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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

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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 neutrophils chemotaxis, eliciting 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 condtions. 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-4 h, -8 h, and -16 h (n = 6 in each group); CLP-4 h, -8 h, and -16 h (n = 10, 6, and 6, respectively); and PDE-4 h, -8 h, and -16 h (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.

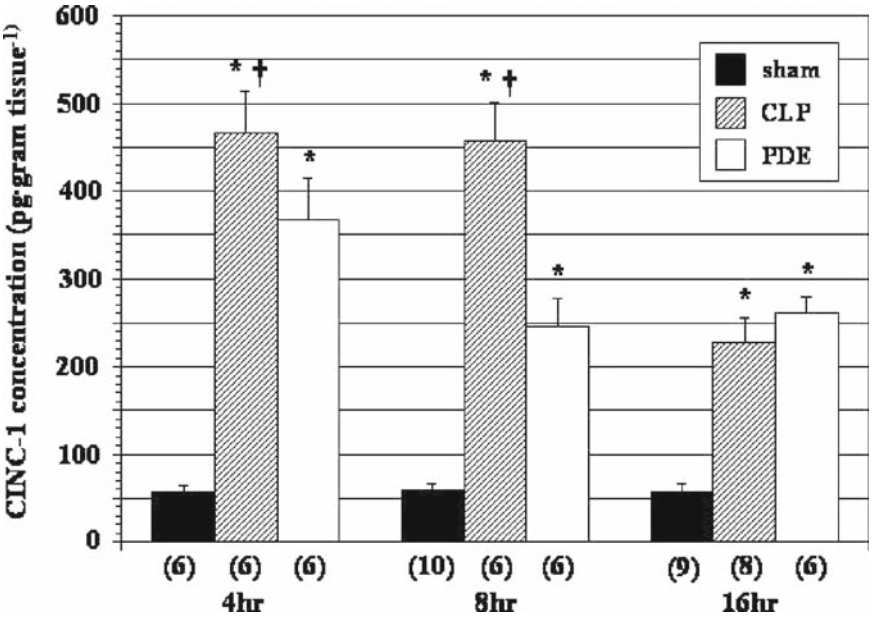

Fig. 1. Cytokine-induced neutrophil chemoattractant-1 (*CINC-1*) concentration in the diaphragm. Data values are expressed as means ± SEM. Numbers in parentheses are numbers of experiments. $^{*}P < 0.01$ compared with sham group; $^{\dagger}P < 0.01$ compared with phosphodiesterase (*PDE*) group. *CLP*, Cecal ligation and perforation group

Results

CINC-1 concentrations in the diaphragm (Fig. 1)

In the sham-operated group, CINC-1 concentrations at 4 h, 8 h and 16 h were 57.0 ± 6.6, 59.3 ± 7.4, and 56.7 ± 9.2 pg·g tissue^{-1}, respectively. No statistically significant differences were observed among these groups. In the CLP group, CINC-1 concentrations at 4 h, 8 h and 16 h were 467.2 ± 47.2, 456.9 ± 44.1, and 226.9 ± 29.4 pg·g tissue^{-1}, respectively. At all three points in time, CINC-1 concentrations in the CLP group were significantly higher than those in the sham group. CINC-1 concentrations in the PDE-4, -8, and -16 h groups were 367.6 ± 46.0, 246.2 ± 32.3, and 260.9 ± 17.6 pg·g tissue^{-1}, respectively. These concentrations of CINC-1 in the PDE group were also significantly higher than those in the sham group.

A comparison of the PDE-4 h group with the CLP-4 h group showed statistically significant differences in CINC-1 concentrations. CINC-1 concentration in the PDE-4 h group was significantly lower than that in the CLP-4 h group. Similarly, CINC-1 concentration in the PDE-8 h group was significantly lower than that in the CLP-8 h group. However, no statistically significant differences were observed between the PDE-16 h and CLP-16 h groups.

Discussion

Sepsis or septic shock leads to multiple organ dysfunction syndrome (MODS) caused by microvascular dysfunction or cytokine activation [11]. Particularly in cases complicated by acute lung injury (ALI) or ARDS, activated neutrophils, which play a leading role in septic shock condition, accumulate in the lung tissue and subsequently activate the cytokine cascade [5].

In patients with abdominal sepsis, several studies have demonstrated that diaphragmatic fatigability is associated with the progression of ALI or ARDS [6,12]. It has been suggested that the diaphragmatic fatigability associated with abdominal sepsis is induced by activated neutrophils in diaphragmatic muscle in which myeloperoxidase (MPO) activity is increased [7].

In septic animal models, both antithrombin and activated protein C have been reported to have beneficial effects on ALI or ARDS [13,14]. However, the objectives of these studies focused on the effects of these agents on the circulatory state and cytokine expression within the lungs. No study has investigated cytokine kinetics in the diaphragm following abdominal sepsis.

