Olprinone decreases elevated concentrations of cytokine-induced neutrophil chemoattractant-1 in septic rats

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Abstract

Purpose. The diaphragm is one of the organs directly affected by abdominal sepsis. Evidence suggests that sepsis induces diaphragmatic fatigability and that activated neutrophils play a crucial role in the development of diaphragmatic fatigability. In the present study, we investigated whether olprinone, a phosphodiesterase inhibitor, influenced the kinetics of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in the diaphragm under abdominal septic conditions.

Methods. Male Wistar rats were randomly assigned to a sham group, a cecal ligation and perforation group, and a phosphodiesterase inhibitor-pretreated group. To measure serial changes in CINC-1 concentrations, the right hemidiaphragm was removed at 4, 8, and 16 h after the surgical procedure in each group.

Result. In the cecal ligation and perforation group, CINC-1 concentrations in the diaphragm were significantly elevated compared with those in the sham group at both 4 and 8 h after the cecal ligation and perforation procedure. In the phosphodiesterase inhibitor-pretreated group, olprinone significantly attenuated the elevated CINC-1 concentrations at both 4 and 8 h after the surgical procedure. However, we observed no statistically significant differences in CINC-1 concentrations between the cecal ligation and perforation group and the phosphodiesterase inhibitor-pretreated groups at 16 h after the surgical procedure.

Conclusion. Olprinone decreases elevated CINC-1 concentration in the diaphragm under septic conditions. This suggests that olprinone may inhibit neutrophil recruitment to the diaphragm.

Key words Abdominal sepsis · Cytokine-induced neutrophil chemoattractant-1 · Phosphodiesterase inhibitor

Introduction

Abdominal sepsis complicated by the leakage of bowel contents is a pathophysiological condition commonly encountered in intensive care units, and physicians are often required to treat patients affected by abdominal sepsis or septic shock [1]. Despite multiple pharmacological and/or physiological interventions, many such cases lead to organ dysfunction followed by severe sepsis [2,3]. The multiple organ dysfunction syndrome associated with abdominal sepsis typically involves respiratory failure, resulting in acute respiratory distress syndrome (ARDS). Such patients require long-term mechanical ventilation assistance, while ARDS itself significantly affects clinical outcomes [4].

Activated neutrophils and the cytokine network play a major role in the development of ARDS induced by abdominal sepsis [5]. Under conditions of abdominal sepsis, the diaphragm plays a significant role as a barrier isolating the lungs from the abdominal organs. Leaked bowel contents not only indirectly target the lungs, mediated by proinflammatory cytokines but also induce inflammation in the diaphragm, resulting in the impairment of diaphragmatic function. Direct and indirect evidence also suggests that ARDS following abdominal sepsis is accompanied by diaphragmatic fatigability, which may be associated with cyclic adenosine monophosphate (cAMP) depletion in diaphragmatic muscle [6]. In addition to the alteration of cAMP content in muscle, neutrophil activation was demonstrated to contribute to the development of diaphragmatic fatigability under abdominal septic conditions, while the intraperitoneal administration of olprinone, a phosphodiesterase (PDE) inhibitor, was shown to improve diaphragmatic contractility and to inhibit neutrophil activation induced in a rat model of abdominal sepsis, although it remains unclear whether olprinone modulated cytokine activation at sites of inflammation [7].
It is also well known that neutrophils play a crucial role in acute-phase responses, including sepsis. The recruitment of neutrophils to an inflammatory site leads to the local release of inflammatory mediators, which results in tissue injury or organ dysfunction. Cytokine-induced neutrophil chemoattractant-1 (CINC-1), which was initially purified from rat kidney epithelioid cells, is a member of the CXC chemokine family [8]. The chemical structure of CXC chemokines is characterized by the feature that the two N-terminal cysteines of these chemokines are separated by one amino acid, termed “X” in this acronym. The appellation of CXC originates from its chemical structure. The major function of CINC-1 in an acute-phase response includes neutrophil recruitment [9]. Additionally, it has also been demonstrated that hepatic CINC-1 functions as an acute-phase protein after brain injury [10].

