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Activation of Cholinergic Anti-Inflammatory Pathway Contributes to the Protective Effects of 100% Oxygen Inhalation on Zymosan-Induced Generalized Inflammation in Mice

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Background. **The 100% oxygen inhalation has been demonstrated to have a protective effect on mice with zymosan-induced generalized inflammation. However, the underlying mechanism is largely unknown. The present study was designed to explore the role of the cholinergic anti-inflammatory pathway in this animal model.**

Methods. **Oxygen inhalation was given to mice at 4 and 12 h after zymosan injection. One group of mice underwent vagotomy 7 d before zymosan injection. The other two groups of mice either received nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine, or α7 nicotinic acetylcholine receptor (α7nAChR) antagonist methyllycaconitine 30 min before oxygen was given.**

Results. **The 100% oxygen treatment significantly decreased the serum level of TNF-α and increased the serum level of IL-10. The pathologic changes of the heart, lung, liver, and kidney were attenuated, as well as the dysfunction of liver and kidney. The 7-d survival rate of zymosan-challenged mice was also improved. Conversely, all these protective effects caused by pure oxygen treatment were abolished in those animals that received anti-cholinergic treatments.**

Conclusions. **The cholinergic anti-inflammatory pathway may be involved in the 100% oxygen protective mechanism against zymosan-induced generalized inflammation in mice.**

Key Words: **zymosan; systemic inflammation; cholinergic anti-inflammatory pathway; inflammatory cytokine; 100% oxygen.**

INTRODUCTION

Systemic inflammatory response syndrome (SIRS) is considered to play an important role in the pathophysiology of sepsis/multiple organ dysfunction syndrome (MODS), which is a leading cause of ICU death [1]. One hundred percent oxygen inhalation/ventilation has been demonstrated to be beneficial to SIRS/shock/sepsis, but its critical mechanism is unclear [2–5]. It is reported that hyperoxia induces the increased parasympathetic tone [6–8]. The cholinergic anti-inflammatory pathway is a mechanism whereby inflammation is modulated by the brain *via* the vagus nerve and nicotinic acetylcholine receptor (nAChR), and the best characteristic of these cholinergic receptors that suppress cytokines is the α7 subunit of the nicotinic AChR (α7nAChR) [6, 9]. So, we hypothesized that hyperoxia might exert its protection against sepsis/MODS *via* activation of the cholinergic anti-inflammatory pathway. Therefore, we investigated the effects of vagotomy, the nAChR antagonist mecamylamine, and the α7nAChR antagonist methyllycaconitine on the protective action

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of 100% oxygen treatment against zymosan-induced generalized inflammation in mice.

MATERIALS AND METHODS

Animals

Male imprinting control region (ICR) mice (specific pathogen free), 6- to 8-wk-old, 20 to 25 g in weight, provided by the Laboratory Animal Center of the Fourth Military Medical University, were used for all experiments. Mice were acclimatized prior to experimentation by being housed in plastic boxes at 20 to 22°C with a constant 12 h light/dark cycle, and given *ad libitum* access to food and water. All experimental and animal handling procedures were performed in accordance with the National Institutes of Health (NIH) guidelines for the use of experimental animals, and approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University.

Zymosan-Induced Generalized Inflammation Model

A 25 mg/mL zymosan (ZY) (Sigma Chemical Co., St. Louis, MO) solution was prepared and sterilized at 100°C for 80 min. All suspensions were freshly made before use. The generalized inflammation models were induced by an aseptic intraperitoneal zymosan injection 1 g per kg body weight [2, 3, 10–13]. The same volume of normal saline (NS) was injected through the same route for the control models.

Oxygen Treatment

The animals were placed inside a sealed Plexiglas chamber. Oxygen was delivered into the chamber through a tube at 4 L/min, and carbon dioxide was removed from the chamber with baralyme. The concentration of oxygen and carbon dioxide at the chamber outlet was continuously monitored by a gas analyzer (Medical Gas Analyzer LB-2, model 40 M; Beckman). The inspired oxygen concentration was maintained at 100% throughout the treatment. The room and chamber temperatures were maintained between 20 to 22°C. Air treatment was done with the control animals by exposing them to room air. Food and water were available *ad libitum* throughout the treatment.

Vagotomy

Left or sham cervical vagotomy was performed on animals anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally). A cervical midline incision was made to expose the left cervical vagus trunk, which was isolated from the surrounding tissue, ligated with a 4-0 silk suture, and transected [14]. In sham-operated mice, the left vagus nerve was exposed but without vagotomy.

