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Beneficial effects of dantrolene on sepsis-induced diaphragmatic dysfunction are associated with downregulation of high-mobility group box 1 and calpain-caspase-3 proteolytic pathway

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ABSTRACT

Background: Intracellular calcium overload is a major contributing factor to diaphragmatic dysfunction triggered by sepsis. In this study, the possible role of dantrolene, a ryanodine receptor inhibitor, in preventing the release of calcium from the sarcoplasmic reticulum in diaphragmatic dysfunction and weakness was explored.

Methods: A middle-grade severity sepsis rat model was established for the effects of treatment with dantrolene, on diaphragm harvested 24 h after cecal ligation and puncture (CLP), and analyzed using functional, histologic, and biomarker assays.

Results: It was found that in septic rats, treatment with dantrolene significantly improved the contractility, relaxation, and fatigue index of the diaphragm in a dose-dependent manner. The benefits are associated with improvement in ultrastructural changes of Z band integrity and myofilament arrangements along with increases both in the ratio of slow-twitch type composition. Moreover, dantrolene effectively inhibits the overexpression of high-mobility group box 1 and reduces the calpain-1-caspase-3 proteolytic activity.

Conclusions: Dantrolene can effectively attenuate the dysfunction of diaphragm in septic rats; Furthermore, the beneficial effects were associated with downregulation of high-mobility group box 1 and calpain-1-caspase-3 proteolytic activity.

1. Introduction

Presently, sepsis is still a worldwide lethal disease that triggers a wide range of systemic inflammatory response leading to dysfunction of organs including the liver, lung, heart, kidney, and skeletal muscles [1–3]. Patients with sepsis typically exhibit respiratory muscle weakness, which may partly contribute to ventilator weaning difficulties and lung injury aggravation [4,5]. Sepsis correlates with a pronounced catabolic response in skeletal muscles, especially degradation of

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myofibrillar proteins such as actin and myosin [6,7]. Recent studies demonstrate that sepsis-induced muscle protein proteolytic cascade mainly depends on calcium-dependent calpain-caspase-3 system [8,9].

Increases of free intracellular calcium level ($[Ca^{2+}]_i$) have been shown in animal models of sepsis and endotoxemia [10]. The elevation of $[Ca^{2+}]_i$ is suggested to be a critical signaling pathway, which may trigger a series of cellular response, including the release of proinflammatory cytokines and proteolysis in sepsis. Dantrolene inhibits the release of calcium from sarcoplasmic reticulum and mitochondria into the cytoplasm. In normal human and animals, dantrolene can decrease the contractility and strength of diaphragm [11], but in septic rats, pretreatment with dantrolene may exert reverse effects [12–14]. A number of studies show that dantrolene has protective effects in metabolic abnormalities associate with septic muscles [12–14]. Treatment of rats with the calcium antagonist dantrolene effectively blocked sepsis-induced muscle proteolysis and reduce muscle protein degradation independent of dexamethasone. Dantrolene also reduces serum tumor necrosis factor-alpha (TNF-α), corticosterone levels, and muscle calcium level and normalizes muscle protein and glucose metabolism in septic rat, indicating the additional anti-inflammatory effects of treatment with dantrolene against sepsis [15]. High-mobility group box 1 (HMGB1) has been recently found to act as late proinflammatory cytokines during sepsis. As a representative member of damage-associated molecular pattern, the elevated HMGB1 in the diaphragm is reportedly associated with compromised contractile performance, and blocking HMGB1 receptor attenuates septic diaphragm dysfunction in rats with peritonitis [16–18].

This study aimed at exploring the possible protective role of dantrolene in the diaphragmatic dysfunction triggered by sepsis, and furthermore, its correlation with calpain-caspase-3 proteolytic system and/or expression of HMGB1 (Fig. 1).

