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The therapeutic effects of lipoxin A_4 during treadmill exercise on monosodium iodoacetate-induced osteoarthritis in rats

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ABSTRACT

Lipoxin A_4 (LXA_4), a kind of adipokines, is a potent stop signal of inflammation. Our preliminary study found that LXA_4 of serum and intra-articular lavage fluid (IALF) was rapidly elevated in 2 h and rapidly reduced to normal level at 4 h after moderate-intensity treadmill exercise. The aim was to confirm the therapeutic effects of LXA_4 during treadmill exercise on rat model of monosodium iodoacetate (MIA)-induced OA and the detailed mechanism of LXA_4 on OA. One hundred and twenty-four male Sprague-Dawley rats were submitted to two different protocols. A single session of treadmill exercise: sixty-four rats were randomly divided into treadmill exercise of different intensities for 60 min only once (n = 4). Formal treadmill exercise: sixty rats were randomly divided into six groups (n = 10): control group (CG), knee OA group (OAG), OA with treadmill exercise of different intensities (OAL, OAM and OAH), and OAM + BOC-2 (an antagonist of LXA_4 receptor). The rats were evaluated by ELISA, histology, immunohistochemistry and western blotting. Fibroblast-like synoviocytes (FLSs) were obtained from knee joint of rats. The effects of LXA_4 on interleukin (IL)-1β induced FLSs were evaluated by western blotting and immunofluorescence. The results of ELISA, histological evaluation, western blotting and immunohistochemistry indicated that OAM had a better treatment which could be suppressed by BOC-2. Moreover, LXA_4 could attenuate the expression of matrix metalloproteinase (MMP)-3 and MMP-13 and suppress the expression of nuclear factor-kappa B (NF-κB) p65 induced by IL-1β in FLSs. The therapeutic effects of LXA_4 during treadmill exercise on MIA-induced OA via inhibiting NF-κB signaling pathway.

1. Introduction

Osteoarthritis (OA) affects a large number of population, and its incidence is showing a growing trend with the increasing life span (Ondrésik et al., 2017). Although physical activity is one of the most common non-pharmacological OA therapies, the duration and type of exercise programs varied widely (McAlindon et al., 2014). The association between physical activity and OA is not well understood, and how physical activity affects OA need further investigation (Barbour et al., 2014). Moreover, there is no unified standard to detect the effect of physical activity on OA treatment. It is noteworthy to find a significant indicator to evaluate it.

Epidemiological studies indicate that inflammatory molecules, including adipokines and cytokines, have been shown to contribute the progression of cartilage loss (Kalunian, 2016; Berenbaum, 2011). Improved understanding of inflammation in OA is required to facilitate therapeutic target discovery (Cicuttini and Wluka, 2016). The role of adipokines, which are produced by adipose tissue and released into the blood where they participate in low-grade inflammation, has been widely studied in recent years (Berenbaum and van den Berg, 2015). Now it is becoming crucial question that studying adipokines as an effective approach toward understanding the pathophysiology of OA. The biological and biomechanical interplay between these tissues that ultimately leads to joint dysfunction remains a topic of high interest (Sandy et al., 2015).

Lipoxin A_4 (LXA_4), a kind of adipokines, is a potent stop signal of inflammation (McMahon et al., 2001; Serhan, 2014). The therapeutic effects of LXA_4 on OA have been confirmed by previous studies (Sodin-Semrl et al., 2000; Chan and Moore, 2010; Conte et al., 2010; Yang et al., 2017). LXA_4 are rapidly formed on cell stimulation, act locally, and are rapidly metabolized and inactivated (Gangemi et al., 2003). And the exogenous LXA_4 could abrogate interleukin (IL)-1β stimulation

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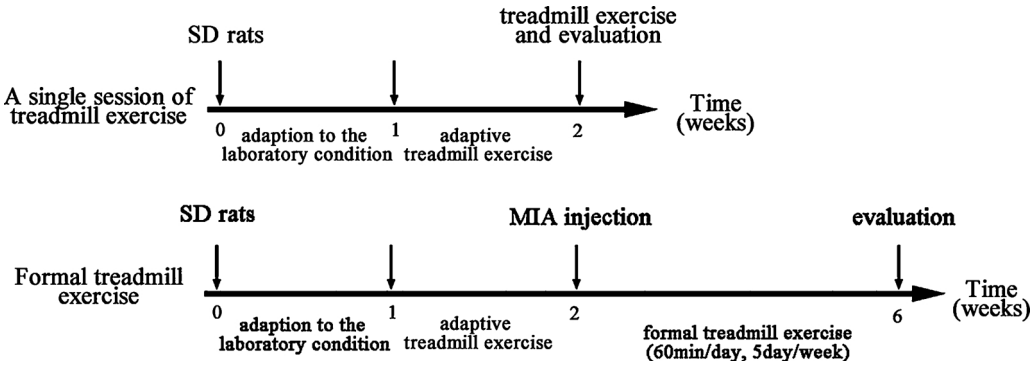

Fig. 1. The design of the treatment schedule and intervals of various parameters.

of fibroblast-like synoviocytes (FLSs) (Sodin-Semrl et al., 2000) which is a key role in inflammation and joint destruction (Mor et al., 2005). Therefore, production of the high LXA_4 level should be regarded as indicator for predictive therapy of OA.

