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Anti-inflammatory effect of dexmedetomidine combined with hypothermia on acute respiratory distress syndrome in rats

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ABSTRACT

Background: To investigate the protective effect of combination of dexmedetomidine and hypothermia on lipopolysaccharide (LPS) induced acute respiratory distress syndrome in rats.

Methods: Fifty male Wistar rats were randomly divided into five groups, with 10 rats in each group. The acute respiratory distress syndrome model was reproduced by LPS injected into the right external jugular vein (L group); only saline was injected into the right external jugular vein for control group (C group). In hypothermia group (T group), the body temperature was lowered to 32.5°C-33.0°C after 1 h of LPS injection, and 10 rats were sacrificed at 8 h. Group dexmedetomidine (D group) and dexmedetomidine combined with hypothermia group (DT group) received intraperitoneal dexmedetomidine 30 min before LPS was injected. The arterial blood gas was determined in all the groups before and 8 h after instillation of saline or LPS, and the oxygenation index (PaO_2/FiO_2) was calculated. The pro-inflammatory cytokines TNF-alpha (TNF-α) and interleukin- 6 (IL-6) levels were determined by enzyme-linked immunosorbent assay. The expression of inflammatory signaling proteins in bronchial alveolar lavage fluid was determined by Western blot.

Results: Compared with group L, TNF-α and IL-6 levels in serum of rats were significantly lower ($P < 0.05$), the expression of toll-like receptors 4 and phosphorylated c-Jun N-terminal kinase was significantly lower ($P < 0.05$), and the p-Akt level was significantly higher ($P < 0.05$). Moreover, the dexmedetomidine combined with hypothermia treated was superior to the single method.

Conclusions: The combination of dexmedetomidine and hypothermia could alleviate acute lung injury in rats.

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Introduction

Acute respiratory distress syndrome (ARDS) is a critical disease with progressive dyspnea and hypoxemia as its main clinical features. In spite of progress in the care of patients with ARDS, morbidity and mortality among these patients remain unacceptably high that have no specific therapy.[1,2] There is an imminent need for an efficacious and novel therapy for ARDS. The pathogenesis of ARDS involves inflammatory response characterized by a predominantly neutrophilic infiltrate and induction of alveolar epithelial and endothelial damage. Reducing inflammatory response syndrome caused by ventilator injury during ARDS treatment is critical for reducing the mortality.[3]

Given the role of neuroprotectivity, hypothermia therapy has been widely used in the neurosurgery, thoracic surgery, and organ transplantation.[4-6] The mechanisms underlying hypothermia's protective effects are complex. An important theory is that hypothermia attenuates secondary injury including inflammation, mitochondrial dysfunction, apoptosis, and free radicals.

Dexmedetomidine is a novel $\alpha 2$ adrenoceptor agonist with high selectivity that can produce a good sedative effect.[7,8] Recent study has shown that dexmedetomidine could reduce the inflammatory response to sepsis with ventilator-induced injury.[9-11] There is little research on the effect of dexmedetomidine combined with hypothermia therapy on the inflammatory response to ARDS.

Considering the above facts, we have analyzed the suitability of the administration of dexmedetomidine combined with hypothermia as a sedative and analgesic for ARDS. In this study, we determined whether dexmedetomidine combined with hypothermia therapy reduces the inflammatory cascade and improves lung function following LPS-induced injury in rat. The application of dexmedetomidine combined with hypothermia therapy may provide novel therapeutic strategies on ARDS.

Materials and methods

Animals

Fifty healthy matured male Wistar rats, weighing 310-370 g, provided by Experimental Animal Center of Guangxi Medical University, were conventionally raised a week after purchase, with a room temperature maintained at 22°C-25°C and 50%-10% relative humidity with a 12 h light/dark cycle. The animals were allowed free access to food and water. All studies involving animals were performed according to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The experimental protocol was reviewed and approved by Guangxi Medical University Institutional Ethical Committee (Guangxi Experimental Animal Center, Guangxi, China, No. SYXK Gui 2014-0003). All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Grouping and modeling

Rats were anesthetized with sodium pentobarbital (50 mg/kg body weight) injected intraperitoneally. Right external jugular vein (infusion) and the left common carotid artery of the rat (blood gas analysis) were isolated, respectively. Respiratory parameters included respiratory frequency, 80/min; tidal volume, 20 mL/kg; and respiratory ratio, 1:2. After 20 min of mechanical ventilation, 200-g/mL LPS (0.5 mL/kg) was injected into the right external jugular vein; 0.5 mL/kg of normal saline was injected into for the control group. Rectal temperature was measured continuously and arterial blood gas analysis was recorded fitfully. When the oxygenation index (PaO_2/FiO_2) is $\leq$ 300-mm Hg, standard model was established.

Rats were divided into five groups randomly, 10 rats in each group: control group (C), in which normal saline was injected into right external jugular vein; model group (L), in which LPS was injected into the right external jugular vein to prepare ARDS model; hypothermia group (T), in which 1 h after LPS was injected into right external jugular vein, both ice bag covered and temperature constant system were used to maintain rectal temperature at 32.5°C-33.0°C; dexmedetomidine group (D), in which dexmedetomidine was administered 30 min before LPS and was injected into the right external jugular vein, and the body temperature was maintained at 37°C; and dexmedetomidine combined with hypothermia group (DT), in which rectal temperature was maintained at 32.5°C-33.0°C, other treatments were the same as group D. Each group was observed for a total of 8 h.

Observation of indexes

Blood gas analysis was recorded before and after 8 h of normal saline, or LPS was added into the trachea. At the end of the observation, eyeballs were removed and the blood was gathered, serum was separated and stored at −80°C. All the animals were sacrificed, and the right lung was taken for bronchoalveolar lavage for bronchial alveolar lavage fluid collection, centrifugal supernatant was stored at −20°C.

Inflammatory mediators in serum

The levels of proinflammatory cytokines TNF-alpha (TNF-α) and IL-6 in serum were assessed by ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Western blot analyses

Right lung was taken for bronchoalveolar lavage and bronchial alveolar lavage fluid collection; alveolar cells were extracted after centrifugation. The supernatant was separated by 12% SDS-PAGE, and was transferred to a polyvinylidene fluoride membrane (Hybond, CA). Membranes were blocked and then incubated overnight at 4°C with antibodies (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) against toll-like receptors 4 (TLR4), phosphorylated c-Jun N-terminal kinase (p-JNK), JNK, phosphorylated Akt (p-Akt), Akt, or β-actin. Next, blots were incubated with secondary antibodies conjugated to