Strain Diversity Of Articular Cartilage Degeneration Induced By Ovariectomy And Forced Running In Mice.

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Disclosures:

Introduction: Osteoarthritis (OA), which leads chronic disability in the elderly people, is considered to be a multifactorial disease with factors such as chronic inflammation, aging, menopause, obesity, mechanical stress, and joint instability [1]. In addition, recent epidemiological analyses indicated racial and ethnic disparities in osteoarthritis phenotypes in humans [2]. Last year we reported a non-invasive experimental OA model, in which we showed that synergistic effects of extensive treadmill exercise and ovariectomy (OVX) on articular cartilage degeneration while exercise and OVX respectively had subtle effects on cartilage degeneration in Balb/c mice (refer miyatake ORS2013 Poster No.1252). Histological and immunohistochemical analysis indicated that exercise and OVX synergistically induced severe synovitis, which was indicated by extensive synovial hyperplasia and macrophage infiltration in synovial membrane. Based on these data, we speculated that synovial inflammation was one of the key factors for primary OA and there was a signal crossstalk between the factors secreted by ovary and mechanical stress on the process of synovial inflammation. We expected that this experimental mouse OA model was suitable for investigating natural occurring primary OA frequently observed in postmenopausal women, since no artificial damage such as meniscectomy and cruciate ligament transection surgery was given inside the knee joint [3]. In this study, to examine the effect of racial and ethnic disparities on articular cartilage homeostasis in mice, we employed the same experimental protocol and examined articular cartilage degeneration in C57BL/6J mice.

Methods: Twenty-eight female 8 week-old C57BL/6J mice were randomly divided into two groups, one was an OVX group (OVX), and the other was a control group (SHAM). Two weeks after ovariectomy or sham operation, all the mice were subjected to forced running for 5 days at 12m/min for 10 minutes followed by 20m/min for 10 minutes to adopt treadmill exercise. Then each group was further randomly divided into two groups, 7 mice were subjected to forced running by treadmill (OVX+Run or SHAM+Run) and the latter 7 were left in cage ad libitum (OVX+Cage or SHAM+Cage). Running group was subjected to forced running for 6 weeks (5 days a week) at 12m/min for 10 minutes followed by 20m/min for 100 minutes. After 6 weeks, both left and right knee joints were harvested. Left knee joints were sliced into 5µm-serial sagittal sections for histology and immunohistochemistry. The areas of the residual proteoglycan in articular cartilage in the sagittal sections stained with safranin-O. To evaluate the loss of proteoglycan from articular cartilage, we developed modified Mankin scoring system. To further compare the severity of articular cartilage damage between the four groups, we analyzed type II collagen expression in articular cartilage. H&E slides were used to evaluate synovial activation by scoring thickening of the synovial lining and cellular influx into joint cavity and synovium. Synovial activation was scored. Right knee joints were subjected for μCT analyses. Both metaphyseal and epiphyseal bone volume per tissue volume (BV/TV) were calculated using Tri/3D-BONE software (Ratoc System Engineering Co., Tokyo, Japan). Statistical analysis was performed using Kruskal-Wallis one-way analysis of variance by ranks followed by Tukey-Kramer methods. P<0.05 was considered as significant.

Results: Safranin O dyeability of articular surface was quite similar between SHAM+Cage, SHAM+Run, OVX+Cage, and OVX+Run groups (Fig.1a). Modified Mankin score indicated no significant difference between the 4 groups (Fig.1b). To further compare the severity of articular cartilage damage, we analyzed type II collagen expression in articular cartilage (Fig.1c). As shown in the figure, type II collagen expression in both femoral and tibial articular cartilage was comparable between the 4 groups. Fig.2a shows H/E staining of sagittal sections of the knee joint. We observed increased cellularity in the synovial membrane in the ovariectomized mice. Synovitis score confirmed that the degree of synovial hyperplasia was significantly increased in both OVX+Cage and OVX+Run groups while treadmill exercise did not affect on synovial hyperplasia (Fig.2b). Micro CT pictures showed no apparent alteration in the bone shape and osteophyte formation between the four experimental groups (Fig.3a). In parallel with previous reports, we observed significant reduction in BV/TV in OVX+Cage group in epiphysis (Fig.3b). Forced running for 6 weeks partially reversed BV/TV in the OVX mice (Fig.3b,3c).

Discussion: In this study, we showed no synergistic effects on extensive treadmill exercise and OVX on articular cartilage degeneration in C57BL/6J mice. This was stark contrast to our previous results observed in Balb/c mice, in which exercise and OVX synergistically accelerated articular cartilage degeneration. Histological analysis indicated that synovial hyperplasia was observed in the ovariectomized C57BL/6J mice, however we did not observe any synergistic effects of exercise on synovitis, which was obvious in Balb/c mice. On the other hand, the effects of exercise and OVX on bone homeostasis were on the same
line between the two mice lines. In both lines, we observed significant loss of metaphysical and epiphyseal trabecular bone volume in the ovariectomized mice. Exercise always increased trabecular bone volume regardless of OVX. These data suggest that strain diversity may mainly affect on the severity of inflammatory response in synovial membrane.

**Significance:** We expect our experimental mice OA model is suitable for investigating the molecular pathogenesis of natural occurring primary OA which is caused by multiple factors such as abnormal mechanical stress, menopause, and genetic diversity.

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