Experimental Design

The animals were randomly divided into 11 groups (Fig. 1). In the ZY+Air, ZY+Air+Sham, ZY+Air+VGX, ZY+Air+Mec, ZY+Air+MLA, ZY+Oxy, ZY+Oxy+Sham, ZY+Oxy+VGX, ZY+Oxy+Mec, and ZY+Oxy+MLA groups. Generalized inflammation was induced by intraperitoneal injection of zymosan, and in the NS+Air group, zymosan was replaced by the same volume of normal saline. In the ZY+Oxy, ZY+Oxy+Sham, ZY+Oxy+VGX, ZY+Oxy+Mec, and ZY+Oxy+MLA groups, oxygen treatment was done by exposing animals to 100% oxygen for 3 h at 4 and 12 h after zymosan injection, respectively, and in the other groups, animals were exposed to room air as the control. For animals of ZY+Oxy+VGX and ZY+Air+VGX groups, vagotomy was performed 7 d before zymosan injection, and for those in the ZY+Oxy+Sham and ZY+Air+Sham groups, left cervical vagus trunk was exposed and isolated from the surrounding tissue, but not transected. In the ZY+Air+Mec, ZY+Oxy+Mec, ZY+Air+MLA, and ZY+Oxy+MLA groups, animals were subcutaneously administered with 1 mg/kg nAChRs antagonist mecamylamine [15] (Sigma Chemicals Co., Poole, UK) or 2 mg/kg α7nAChRs antagonist methyllycaconitine [16] (Sigma-Aldrich, St. Louis, MO) at 30 min before oxygen inhalation, respectively. All drugs were dissolved in sterile saline solution immediately before use. The survival rate was recorded on day 0.5, 1, 2, 3, 5, and 7 after zymosan or normal saline injection.

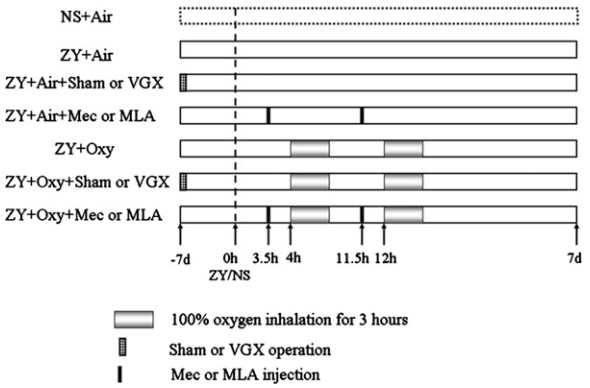

FIG. 1. The schematic diagram for grouping methods and experimental protocols. The animals were randomly divided into 11 groups: NS+Air, ZY+Air, ZY+Air+Sham, ZY+Air+VGX or Mec or MLA, ZY+Oxy, ZY+Oxy+Sham, and ZY+Oxy+VGX or Mec or MLA groups. In the ZY+Air, ZY+Air+Sham, ZY+Air+VGX or Mec or MLA, ZY+Oxy, ZY+Oxy+Sham, ZY+Oxy+VGX or Mec or MLA groups, generalized inflammation was induced by intraperitoneal injection of zymosan, and in the NS+Air group, the same volume of normal saline was given. In the ZY+Oxy, ZY+Oxy+Sham, ZY+Oxy+VGX or Mec or MLA groups, oxygen treatment was done with animals exposed to 100% oxygen for 3 h at 4 and 12 h after zymosan injection respectively, and in the other groups, animals were exposed to room air as the control. For animals of ZY+Oxy+VGX and ZY+Air+VGX groups, vagotomy was performed 7 d before zymosan injection, and for those in the ZY+Oxy+Sham and ZY+Air+Sham groups, left cervical vagus trunk was exposed and isolated from the surrounding tissue, but not transected. In the ZY+Air+Mec, ZY+Oxy+Mec, ZY+Air+MLA, and ZY+Oxy+MLA groups, animals were subcutaneously administered with 1 mg/kg nAChRs antagonist mecamylamine or 2 mg/kg α7nAChRs antagonist methyllycaconitine at 30 min before oxygen inhalation respectively. All drugs were dissolved in sterile saline solution immediately before use. The survival rate was recorded on day 0.5, 1, 2, 3, 5, and 7 after normal saline or zymosan injection. h = hour; d = day; NS = normal saline; ZY = zymosan; Oxy = oxygen; VGX = vagotomy; Mec = mecamylamine, MLA = methyllycaconitine.

To further study the effects of vagotomy, nAChRs antagonist and α7nAChRs antagonist on zymosan-challenged mice with 100% oxygen treatment, additional animals were used. The grouping method and experimental protocols were the same as described above. At 24 h after zymosan or normal saline injection, blood samples and organs were collected to measure changes of serum biochemical parameters, inflammatory cytokines, and organ histopathology.

Arterial Blood Gas Analysis

Arterial blood sample was immediately collected from the carotid artery at the end of oxygen treatment (15 h after zymosan or normal saline). All arterial blood gas analyses were performed using a GEM Premier 3000 gas analyzer (Instrumentation Laboratory, Milan, Italy).

Serum Biochemical Parameter Assay

At the predetermined time points, animals were anesthetized in order to collect blood samples by cardiac puncture, and then clotted for