In the present study, we chose the CLP model to reproduce clinical abdominal sepsis, and we investigated the kinetics of CINC-1, the chemokine responsible for inflammatory reactions and the recruitment of neutrophils, because it has been demonstrated that CLP offered a reproducible septic model [15]. Additionally, it has also been shown that several cytokines were continuously released from immune-competent cells [16].

Divangahi et al. [17] reported that the expression of proinflammatory cytokine genes, including interleukin-1β (*IL-1*β), tumor necrosis factor-α (*TNF*-α), and *IL-6* was upregulated in the diaphragm after pulmonary infection induced by *Pseudomonas aeruginosa*. The results of their study suggested that the expression of various cytokines might be associated with the develop-

ment of diaphragmatic fatigability or dysfunction. However, Janssen et al. [18] demonstrated that in vitro recombinant human IL-6 administration did not alter diaphragmatic contractility. The inconsistency between these results may be a result of the complexity of the cytokine network. Therefore, we have focused on neutrophil migration and activation associated with organ dysfunction in septic rats. Our previous study showed that the degree of diaphragmatic fatigability paralleled MPO activity in the diaphragm [7]. This suggests that neutrophil activation is essential for the development of diaphragmatic fatigability. Therefore, it is necessary that the production of CINC-1 in the diaphragm is increased in order that neutrophil recruitment and activation may occur.

CINC-1 possesses potent chemotactic activity for neutrophils, and this chemokine is released from macrophages, mast cells, and inflammatory fibroblast cells [9,19,20]. Shibata et al. [21] demonstrated that the production of CINC-1 by cultured rat macrophages stimulated by lipopolysaccharide (LPS) reached a maximum at 12 h after LPS stimulation. Meanwhile, it was also demonstrated that, in rats with LPS–induced sepsis, CINC-1 concentration in the air-pouch exudate reached a maximum at 4 h after subcutaneous LPS injection [9]. It was speculated that the difference in time to reach maximum CINC-1 production between in vivo and in vitro experiments could be attributed to the different responses of inflammatory cell types, i.e., macrophages and fibroblasts.

In our study, the diaphragmatic concentration of CINC-1 in the CLP group, compared with that in the sham group, was significantly elevated at 4 h after the CLP preparation. This elevated concentration persisted for 12 h thereafter. However, in both the PDE-4 h and -8 h groups, olprinone significantly reduced CINC-1 concentrations compared with those in the CLP-4 h and CLP-8 h groups, respectively. However, there were no significant differences in CINC-1 concentrations between the CLP-16 h and PDE-16 h groups. This suggests that a single administration of olprinone may provide an inhibitory effect on CINC-1 production during only a short period. Therefore, in the present study, a continuous infusion of olprinone might be a better administration method than a single injection. However, we speculate that, for the first several hours after an insult, the inhibition of CINC-1 production provided by a single dose of olprinone may lead to consecutive neutrophil recruitment and activation in an injured organ, resulting in organ protection.

In the present study, trends in diaphragmatic CINC-1 concentrations were consistent with the trends in MPO observed in our previous study, wherein we demonstrated that olprinone improved diaphragmatic contractility in a model of abdominal sepsis [7]. The results of our present study support the concept that olprinone suppresses proinflammatory cytokines originating from activated neutrophils in abdominal sepsis. Other PDE inhibitors, amrinone and milrinone, have been reported to affect the cytokine network mediated by nuclear factor kappa B (NFκB) [22]. It has also been demonstrated that the inhibition of PDE itself by pentoxifylline reduces NFκB activation [23]. These results suggest that olprinone also suppresses the cytokine cascade. While the mechanism underlying this effect of olprinone remains unclear, it is partially explained by recognizing that olprinone may inhibit NFκB activation, subsequently inhibiting neutrophil recruitment and activation. Although, in clinical circumstances, olprinone is generally applied for cardiac dysfunction, including congestive heart failure, olprinone may reduce the duration required for mechanical ventilation support by alleviating diaphragmatic fatigability, and thereby it may reduce mortality and morbidity in patients with sepsis.

In conclusion, olprinone decreases elevated CINC-1 concentrations in the diaphragm during abdominal sepsis after CLP. We speculate that olprinone may inhibit neutrophil recruitment to the diaphragm, and consequently may modify the cytokine cascade.

