Original Article
Effects of dexmedetomidine on the protection of hyperoxia-induced lung injury in newborn rats

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Abstract: Dexmedetomidine (Dex) is a specific agonist of α2-adrenoceptor and was reported to have protective effect on a variety of organs, however the effect of Dex on hyperoxia-induced lung injury remains unknown. In the present study, Dex was administrated to newborn rats and its effect against hyperoxia-induced lung injury was examined. The results showed that, Dex significantly attenuated the aberration, macrophage infiltration, inflammatory responses and pulmonary edema induced by hyperoxia. In addition, the down-regulation of AQP1 was also reversed by Dex. These data indicate that Dex may be a potential therapy in the prevention of hyperoxia-induced lung injury in infants.

Keywords: Dexmedetomidine, hyperoxia-induced lung injury, inflammatory responses, pulmonary edema, AQP1

Introduction

With the development of perinatal medicine and neonatal intensive care, the survival rate of premature or low-birth weight infant has been raised significantly. Mechanical ventilation with hyperoxia as the primary treatment also leads to lung injury and causes complications such as bronchopulmonary dysplasia (BPD), that influence the long-term outcome of children [1]. Pathological changes of BPD mainly characterized pulmonary fibrosis in acute phase and pulmonary edema in chronic phase [2]. The pathogenesis of BPD is complex and no effective clinical treatment has been developed.

Dexmedetomidine (Dex), a highly specific agonist of α2-adrenoceptor with sedative [3], analgesic [4, 5], anxiolytic [6] and neuroprotective [7] physiological effects. As a sedative, Dex has several unique properties such as attenuates respiratory depression [8], reduces hemodynamic change [9], stable sedative and awake effect [10] and diminishes opioid requirements [11]. Recently, Dex has also been reported to have a protective effect on lung, liver, heart and kidney through the inhibition of inflammatory and oxidative stress reaction that induced by endotoxemia or ischemia reperfusion [12-16].

A previous study showed that high dose Dex significantly attenuates ventilator-induced lung injury via the regulating of pulmonary inflammation [17]. In addition, treated with Dex result in a lower inflammation, hemorrhage and edema score in acute lung injury in rats induced by α-naphthylthiourea [18]. Therefore, in the present study, we hypothesized that administration of Dex would attenuate hyperoxia-induced lung injury. Our result showed that Dex relieved the aberration, inflammation and edema in lungs of newborn rats and provides a new way for the treatment of hyperoxia-induced lung injury.

Materials and methods

Animal model

Pregnant Sprague-Dawley rats were purchased from Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). All animal experiments were approved by the Animal Use and Care Committee of Harbin Medical University. Naturally delivered newborn rats were randomly divided into five groups within 24 hours of birth, hyperoxia (Hyp, n = 8); hyperoxia plus dexmedetomidine (Hyp+Dex, n = 8); hyperoxia plus yohimbine (Hyp+Yoh, n = 8); hyperoxia plus dexmedetomidine plus yohimbine (Hyp+Dex+Yoh, n
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Table 1. Primers used in real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’-3’)</th>
<th>Produce size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>F: GTTGCCTTCTTGGGACTGATG</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>R: TACTGGTCTGTGGGGTGTTT</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>F: GCCACACGCTCTTAGTC</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>R: GCTACGGGGCTGTCACCTG</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>F: TCCAGTGCGGCTCTTTG</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>R: CGAGATGCTGCTGTGAGATT</td>
<td></td>
</tr>
<tr>
<td>AQP1</td>
<td>F: ACCCGCAACTTCTCAAC</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>R: CAGGTCATACTCCTCAGTT</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>F: GGAGATTACGTGCGGTGCTCTAGC</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>R: GGCCGGACTCGTACTCCTGT</td>
<td></td>
</tr>
</tbody>
</table>

= 8); Normoxia control (Nor, n = 8). Hyperoxia treatment was performed as previously described [19], rats were placed in oxygen chambers with a continuous oxygen supply (FiO₂ = 0.90, monitored by oxygen recorder) and constant temperature (25°C) and humidity (50%-70%). The chambers were opened for 1 hour everyday at fixed time to add water, food and inject drug, maternal rat was exchanged daily with control group to avoid poor feeding caused by oxygen toxicity. Dexmedetomidine or yohimbine was injected through intraperitoneal, 500 μg/kg every day. Pups in normoxia control group were exposed to normoxia (FiO₂ = 0.21) and administration with the same volume of solvent. Animals were sacrificed on day 7, freshly harvested lung samples from each group were weighed and then placed in a thermostatic oven. The dry weight was detected until the weight does not change, then the wet/dry weight ratio was determined. The remaining lung tissues were fixed by 4% paraformaldehyde for histological analysis or immediately frozen in liquid nitrogen and stored at -80°C for biochemistry and molecular biology detection.