In order to produce a septic animal model, the cecal ligation and perforation (CLP) technique was applied to an animal model, because this type of septic model is an established experimental model of abdominal sepsis.

In the present study, we hypothesized that an increase in CINC-1 concentration in an injured organ would be essential for neutrophil recruitment under septic conditions. The purposes of this study were as follows: (1) to investigate the kinetics of CINC-1 in diaphragmatic muscle after CLP, and (2) to evaluate the effects of intraperitoneal olprinone on CINC-1 kinetics.

Materials and methods

Preparation of animals for abdominal sepsis

All experimental protocols were approved prior to the study by the Oita University Animal Care and Use Committee. The care and handling of the animals were in accordance with the United States National Institutes of Health guidelines.

Male Wistar rats, weighing 250 to 350 g, were made to fast overnight and were assigned to the following three groups: (1) a sham group; (2) a CLP group; and (3) a pretreatment with PDE inhibitor group (PDE group). To determine serial changes in CINC-1 concentrations in the diaphragm, we then randomly assigned rats in each group to subgroups which were killed 4, 8, and 16 h after the surgical procedure. Finally, the following nine subgroups were prepared: sham-4h, -8h, and -16h (n = 6 in each group); CLP-4h, -8h, and -16h (n = 10, 6, and 6, respectively); and PDE-4h, -8h, and -16h (n = 9, 8, and 6, respectively).

Following anesthetization with sevoflurane, rats in the sham group were subjected to laparotomy through a midline abdominal incision. The cecum was minimally manipulated but neither ligated nor perforated. After manipulation of the bowel, the abdominal incision was closed with 3-0 silk thread. Laparotomy was also performed on rats in the CLP group, after which the cecum was ligated just below the ileocecal valve with 3-0 silk thread. Following ligation, the cecum was perforated three times with an 18-gauge needle, then gently compressed to extrude feces through the perforations. The small intestine and cecum were returned to the abdomen and the incision closed. In the PDE group, olprinone (Eizai Pharmaceutical, Tokyo, Japan) 15 mg·kg⁻¹, a representative PDE inhibitor, was administered intraperitoneally 60 min before CLP preparation. At the same time, an identical volume of saline was administered intraperitoneally at the same site in both the sham and the CLP groups. After each rat was killed, the right hemidiaphragm was removed and stored at −80°C until the measurement of CINC-1 concentrations in the diaphragm was carried out.

Determination of CINC-1 concentrations in the diaphragm

The removed diaphragms were weighed and homogenized with a homogenizer (Polytron PT-MR2100; Kinematica, Lucerne, Switzerland) in a 10% (wt/vol) homogenization buffer, which consisted of 50 mmol·l⁻¹ of phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO, USA), and which was sonicated for 20 s. CINC-1 concentrations in the diaphragm were measured with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (GRO/CINC-1; Rat Biotrak ELISA System; Amersham Biosciences UK, Buckinghamshire, UK), and assays of optical density were performed for each sample with a commercially available microplate reader (Bio Rad Model 680; Bio Rad Laboratories, Hercules, CA, USA).

The measurements of CINC-1 concentrations in each sample were performed according to the instructions provided with the commercial kit. However, the CINC-1 concentrations in diaphragmatic tissue were found to be higher than those in serum. Therefore, each homogenized sample was prediluted to a ratio of 1:5 with assay buffer. The CINC-1 concentration measurements obtained were expressed in picograms per gram of diaphragmatic muscle.

Statistical analysis

Values for all results were expressed as means ± SEM, with the number of experiments as n. The data were statistically analyzed with one-way analysis of variance (ANOVA), followed by Fisher’s least-significant difference test. P values of less than 0.05 were considered significant.