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Low-intensity treadmill exercise enhances fast recovery from bupivacaine-induced muscle injury in rats

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ABSTRACT

Background: Although bupivacaine has been used to study muscle degeneration and regeneration, the potential enhancement of muscle injury by exercise has not been well examined. The purpose of this study was to determine whether low-intensity treadmill exercise enhances fast recovery from bupivacaine-induced muscle injury and to examine concomitant changes in heat-shock protein 70 (HSP70) expression during regeneration process.

Methods: In this study, Sprague-Dawley rats were randomly divided into the following four groups: the control group (CON), the sham group (SHAM), the injury group (INJ), and the injury and exercise group (EX) ($n = 14$ in each group). Expressions of HSP70, inducible nitric oxide synthase (iNOS), and caspase-3 were determined at 1 and 7 days after bupivacaine-induced muscle injury in gastrocnemius.

Results: Results showed that bupivacaine-induced muscle injury (1 day) significantly increased the expressions of HSP70 and iNOS. At 7 days after the muscle injury, HSP70 expression was higher in the EX group compared with that in the INJ group and elevated level of HSP70 by exercise is concomitant with downregulation of iNOS and the decreased number of caspase-3-positive cells as a marker of apoptosis. Fewer necrosis of myofibers were also found in the EX group compared with the INJ group.

Conclusion: Our results suggest that low-intensity treadmill exercise may enhance fast recovery from bupivacaine-induced muscle injury in rat partly by HSP70 upregulation.

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1. Introduction

For the study of muscle injury and its mechanisms, denervation, ischemia, and injection of neuromyotoxic agents have been adopted as indirect methods.[1,2] Among them, local bupivacaine has been widely used to examine skeletal muscle degeneration and regeneration because even a single injection of this agent can rapidly induce necrosis in muscle fibers.[1] It thus appears that a bupivacaine injection is a suitable method to study histological and cytochemical changes associated with muscle injury.[3,4]

Previous studies on the recovery process of muscle injury have been focused on the structural changes,[5–7] whereas recent studies have focused on the identification and characterization of stress proteins, which play a pivotal role in cellular responses of the regeneration process. Recently, growing evidence showed that heat-shock proteins (HSPs) are involved in the recovery process of injured skeletal muscle.[8–10] Because these molecular chaperones play a pivotal role in cytoprotection,[11,12] HSPs are thought to be as one of the most important molecular factors in skeletal muscles response to injuries and subsequent regeneration.

Among the HSPs family, HSP70 is considered to be one of the most responsive molecular chaperones that plays a role in repairing folded peptides during physiological challenges in skeletal muscles.[13,14] Recent reports showed that HSP70 has the capacity to suppress the injury process and apoptosis in response to a variety of stimuli including heat, DNA damage, and death receptor ligation,[15] suggesting the potential survival-promoting effects of HSP70. Regarding the expression of HSP70 in skeletal muscle injury, previous studies reported a potential protective role of HSP70 in skeletal muscle injury models including ischemia reperfusion,[8] denervation,[10] and eccentric contraction.[9] A recent study also strongly supported this notion by demonstrating that overexpression of HSP70 prevented the specific force deficit and protected against muscle damage.[16] However, previous studies reported inconsistent expressions of HSP70 in the recovery process from muscle injury, and the reasons for such differences are mainly due to different muscle injury models, type of muscle fiber, and duration of injury period. Furthermore, as far as bupivacaine-induced muscle injury is concerned, currently there is no report demonstrating the potential protective role of HSP70 expression.

Skeletal muscle has a capability to adapt to a variety of stresses including contractile activity through induction of cytoprotective proteins such as HSP70. It is relatively well documented that exercise substantially induces production of HSP70 in the skeletal muscle and it may provide an insight into the underlying mechanisms by which regular exercise can protect against related and not-related stressors including muscle injury.[17,18] Nonetheless, the effect of exercise-induced HSP70 expression on skeletal muscle injury and recovery has not been studied yet. Thus, in this study, the effect of low-intensity treadmill exercise on bupivacaine-induced muscle injury and the potential role of HSP70 expression in the recovery process were investigated.

2. Methods

2.1. Animal model

A total of 56 male Sprague-Dawley rats, weighing 301.7 ± 3.4 g, were obtained from a commercial breeder (Orient Co., Seoul, Korea) for the experiments. All surgery and experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health and the Korean Academy of Medical Sciences. The rats were housed in standard stainless steel wire-mesh cages in a room with a 12-hour light/dark cycle (light period: 7:00 AM–7:00 PM;temperature:20 ± 2 °C). All animals had *ad libitum* access to food (rat chow) and water.

2.2. Experimental design

The animals were randomly divided into the following four groups: the control group (CON), the sham group (SHAM), the injury group (INJ), and the injury and exercise group (EX) ($n = 14$ in each group). The rats in the INJ group received bupivacaine into the left gastrocnemius muscle, whereas the rats in the SHAM group received an equivalent amount of NaCl.

Six hours after bupivacaine injection, two animals from each of the CON, SHAM, and INJ groups were killed to determine the level of tumor necrosis factor-alpha (TNF-α) as a marker of inflammation using reverse transcription-polymerase chain reaction (RT-PCR). Half of the animals in each group ($n = 6$) were killed at 24 hours after receiving a bupivacaine injection, whereas the others in each group ($n = 6$) were killed at 7 days after receiving a bupivacaine injection. Immediately after killing, the left hind limb was shaved and gastrocnemius muscles were carefully dissected. For the histological and immunohistochemical examinations, some parts of the gastrocnemius muscles were fixed in O.C.T. Compound (Sakura, Japan) and quickly frozen at –70 °C until analysis.

2.3. Muscle injury induced by bupivacaine injection

The animals were anesthetized by inhalation of diethyl ether, following which 0.1 mL of 1% bupivacaine hydrochloride prepared in 0.9% saline solution was injected six times into the left gastrocnemius muscles using a syringe equipped with a 26G needle (total injection volume: 0.6 mL).[19] The sham group received 0.1 mL of 0.9% saline solution six times (total injection volume: 0.6 mL). The rats were allowed to recover in their cages in a warm environment.

2.4. Exercise protocol

The rats in EX were forced to walk 24 hours after receiving the bupivacaine injection on a motor-driven treadmill. Exercise was performed for 30 minutes either one time (1 day) only or once daily for 7 consecutive days to compare the acute effect and relatively short-term training effect. The exercise load for the EX consisted of walking at a speed of 8 m/minute, at 0 degree of inclination. This exercise regimen is a low-intensity treadmill exercise.[20] Electric shock to stimulate the animals to run was not used; however, uncooperative rats