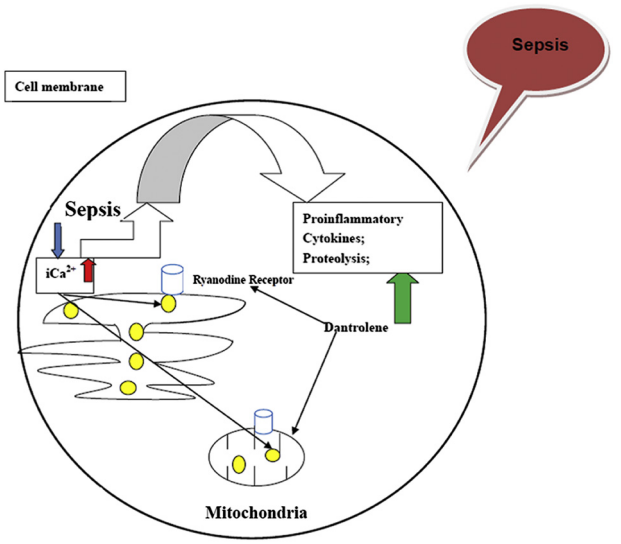

Fig. 1 – The hypothesis of this study. In this figure, it is hypothesized that sepsis-induced cellular calcium overload in the diaphragm triggered a cascade of release of proinflammatory cytokines and promoted myofilament proteolysis, whereas dantrolene, a specific ryanodine receptor antagonist, can reduce the leakage of calcium from mitochondria and sarcoplasmic reticulum and therefore produce the beneficial effects. (Color version of figure is available online.)

2. Materials and methods

2.1. Animals and experimental design

Experimental protocol: This study was approved by the Institutional Animal Care and Use Committee in the First People's Hospital affiliated to Shanghai Jiaotong University. Experiments were performed in adult male Sprague–Dawley rats (body weight, 200–220 g). Animals were allowed free access to food and water and housed at ambient temperature (22°C) with 12 h light-dark cycles. Rats were randomized into six groups ($n = 20$ per group): (1) Sham group: Control rats underwent only sham operation of laparotomy and manipulation, without ligation or puncture of the cerum. (2) CLP group: Sepsis was induced by cecal ligation and puncture (CLP). Critical steps in the CLP procedure as follows [19]: (1) After being anesthetized, skin midline incision. (2) Exposure of the cecum, ligation at half the distance between distal pole, and the base of the cecum. (3) Cecal puncture ("through-and-through") from mesenteric toward antimesenteric direction with 18 G needle. After removing the needle, extrude a small amount of feces from the penetration holes to ensure patency. (4) Wound closure and resuscitation with prewarmed saline. (3) CLP+2 mg/kg dantrolene group (CLP + DAN2): dantrolene (Sigma Chemical Co.) was administered intraperitoneally (i.p.) in doses of 2 mg/kg 1 h before and 8 h after surgical procedure. (4) CLP+6 mg/kg dantrolene group (CLP + DAN6): dantrolene was administered at 6 mg/kg, i.p. (5) CLP+10 mg/kg dantrolene group (CLP + DAN10): dantrolene was administered at 10 mg/kg, i.p. (6) Sham group plus dantrolene 2 mg/kg (Sham + DAN2). Immediately after surgical procedure, 1% ropivacaine was administered by wound infiltration to alleviate postoperative pain. All rats were resuscitated with administration of saline at 5 mL/100 g body weight subcutaneously on the back at the time of surgery. Rats had free access to water and food after surgery and were observed every 2 h until 24 h postoperation.

2.2. Contractile characteristics of diaphragmatic strips

At 24 h after surgery, all rats that survived were anesthetized using pentobarbital (50 mg/kg i.p.), and then, diaphragm was instantly excised and perfused with a Krebs solution (mM: NaCl 118, KCl 4.9, $CaCl_2$ 2.5, $MgSO_4$ 1.18, KH_2PO_4 1.18, $NaHCO_3$ 25, and dextrose 11 with the addition of 95% oxygen and 5% carbon dioxide mixture at a constant temperature of 37°C) and a pH of 7.40 (ALC-MPA2000 m; Alcott Biotech Co, Shanghai, China). Muscle tissue strips with a width of approximately 8 mm, without phrenic nerves, were cut from the left costal diaphragm, and a bundle of central tendon was connected to a force transducer. Electrical stimulation via