In addition, meconium contains various pro-inflammatory substances [2] that enhance chemotactic activity of polymorphonuclears (PMNs), particularly of neutrophils, and stimulate their adhesion to endothelium and trigger a leak through the alveolocapillary membrane [3,4]. Monocytes/macrophages are important in meconium-induced lung inflammation, as well [5]. Alveolar macrophages play a key role during the progression of inflammation in the lungs providing a first line of defense in the lower respiratory tract against inhaled particles such as meconium. In an *in vitro* study, meconium interfered with alveolar macrophages by inducing oxidative stress and apoptosis [5]. Thus, aspirated meconium may induce inflammation directly or indirectly through the release of biologically active substances from the activated cells [3]. The presence of meconium in the lungs facilitates an expression of cytokines (e.g. TNFα, IL-1, -6, -8), pro-inflammatory enzymes (phospholipase A_2, proteases) and other substances like derivatives of arachidonic acid, endothelin-1,

* Corresponding author. Tel./fax: +421 434131426.
E-mail address: mokra@jfmed.uniba.sk (D. Mokra).

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platelet activating factor, etc. Furthermore, activated cells create large amounts of reactive oxygen species (ROS). All the mentioned substances then participate in surfactant dysfunction, parenchymal damage, edema, pulmonary vasoconstriction and contraction of the bronchial smooth muscle [1].

Based on this knowledge, various anti-inflammatory drugs (glucocorticoids, inhibitors of phosphodiesterases, inhibitors of angiotensin-converting enzyme, inhibitors of cyclooxygenase, antioxidants etc.) have been tested as possible treatment of MAS [1]. In our recent experiments, the non-selective phosphodiesterase (PDE) inhibitor aminophylline has shown significant anti-inflammatory and antioxidative effects on meconium-injured lungs [6]. Because of the possibility of less side effects, selective PDE inhibitors (particularly PDE3, 4, 5 and 7) have been introduced in the treatment of respiratory disorders with bronchoconstriction and inflammatory components [7–11]. In MAS, a PDE5 inhibitor sildenafil reversed a meconium-induced increase in pulmonary vascular resistance in a piglet model [12] and milrinone, a PDE3 inhibitor, has improved oxygenation and the survival of four neonates with MAS [13].

PDE3 inhibitors selectively inhibit the action of the PDE3 enzyme. PDE3 inhibitors are commonly used in the therapy of acute heart failure and cardiogenic shock due to their positive inotropic effect, as they increase intracellular concentration of cAMP and enhance Ca^{2+} influx into the cardiac muscle cells [14,15]. PDE3 inhibitors also have a vasodilation effect, as they cause the relaxation of smooth muscle cells in peripheral vessels decreasing cytosolic Ca^{2+} [11,16]. In addition, PDE3 inhibitors may also be useful in the treatment of respiratory diseases due to their bronchodilation and antitussive effects [10,15,17]. Besides these effects, there is increasing evidence of an anti-inflammatory and antioxidative action of PDE3 inhibitors [9,18,19].

Among PDE inhibitors, the most potent anti-inflammatory action was observed with PDE4 inhibitors [7,8,10]. On the other hand, older PDE3 inhibitors (amrinon, milrinon, cilostamide) have shown little or no anti-inflammatory action. However, they enhance the anti-inflammatory effect of PDE4 inhibitors [20,21]. The anti-inflammatory action of olprinone – a newly developed PDE3 inhibitor - was found accidentally after administration in patients with cardiopulmonary bypass [22] and acute heart failure [18]. Recently, these effects have also been proven in rats with lipopolysaccharide-induced acute lung injury [9].

As our assumption is that PDE3 inhibitors, alongside their modulatory effects on inflammation, may also reduce the formation of ROS and the oxidative damage, the effects of olprinone – a representative of selective PDE3 inhibitors – were evaluated in a model of meconium-induced oxidative lung injury. The anti-oxidative action of olprinone was determined particularly in the lung mitochondria, as the oxidation processes are most prominent in the mitochondria. To assess the meconium-induced oxidation as well as the antioxidative effect of the treatment on the systemic level, concentrations of thiobarbituric acid-reactive substances (TBARS) as a marker of lipid peroxidation, and TAS were also estimated in the blood plasma. In addition to oxidation markers, the total and differential white blood cell (WBC) counts in the arterial blood and differential WBC count in the BAL fluid, as well as wet/dry lung weight ratio expressing the extent of lung edema were determined to evaluate the effect of olprinone on lung inflammation.

