EFFECT OF LIPOPOLYSACCHARIDE (LPS) ON SKELETAL MUSCLE REGENERATION IN MICE

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I. INTRODUCTION

Skeletal muscle tissue shows the remarkable ability to initiate a rapid and extensive repair process upon muscle injury [1]. Skeletal muscle repair is a highly synchronised process involving the activation of various cellular responses. The initial phase of muscle repair is characterised by necrosis of the damaged tissue, activation of an inflammatory response, and increased reactive oxygen species (ROS) [2]. Shelar *et al*. [3] reported that genetic ablation of Nrf2, master anti-oxidative transcription factor, aggravates muscle regeneration in mice [3]. These reports led us to hypothesize that excessive ROS during muscle injury inhibits appropriate muscle regeneration. In this study, we aimed to elucidate the effect of Lipopolysaccharide (LPS), a inducer of muscular ROS [4], on muscle regeneration. This study contributes to our understanding of relation muscle atrophy and oxidative stress in muscle injury and lead to more efficient protein production in livestock.

II. MATERIALS AND METHODS

Eight-week-old C57BL/6J male mice (n=4) were used. To induce muscle injury, 10 μM cardiotoxin (CTX) in PBS was injected into tibialis anterior (TA) muscle. From the day of CTX treatment, 1 mg/ml/kg/day LPS was administered intraperitoneally. TA muscles were harvested on 0, 3, 7 and 16 days after CTX treatment for histological and biochemical analyses. Histological analysis: muscle tissues were embedded and frozen for cryosection. HE staining and immunohistochemistry were performed. Biochemical analysis: Total RNA was extracted and qPCR was performed. The mRNA expressions of muscle regeneration- and oxidative stress-related genes were analysed. Statistical analysis was performed using Student’s t-test.

III. RESULTS AND DISCUSSION

LPS administration significantly reduced diameter of muscle fibers in day 16 compared with control group (P=0.045). The number of muscle fibers with central nuclei, a marker of muscle regeneration were significantly decreased in LPS group at day7 (P=0.041) and 16 (P=0.024) (Figure 1A and B). Decreased MyoZ1-positive fibers and Myoz1 mRNA expression were observed in both immunohistochemistry (P=0.013) (Figure 2A and B) and qPCR analysis (P=0.018), suggesting delay of muscle regeneration. These data demonstrated that LPS administration induces delayed muscle regeneration and skeletal muscle atrophy after regeneration.

IV. CONCLUSION

We found that LPS induces delay of muscle regeneration in mice. This effect may relate increased ROS level in injured muscle; however, further studies are required to clarify the detailed mechanism underlying the effects of ROS on skeletal muscle regeneration.

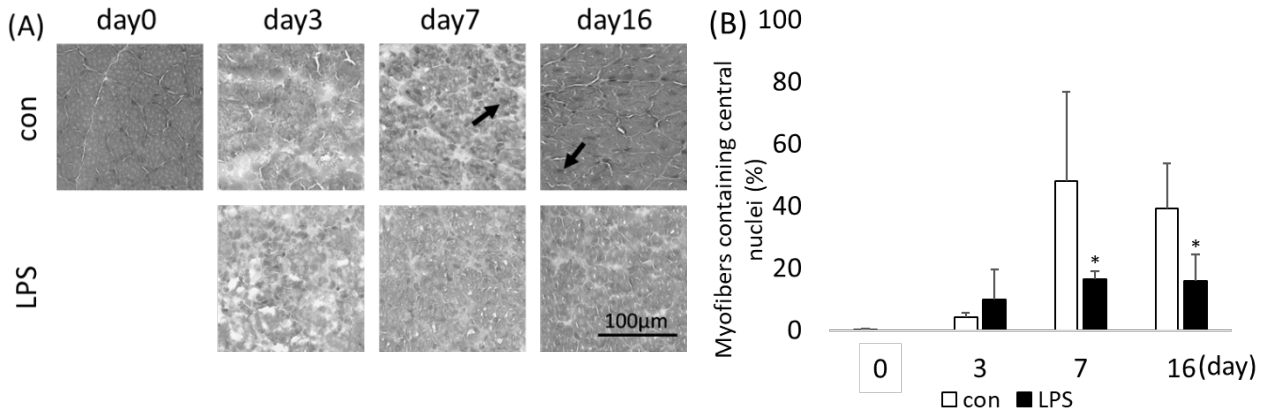

Figure 1: The ratio of muscle fibers with central nuclei.

(A) HE-stained images of TA muscle sections for each day after injury. Arrows in the figure indicate central nuclei, a marker of muscle regeneration. (B) The percentage of number of muscle fibers with central nuclei per number of total muscle fibers. Values are expressed as mean ± SE (n = 4). *: P < 0.05 vs. respective control.

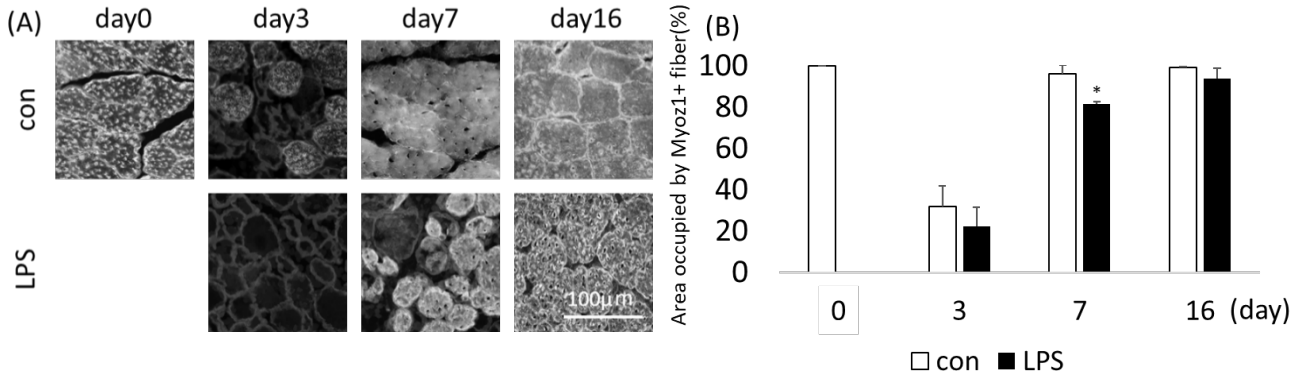

Figure 2: The ratio of MyoZ1 positive fibers.

(A) Images of fluorescence immunostained sections of TA muscle after CTX injury. GFP: MyoZ1, TexasRed: Laminin, DAPI: nuclei. (B) The ratio of MyoZ1-positivefibers was determined by the number of MyoZ1-positivefibers per number of Laminin-positive myofibres. Values are expressed as mean ± SE (n = 4). *: P < 0.05 vs. respective control.

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