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References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR (2001) Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 29:1303–1310
2. Baker RA (2004) Optimum treatment of severe sepsis and septic shock: evidence in support of the recommendations. Dis Mon 50:168–213
3. Crouser ED (2004) Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. Mitochondrion 4:729–741
4. Goss CH, Brower RG, Hudson LD, Rubenfeld GD (2003) Incidence of acute lung injury in the United States. Crit Care Med 31:1607–1611
5. Ware LB, Matthay MA (2000) The acute respiratory distress syndrome. N Engl J Med 342:1334–1349
6. Fujimura N, Sumita S, Aimono M, Masuda Y, Shichinohe Y, Narimatsu E, Namiki A (2000) Effect of free radical scavengers on diaphragmatic contractility in septic peritonitis. Am J Respir Crit Care Med 164:2159–2165
7. Miyakawa H, Oishi K, Hagiwara S, Kira S, Kitano T, Iwasaka H, Noguchi T (2004) Olprinone improves diaphragmatic contractility and fatigability during abdominal sepsis in a rat model. Acta Anaesthesiol Scand 48:637–641
8. Watanabe K, Konishi K, Fuioka M, Kinoshita S, Nakagawa H (1989) The neutrophil chemoattractant produced by the rat kidney epithelioid cell line NRK-52E is a protein related to the KC/gro protein. J Biol Chem 246:19559–19563
9. Shibata F, Kato H, Konishi K, Okumura A, Ochiai H, Nakajima K, AL-Mokdad M, Nakagawa H (1996) Differential changes in

the concentrations of cytokine-induced neutrophil chemoattractant-1 (CINC-1) and CINC-2 in exudates during rat lipopolysaccharide-induced inflammation. Cytokine 8:222–226
10. Campbell SJ, Hughes PM, Iredale JP, Wilcockson DC, Waters S, Docagne F, Perry VH, Anthony DC (2003) CINC-1 is an acute-phase protein induced by focal brain injury causing leukocyte mobilization and liver injury. FASEB J 17:1168–1170
11. Vincent JL, DeBacker D (2005) Microvascular dysfunction as a cause of organ dysfunction in severe sepsis. Crit Care 9 (Suppl 4): S9-S12
12. Fujimura N, Sumita S, Narimatsu E (2000) Alteration in diaphragmatic contractility during septic peritonitis in rats: effect of polyethylene glycol-absorbed superoxide dismutase. Crit Care Med 28:2406–2414
13. Isobe H, Okajima K, Uchiba M, Harada N, Okabe H (2000) Antithrombin prevents endotoxin-induced hypotension by inhibiting the induction of nitric oxide synthase in rats. Blood 99: 1638–1645
14. Isobe H, Okajima K, Uchiba M, Mizutani A, Harada N, Nagasaki A, Okabe K (2001) Activated protein C prevents endotoxin-induced hypotension in rats by inhibiting excessive production of nitric oxide. Circulation 104:1171–1175
15. Wichterman KA, Baue AE, Chaudary IH (1980) Sepsis and septic shock. A review of laboratory models and a proposal. J Surg Res 29:189–201
16. Remick DG, Newcomb DE, Bolgos GL, Gall DR (2000) Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs cecal ligation and puncture. Shock 13:110–116
17. Divangahi M, Demoule A, Danialou G, Yahiaoui L, Bao W, Xing Z, Petrof BJ (2007) Impact of IL-10 on diaphragmatic cytokine expression and contractility during Pseudomonas infection. Am J Respir Cell Mol Biol 36:504–512
18. Janssen SPM, Gayan-Ramirez G, Bergh AVD, Herijgers P, Maes K, Verbeken E, Decramer M (2005) Interleukin-6 causes myocardial failure and skeletal muscle atrophy in rats. Circulation 111:996–1005
19. Nakagawa H, Ikesue A, Hatakeyama S, Kato H, Gotoda T, Komorita N, Watanabe K, Miyai H (1993) Production of an interleukin-8-like chemokine by cytokine-stimulated rat NRK-49F fibroblasts and its suppression by anti-inflammatory steroids. Biochem Pharmacol 45:1425–1430
20. Ramos CDL, Heluy-Neto NE, Ribeiro RA, Ferreira SH, Cunha FQ (2003) Neutrophil migration induced by IL-8-activated mast cells is mediated by CINC-1. Cytokine 21:214–223
21. Shibata F, Shibata Y, Yoshimoto Y, Nakagawa H (2000) The expression of three types of CINCs by lipopolysaccharide-stimulated rat macrophages is inhibited similarly by anti-inflammatory steroids. Inflamm Res 49:80–85
22. Chanai NK, Cowan DB, Takeuchi K, Poutias DN, Garcia LM, del Nido PJ, McGowan JX Jr (2002) Differential effects of amrinone and milrinone upon myocardial inflammatory signaling. Circulation 106:284–289
23. Coimbra R Melbostad H, Loomis W, Tobar M, Hoyt DB (2005) Phosphodiesterase inhibition decreases nuclear factor-κB activation and shifts the cytokine response toward anti-inflammatory activity in acute endotoxemia. J Trauma 59:575–582