2. Methods

2.1. General design of experiments

Fresh meconium was collected from 20 healthy neonates, lyophilized and stored at −20 °C. Before use, meconium was suspended in 0.9% NaCl at a concentration of 25 mg/ml.The design of the experiments was approved by Local Ethics Committee of Jessenius Faculty of Medicine. Adult rabbits (chinchilla) of 2.7 ± 0.4 kg were anesthetized with intramuscular ketamine (20 mg/kg; Narkamon, Spofa, Czech Republic) and xylazine (5 mg/kg; Rometar, Spofa, Czech Republic) followed by a continuous infusion of ketamine (20 mg/kg/h). A tracheotomy was performed and catheters were inserted into the femoral artery and right atrium for sampling arterial and mixed venous blood, and into the femoral vein to administer anesthetics. The animals were then paralyzed with pipecuronium bromide (0.3 mg/kg/30 min; Arduan, Gedeon Richter, Hungary) and subjected to a pressure-controlled ventilator (Beat-2, Chirana, Slovakia). All the animals were ventilated with a frequency of 30/min, fraction of inspired oxygen (FiO_2) of 0.21, inspiration time 50%, peak inspiratory pressure (PIP) to keep a tidal volume (V_T) between 7 and 9 ml/kg and no positive end-expiratory pressure (PEEP) at this stage of the experiment. After the stabilization, ventilatory parameters were recorded and samples of blood were taken for blood gas analysis and an estimation of hemoglobin by blood analyzer (Rapidlab™348, Bayer Diagnostics, Germany). Then, 4 ml/kg of saline (Sal group, $n = 7$) or meconium suspension (25 mg/ml) were instilled into the tracheal tube homogenously, into the right and left lungs during the positioning of the animal. From this moment on, all animals were oxygen-ventilated (FiO_2 1.0, PEEP 0.3 kPa). Within 30 min after meconium instillation, respiratory failure developed, defined as >30% decrease in dynamic lung-thorax compliance (C_{dyn}) and PaO_2<10 kPa at FiO_2 1.0. Blood samples were taken and parameters recorded again. Meconium-instilled animals then received intravenously a single dose of 0.2 mg/kg olprinone (Olprinone hydrochloride, Sigma–Aldrich, Germany; Mec + Olp group, $n = 8$) 0.5 h after meconium instillation or were left without treatment (Mec group, $n = 8$). Animals were oxygen-ventilated for an additional 5 h.

Tracheal airflow and V_T were measured by a Fleisch head connected to a pneumotachograph. Airway pressure was registered via a pneumatic catheter placed below the tracheal tube and connected to an electromanometer. C_{dyn} was calculated as a ratio between V_T adjusted per kg b.w. and airway pressure gradient (PIP-PEEP). Samples of arterial blood were taken before meconium instillation and after 1, 3 and 5 h of the treatment and the total and differential WBC count were determined microscopically and by hematology analyzer Mindray BC-5500 (Mindray Medical International Ltd., China).

At the end of experiments, 4 ml of arterial blood was taken and centrifuged at 3000 rpm for 15 min. Blood plasma was stored at −70 °C till the biochemical analyses were performed. Then, the animals were killed by an overdose of anesthetics and the lungs were excised. The left lungs were lavaged by saline (0.9% NaCl, 37 °C) 3 × 10 ml/kg, bronchoalveolar lavage (BAL) fluid was centrifuged at 1500 rpm for 10 min and the differential WBC count in the sediment was evaluated microscopically after staining by Pappenheim. The right lungs were cut, strips of the tissue were weighed and dried at 60 °C for 24 h to determine the wet/dry weight ratio, or were stored at −70 °C for biochemical analyses.

2.2. Biochemical analyses

Lung tissue was washed, minced and homogenized in 50 mmol of phosphate buffer (pH = 7.4) and 1 mmol of butylated hydroxytoluene (BHT) in a ratio 1:5 using homogenizer Potter (B. Braun Melsungen A.G., Germany) at a temperature 0–4 °C. A protein assay was performed by method of Lowry et al. [23]. For the dilution of the homogenate, 1% sodium dodecyl sulfate (SDS) was used. The concentration was calculated using the bovine serum albumine (BSA) as a standard.