Histological analysis

Lung tissues from each group were paraffin-embedded by conventional procedures, then sectioned into slices of 5-μm thickness. Hematoxylin and eosin staining was conducted according to the standard procedure. For immunohistochemistry analysis, the lung tissue sections were rinsed with 1% phosphate buffered saline for several times and endogenous peroxidase was blocked by 3% H₂O₂. After that, 10% normal goat serum was used to block nonspecific binding. Then, the slices were incubated with a primary antibody (AQP1, dilution 1:100, Boster, Wuhan, China; F4/80, dilution 1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight, negative control was incubated with PBS. After washing, the sections were incubated with a biotinylated goat anti-rabbit IgG (Beyotime, Haimen, China) at 37°C for 30 min followed by incubating with avidin-horseradish peroxidase complex (Beyotime). Finally, the sections were visualized with 3,3’-diaminobenzidine (Beyotime) and counterstained with hematoxylin (Solarbio, Beijing, China).

Real-time PCR

Total RNA of lung tissues were extracted by using RNA Simple Total RNA Kit (TianGen, Beijing, China) and reversely transcribed into cDNA using Super M-MLV Reverse Transcriptase Kit (Biotek Corporation, Beijing, China). Primers were synthesized as Table 1. Real-time PCR was performed using SYBR Green (Solarbio) on Exicycler TM 96 (Bioneer, Daejeon, Korea). β-actin was used as an internal control. Data were analyzed via the comparative threshold cycle (Cₜ) method.

Western blot

Protein from lung homogenates was extracted by NP-40 lysis buffer (Beyotime) and the concentration was determined using bicinchoninic acid (BCA) method. After that, 40 μg of protein were separated on sodium dodecyl sulfate polyacrylamide gel and electrically transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% non-fat dry milk and then incubated with antibody against AQP1 (dilution 1:400, Boster) overnight at 4°C. After incubation with horseradish peroxidase labelled goat anti-rabbit IgG (Beyotime) at 37°C for 45 min, the membranes were visualized with an enhanced chemiluminescence (ECL) kit (Qihai Biotecnology, Shanghai, China). Densitometric analysis was conducted with Gel-Pro Analyzer Version 3.0 (Media Cybernetics, Silver Spring, MD, USA) and β-actin was used as an internal control to calculate the relative protein levels.

Inflammatory reaction

Total TNF-α, IL-1β and IL-6 protein concentration in lung homogenates was analyzed by commercial enzyme-linked immunosorbent assay kits according to the manufacturer's instruc-
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Myeloperoxidase (MPO) activity was determined by Myeloperoxidase activity assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

**Statistical analysis**

Data were showed as means ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni
tions (USCN Life Science, Wuhan, China). Myeloperoxidase (MPO) activity was determined by Myeloperoxidase activity assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

**Figure 1.** Dex attenuates lung morphological changes induced by hyperoxia. Representative pathologic changes of lung tissues from each group determined by hematoxylin and eosin staining. Scale bar = 100 μm.

**Figure 2.** Dex inhibits macrophage infiltration in lungs induced by hyperoxia. F4/80 expression of lung tissues were identified by immunohistochemistry, representative results are shown and scale bar = 50 μm. Optical density was analyzed by IPP6.0 software. Data were expressed in means ± SD, **P < 0.01 vs. Nor group, ***P < 0.01 vs. Hyp group.
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Results

Dex attenuates lung morphological changes induced by hyperoxia

The morphological changes of lung tissues were observed by hematoxylin and eosin staining (Figure 1). The alveolar structure has been initially formed in Nor group at day 7, whereas alveoli in Hyp group was larger and the interstitial thickness was decreased, suggested that the formation of alveolar was arrested by hyperoxia. The changes in lung morphology were alleviated by administration of Dex, including the thickness of interstitial, the diameter of alveolar cavity and the number of alveoli. These results demonstrate that Dex prevented hyperoxia-induced changes in lung morphology.

Dex inhibits the infiltration of macrophage

F4/80 is a marker of macrophage [20], to assess the effect of Dex on the infiltration of macrophage, we further detected the expres-
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Figure 4. Dex reduces lung edema induced by hyperoxia. Pulmonary edema was examined by wet to dry weight ratio. Freshly harvested lung samples were weighed and recorded as wet weight, dry weight was detected until the samples were dried out in thermostatic oven. Data were expressed in means ± SD, ***P < 0.01 vs. Nor group, *P < 0.05 vs. Hyp group.

The expression of F4/80 by immunohistochemistry (Figure 2). The alveolar interstitium of lungs in Nor group was almost unstained, but it was mostly dark brown in Hyp group and became moderate in Hyp+Dex group. These results were further identified by optical density analysis. Hence, the massive infiltration of macrophage induced by hyperoxia was inhibited by Dex.

Dex alleviates the inflammatory reaction

To investigate the inflammatory response in lung, the expression of TNF-α, IL-1β, IL-6 was detected by real-time PCR and enzyme-linked immunosorbent assay (Figure 3). The levels of TNF-α, IL-1β and IL-6 in Hyp group were significantly higher than Nor group, and dramatically down-regulated in Hyp+Dex group, which in accordance with mRNA expression. The activity of MPO, an indicator of neutrophil accumulation, was also up-regulated by hyperoxia and reversed by the administration of Dex. These findings suggest that Dex is protective against hyperoxia-induced inflammatory reaction by the inhibition of inflammatory cytokines secretion and neutrophil accumulation.

