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Sepsis induced denervation-like changes at the neuromuscular junction

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ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form
12 August 2015
Accepted 9 September 2015
Available online 18 September 2015

Keywords:
Sepsis
Muscle denervation
Neuromuscular junction
Receptors
Nicotinic
Electromyography

ABSTRACT

Background: The aim of this study was to determine the functional and biochemical changes at the neuromuscular junction (NMJ) induced by sepsis.

Materials and methods: Male Sprague–Dawley rats were divided into three groups as follows: control, denervation, and sepsis. The rats were subjected to cecal ligation and puncture (CLP) or tibias nerve transection. NMJ function and the area of end plates were assessed, and the protein level of acetylcholine receptors and axonal neuregulin-1 was evaluated on postoperative days 1, 7, and 14.

Results: In the control group, the amplitude of compound muscle action potential (CMAP) was 16.51 ± 2.53 mV. In the sepsis group, the amplitude of CMAP decreased, and duration was prolonged on postoperative days 7 and 14 ($P < 0.01$). Meanwhile, motor conduction velocity decreased significantly ($P < 0.01$). CMAP was lost in the denervation group. The twitch tension magnitude gradually declined ($P < 0.05$) in the sepsis group, although it could not be recorded after lesion. Sepsis and denervation upregulated the expression of γ-nicotinic acetylcholine receptor (nAChR) and α7-nAChR in muscle membrane, compared with those in normal NMJ (261.4 ± 26.5 μm^2). The NMJ area decreased from 254.6 ± 23.8 μm^2 (1 d after CLP) to 275.4 ± 22.6 μm^2 (7 d after CLP) to 322.7 ± 34.4 μm^2 (14 d after CLP). The postsynaptic NMJ had more discrete fragments (3.84 ± 0.6) compared with the control group (2.13 ± 0.4; $P < 0.01$). After denervation, NMJ underwent fragmentation and the number of discrete fragments increased (5.57 ± 1.2; $P < 0.01$). NMJ area increased from 254.6 ± 23.8 μm^2 (1 d after CLP) to 275.4 ± 22.6 μm^2 (7 d after CLP) to 322.7 ± 34.4 μm^2 (14 d after CLP). Sepsis induced neuregulin-1 to decrease from 1 d up to 2 wk compared with the control group ($P < 0.05$).

Conclusions: Chronic sepsis has a denervation-like effect on the NMJ, which was indicated by upregulation of heterogeneous nAChRs, the increased area of end plates, and demyelination of the motoneuron axon.

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0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2015.09.012

1. Introduction

Sepsis is a major infection-induced syndrome with high mortality ranging from 28%–47% [1,2]. Sepsis promotes failure of vital parenchymal organs such as the lung, brain, liver, and kidney. It can also cause damage or dysfunction of peripheral nerves and skeletal muscle [3]. Sepsis-related dysfunction of the peripheral nerves and muscle weakness are termed critical illness polyneuropathy and myopathy (CIPNM) [4,5]. In addition to prolonging mechanical ventilation and hospitalization, it increases hospital mortality and causes chronic disability in survivors of serious illnesses [6]. The pathogenesis of CIPNM associated with sepsis remains unclear. Thus, it is worthwhile to elucidate the mechanism of CIPNM to establish effective strategies for the prevention and treatment of this disorder.