As a critical transcriptional factor in promoting the expression of a number of pro-inflammatory genes, NF-κB is a key signaling molecule in the control of OA (Olivotto et al., 2015). Activated NF-κB signaling pathway boosts the expression of many inflammation-related cytokines. Therefore, the suppression of NF-κB signaling pathway is one of the potential therapeutic OA targets (Ni et al., 2015).

Our preliminary study found that LXA_4 of serum and intra-articular lavage fluid (IALF) was rapidly elevated in 2 h and rapidly reduced to normal level at 4 h after moderate-intensity treadmill exercise. LXA_4 could inhibit the synthesis of inflammatory cytokines, such as IL-1β and TNF-α in the articular cavity (Yang et al., 2017). But we couldn't confirm the therapeutic effects of LXA_4 during treadmill exercise in OA, and the detailed mechanism of LXA_4 on OA need further investigation, such as on fibroblast-like synoviocytes (FLSs).

In present study, we used BOC-2, an antagonist of LXA_4 receptor, to evaluate the effects of LXA_4 during treadmill exercise of different intensities in rat model of MIA-induced OA. And we explored the therapeutic effects of LXA_4 focusing on the inhibition of NF-κB signaling pathway and its effects. The results provide new insight that LXA_4 was a significant indicator to detect the effect of treadmill exercise on OA treatment.

2. Materials and methods

2.1. Experimental animals

One hundred and twenty-four male Sprague-Dawley (SD) rats (230 ± 10 g, 8 weeks of age, and specific-pathogen-free) were obtained from HFK Bioscience Co. Ltd. (Beijing, China). Maintenance and care of the experimental rats followed the guidelines of the Ethics Committee of China Medical University, and the study was approved by this Ethics Committee. Rats were kept in individual plastic cages on sawdust bedding, a 12:12 h light: dark cycle with the lights on from 6:00 a.m. to 6:00 p.m., a controlled temperature of 22 ± 2 °C, and 70% humidity. They had free access to food and water. Body weight was recorded at regular intervals. They were adapted to laboratory conditions for 1 week prior to the experimental procedures. And then, all rats were habituated to the ZH-PT treadmill exercise (Zhongshidichuang Science & Technology Development Co. Ltd., Beijing, China) for 1 week at a speed of 10 m/min for 10 min/day to reduce stress. All rats adapted to the treadmill exercise. (Fig. S1)

2.2. OA model and treadmill running protocols

After the adaptive treadmill exercise, all rats were submitted to two different exercise protocols: a single session of treadmill exercise or formal treadmill exercise with appropriate photostimulation (300 lx), acoustic stimulation (80 DB) and electric stimulation (100 Hz, 0.18 mA).

A single session of treadmill exercise (only 60 min): Sixty male SD rats were randomly divided into three groups (n = 20): 15.2 m/min with 0° of inclination (low-intensity treadmill exercise, LIT); 19.3 m/min with 5° of inclination (moderate-intensity treadmill exercise, MIT); and 26.8 m/min with 10° of inclination (high-intensity treadmill exercise, HIT) respectively. All rat exercise last for 60 min only once. Rats were evaluated immediately after the exercise (A.E.), and 1, 2, 3, 4 h after treadmill exercise (n = 4). The other four male SD rats were no treadmill exercise named as basal group.

Formal treadmill exercise: sixty rats were randomly divided into six groups (n = 10): control group (CG), knee OA model (OAG), OA with low-intensity treadmill exercise: 15.2 m/min with 0° of inclination, 60 min/day, 5 days/week for 4 weeks (OAL), OA with moderate-intensity treadmill exercise: 19.3 m/min with 5° of inclination, 60 min/day, 5 days/week for 4 weeks (OAM), OA with high-intensity treadmill exercise: 26.8 m/min with 10° of inclination, 60 min/day, 5 days/week for 4 weeks (OAH) (Ni et al., 2013, 2012; Li et al., 2017), and OAM + BOC-2 (abs45120618, absin) (BOC-2, an antagonist of LXA_4 receptor, was administered by intraperitoneal injection 50 μg/kg (Li et al., 2013; Brigatte et al., 2016) before every treadmill exercise). After the adaptive exercise, all rats were anesthetized with 1.5% pentobarbital sodium (30 mg/kg, intraperitoneal injection). Knee OA was induced by intra-articular injection of MIA (1 mg per cavity in 50 μl sterile saline) by micro syringe through the infrapatellar ligament and into the bilateral knee joint cavity. The CG rats received an intra-articular injection of 50 μl sterile saline. The CG and OAG rats were kept sedentary after injection, but other groups need 24 h to recover before being placed into treadmill paradigm. Rats were evaluated after completing the treadmill exercise program. (Fig. 1)

2.3. Sampling and tissue preparation

After a single session of treadmill exercise, rats were anesthetized and sacrificed by cervical dislocation. Blood samples were obtained 5 min after the animals were anesthetized, and centrifuged at 3000 × *g* for 10 min to obtain serum. IALF was obtained from the synovial cavity of the right knee by injection and recovery of 0.1 ml of phosphate-buffered saline (PBS) using a 1-ml syringe 10 min after anesthetized.

After the last session of formal treadmill exercise, serum and IALF were also collected. The left knee joints of all rats were dissected and fixed in 4% paraformaldehyde solution. Articular cartilage and synovium were collected from the right knee joint. After anesthetizing and sacrificing the rats by cervical dislocation, we used surgical scissors to remove the skin and muscles along the patellar ligament to open the capsula articularis, being careful to avoid damage to the cartilage and synovium. Next, articular cartilage specimens were removed from the weight-bearing area of the condyles of the right femur and tibia using a