Dex reduces lung edema

The lung wet-to-dry-weight ratio was detected in order to investigate the effect of Dex on lung edema (Figure 4). It was significantly increased in Hyp group and Hyp+Yho group, but animals treated with Dex exhibited a significantly lower lung wet-to-dry-weight ratio as compared with the Hyp group. However, the effect of Dex was reversed by yohimbin (a potent α,2-adrenergic receptor antagonist), suggesting that Dex reduced hyperoxia-induced lung edema partly through the activation of α,2-adrenoceptor.

Dex up-regulates AQP1 expression

AQP1, one of the mainly expressed aquaporin in lung, has an important effect in water transporting [21], we therefore examined the expression of AQP1 (Figure 5). Our data demonstrated that both mRNA and protein levels of AQP1 were evidently decreased in Hyp group, however the levels in Hyp+Dex group were significantly increased compared to Hyp group. Immunohistochemistry showed that AQP1 was located in pneumocytes and the levels of AQP1 in each group were paralleled to the real-time PCR and western blot results. Collectively, these findings implied that Dex protects lung from edema by the regulation of AQP1.

Discussion

Dexmedetomidine is a selective and potent α,2-adrenoceptor agonist originally used for sedation in ICU patients. Studies have tested Dex as a protected agent in several lung injury models [17, 18, 22, 23], but whether Dex has protective effect against hyperoxia-induced lung injury remains unknown. We demonstrated for the first time that Dex treatment effectively attenuates hyperoxia-induced morphological changes, macrophage infiltration, lung edema, and the expression of inflammatory factors in lungs of infant rats. In addition, hyperoxia-mediated down-regulation of AQP1 was also inhibited by Dex. These findings suggest a protective role of Dex in hyperoxia-induced lung injury.

Alveolar injury is one of the major pathologic changes in lung injury induced by hyperoxia, its main features are endothelial cell necrosis, hemorrhage, edema and inflammatory response [1]. In infants, the injury occurs during the alveolar development stage, so hyperoxia often lead to persistent abnormalities in lung morphology [24]. In our study, newborn rats exposed in hyperoxia for 7 days showed obvious aberrations in postnatal lung development, such as larger alveolar, thinner interstitial and
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Figure 5. Dex up-regulates AQP1 expression in lungs of hyperoxia housed rats. A: AQP1 mRNA expression determined by real-time PCR. B: Representative protein bands analyzed by western blot and relative protein level were calculated as a ratio to β-actin. C: AQP1 expression of lung tissues were identified by immunohistochemistry, representative results are shown and scale bar = 50 μm. Data were expressed in means ± SD, **P < 0.01 vs. Nor group, ##P < 0.01 vs. Hyp group.

Reduced alveolar count, that was consistent with previous studies [25, 26]. Hyperoxia also resulted in massive macrophage infiltration, severe inflammatory reaction and pulmonary edema. The above changes demonstrated that the animal models established in our experiment were suitable for the investigation of hyperoxia-induced lung injury.

Inflammatory reaction mediated by inflammatory cytokines and neutrophils plays an important role in the development of hyperoxia-induced lung injury [25, 27]. Results from this study is in concert with previous results [1], that exposed in hyperoxia induced significant inflammatory changes including the up-regulation of TNF-α, IL-1β, IL-6 and neutrophil acu-
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mulation. The anti-inflammatory capacity of Dex has been widely reported [28-30], our results further confirmed that Dex can alleviate the inflammatory response in lung induced by hyperoxia, that evidenced by the inhibition of TNF-α, IL-1β, IL-6 expression and MPO activation.

AQP1 is expressed in peribronchiolar and micro-vascular endothelial cells in lung and plays an important role in water transporting [21, 31]. Bai et al. [32] reported that the permeability of water in the lung of AQP1 knockout mice was significantly decreased, suggested that AQP1 is closely related to pulmonary edema. In the current study, the lung wet to dry weight was dramatically elevated in Hyp group and accompanied by the down-regulation of AQP1. Interestingly, these changes were remarkably abolished by the administration of Dex. Thus, we propose that Dex possesses a protective effect against pulmonary edema, similar results have also been demonstrated in an acute lung injury rat model [18].

Yang et al. [17] showed that Dex mitigated ventilator-induced lung injury through α2-adrenergic receptors. In accordance with previous results, our experiment showed that the pulmonary protective effect of Dex was weakened by the α2-adrenergic receptor antagonist yohimbine. Therefore, the therapeutic effects of Dex were mediated, at least in part, by α2-adrenoceptor.

In conclusion, the present study demonstrated that Dex effectively protects lung injury from hyperoxia induced aberration, macrophage infiltration, inflammatory response, edema and the depletion of AQP1. In addition, the effects exerted by Dex may be associated with the activation of α2-adrenoceptors. The data presented here also imply that the administration of Dex may be an effective novel strategy against hyperoxia-induced lung injury.

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Disclosure of conflict of interest

None.

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References

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