Recently, the vast majority of experimental sepsis models demonstrated only neuromuscular dysfunction without organ damage at an early stage. Nayci *et al.* [7] showed that electrophysiological abnormalities are present after 6 h with worsening after 24 h in septic rats. On the other hand, histopathologic examination shows healthy muscular fibers and focal slight myelin degeneration of the phrenic nerve fibers [8]. In addition, qualitative and quantitative changes of nicotinic acetylcholine receptors (nAChRs) have been found on the muscle membrane [9]. Tsukagoshi *et al.* [10] showed that cecal ligation and puncture (CLP)–associated peritonitis results in downregulation of nAChRs. Preliminary or indirect evidence indicates that the fetal-type (γ-nAChR) and the neuronal α7-type nicotinic acetylcholine receptor (α7-nAChR) could be reexpressed throughout the muscle membrane under pathologic conditions (e.g., denervation, burn, or immobilization) [11–13]. Normally, the γ- and α7-nAChR are only present (scattered) throughout the uninnervational muscle membrane at the early fetal stage. In adults, however, ε-nAChR instead of the two other isoforms is synthesized to maintain the neuromuscular transmission [11,14]. Reexpression of γ- and α7-nAChR in a mature neuromuscular junction (NMJ) leads to dysfunction because these receptors have different electrophysiological characteristics and sensitivity to both agonists and antagonists [15–17]. The stability of NMJ is thought to be central to the neuromuscular function. Stability is characterized by maintenance of the motor end plate and expression of the relevant nAChR subtypes. Thus, we hypothesized that denervation-like changes will occur at the septic NMJ, which will lead to muscle weakness. The denervation-like changes in the skeletal muscle, which are produced by local anesthetic bupivacaine, were first described by Libelius in 1970 [18]. The term denervation-like can be interpreted as some changes similar to those in the denervated muscle, including changes in electrophysiology, expression of nAChRs, and, from a morphologic point of view, the results obtained in the innervated muscle [19].

The present study in rats tested the hypothesis that chronic sepsis can induce denervation-like changes at NMJ, such as changes in electromyography, quantity and quality of nAChR, and morphology of the end plate. We used surgical peritonitis to set up a chronic sepsis model, and tibial nerve transection was used to produce surgical denervation. Comparing these two animal models, we aimed to investigate the different functional and biochemical alterations of NMJ to elucidate the pathogenesis via which sepsis causes muscle weakness.

2. Materials and methods

Male Sprague–Dawley rats (2–3 mo old, weight range 200–250 g) were obtained from the Experimental Animal Center of Chongqing Medical University (Chongqing, China). All rats received humane treatment according to the regulations of the Institutional Animal Care and Use Committee of Chongqing Medical University. One week before the experiments, rats were housed in a specific pathogen-free laboratory in an acclimatized room at standard room conditions (25°C ± 2°C, 55% humidity), with a 12-h light–dark cycle. Rats were allowed free access to water and standard chow. All experimental procedures involving animals were approved by the Animal Ethics and Use Committee of Chongqing Medical University.

For surgical intervention, all the rats were anesthetized with 10% chloral hydrate (350 mg/kg) administered intraperitoneally. All rats were randomly divided into three groups as follows: (1) a control group, in which no operation was performed; (2) the sepsis group, which underwent a CLP operation; and (3) the denervation group, where tibial nerve transection was performed. Given the time frame of the animal model, the rats in each group were assigned to three subgroups as follows: day 1, day 7, and day 14. Group assignment is shown in Figure 1.

In the sepsis group, sepsis was induced by means of CLP, which was performed as described previously. In this sepsis model, rats are considered septic 6 h after CLP [20,21]. The midline laparotomy was performed to expose the cecum. The cecum was ligated tightly using a 3-0 silk suture at its base below the ileocecal valve and punctured with a 24-gauge needle to prevent bowel obstruction. A small amount of feces was extruded by applying gentle pressure on the ligated cecum. Then, cecum was returned to the peritoneal cavity.

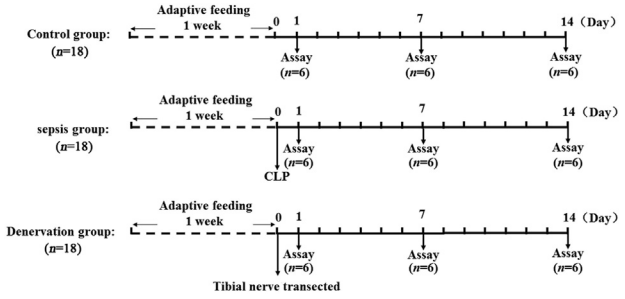

Fig. 1 – A flowchart of the experiments. Sixty-six rats were assigned to the control group ($n = 18$), sepsis group ($n = 30$), and denervation group ($n = 18$). The rats in each group were divided into three subgroups: day 1, day 7, and day 14. Given the exclusion of 11 rats because of death or hemodynamic and metabolic instability, the final statistical analysis included 19 animals in the sepsis group.