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ORIGINAL ARTICLE

Evaluation of osteoarthritis induced by treadmill-running exercise using the modified Mankin and the new OARSI assessment system

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Abstract To apply the Osteoarthritis Research Society International (OARSI) assessment system to an osteoarthritis model, 44 Wistar rats were randomized into treadmill-running exercise or control group. At 6, 8, and 10 weeks, medial knee joints were histopathologically evaluated, and aggrecan neoepitope and TUNEL staining were performed. Cartilage changes in exercise group were histopathologically and histochemically compatible with early OA. Total modified Mankin system (MMS) scores were significantly higher at all time points (each $P \leq 0.01$) in exercise than in control group. However, only tibial OARSI scores of runners were higher at 10 weeks ($P < 0.05$), although OARSI scores were found to be significantly correlated with MMS scores. Both total MMS (Spearman's coefficient $\rho = 0.786$) and OARSI scores ($\rho = 0.443$ for femoral; $\rho = 0.604$ for tibial) were significantly associated with the exercise duration. In conclusion, the OARSI system may not be sensitive to early OA changes induced by treadmill exercise.

Keywords Osteoarthritis · Treadmill · OARSI osteoarthritis score · Modified Mankin score · Wistar rats

Introduction

Since 1971, when Mankin et al. [1] developed a microscopic system for grading osteoarthritic cartilage, the original or modified Mankin grading system has been applied to many experimental osteoarthritis (OA) models. However, Mankin system was initially designed to assess human articular cartilage, and it has not been validated in a rodent OA model. Recently, the Osteoarthritis Research Society International (OARSI) working group proposed a new histopathological assessment system for clinical and experimental OA models, especially for the assessment of early or mild OA cartilage changes [2]. Custers et al. [3] demonstrated that the reliability of OARSI system was higher than Mankin system but the reproducibilities of both systems were similar in their metallic implant-induced OA goat model. Nevertheless, it remains to be elucidated whether the OARSI system is applicable to early OA in rodents.

Many studies have been conducted on experimental OA models including chemical-induced, surgically injured, and spontaneous OA models [4, 5]. However, because primary OA occurs in human beings without a definite mutation or history of injury, the pathogenesis of OA induced in surgically or chemically injured models may not be representative of

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human primary OA. In addition, surgical animal models can be dependent on the skills of investigators. Therefore, other interventions such as exercise are needed to simulate the human disease. Furthermore, rodent models can be used to produce OA lesions more rapidly and less expensively than large animal models, and although rats have the limited number of transgenic strains when compared to mice, rat models provide adequate amounts of tissue and rats are easy to handle.

Thus, we devised a running exercise–based Wistar rat model of OA using a motorized multi-track treadmill and evaluated histological OA cartilage changes using both the modified Mankin and OARSI scoring systems.

Methods and materials

Experimental animals and exercise protocols

Forty-four Wistar rats (13–14 week old, weight 250–300 g) served as subjects and randomized equally to an exercise or control group. In both groups, food and water were provided ad libitum and their spontaneous physical activity was not limited. For the running exercise, we designed a motorized multi-track treadmill, which had 5 lanes of length 55 cm. The angle of lane could be varied from 0 to 15 degrees, and the range of velocity was 1–30 m/min. Exercise was conducted for 1 h/day at the frequency of 5 days/week. The speed and inclination of the treadmill were 1.5 km/h and at 5° during the intervention. This inclination was designed to cause the rats to more flex and extend both hind-limb knee joints on the lane than on the flat ground. Mild electric shock was applied at the starting position of each lane when rats were resistant to run on the inclined lanes. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Wonkawng University College of Medicine.

Processing for histopathology and histomorphological evaluations

After six ($n = 8$), eight ($n = 6$), and ten ($n = 8$) weeks of exercise, the animals were killed using CO_2 asphyxiation. Both knee joints were fixed in 10% buffered formalin (pH 7.4) for 24 h and then decalcified in 10% EDTA solution. Paraffin-embedded left knee joint tissues were coronally sectioned serially and the assessed area consisted of the medial cartilage residing between the frontal planes of the insertions of the anterior and posterior cruciate ligaments, that is, the central area of the medial tibial condyle and the corresponding area of the femoral condyle. Hematoxylin and eosin (H&E) and safranin-O stains were applied. A semi-quantitative modified Mankin system (MMS) was used to grade histological changes, as previously described [6]. Histological cartilage changes were subgrouped into mild (1–4), moderate (5–9), or severe (10–13) OA using total MMS scores. In addition, the OARSI scoring method was applied separately according to the OARSI cartilage OA histopathology grading system [2]. A pathologist (Kim BK), who was blind to the exercise status and age, scored three consecutive sections of every knee joint.

Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling (TUNEL) stain and immunohistochemistry for aggrecan neoepitope

After tissue sections had been deparaffinized, apoptotic chondrocytes were labeled in situ using a commercial TUNEL assay kit (ApopTag Peroxidase in situ Apoptosis Detection Kit, Chemicon International, Temecula, California, USA) and visualized using diaminobenzidine (DAB), according to the manufacturer's instructions. To evaluate aggrecan degradation by matrix metalloproteinases and aggrecanases, immunostaining for aggrecan neoepitope 374ARGSV was performed using BC-3 monoclonal antibody (Abcam Inc., Cambridge, Massachusetts, USA) [7]. The slides were counterstained with safranin-O.

Statistical analysis

The Mann–Whitney test was used to compare the control and exercise groups with respect to femoral and tibial OARSI scores, subtotal femoral and tibial MMS scores, and total MMS scores. The Kruskal–Wallis test was used to compare longitudinal inter-group total MMS scores in each group. Spearman's correlation coefficients were used to analyze for associations between exercise duration and histological scores. The Fisher's exact test was used to compare dichotomous variables. A P value < 0.05 was considered as statistically significant.

Results

Histopathological and histochemical findings in control and exercise groups

In the histopathological findings of the H&E and safranin-O slides, the running exercise group showed more severe OA changes than the control group (Fig. 1a). But there were no definite findings suggestive of advanced OA changes such as deep vertical fissures, erosion, denudation, or osteophyte formation. In the TUNEL assay, TUNEL-positive chondrocytes had been observed in both groups since the 8th week, but the exercise group had more TUNEL-positive chondrocytes than the controls (Fig. 1b). Furthermore, immunohistochemistry revealed greater intensities and frequencies of

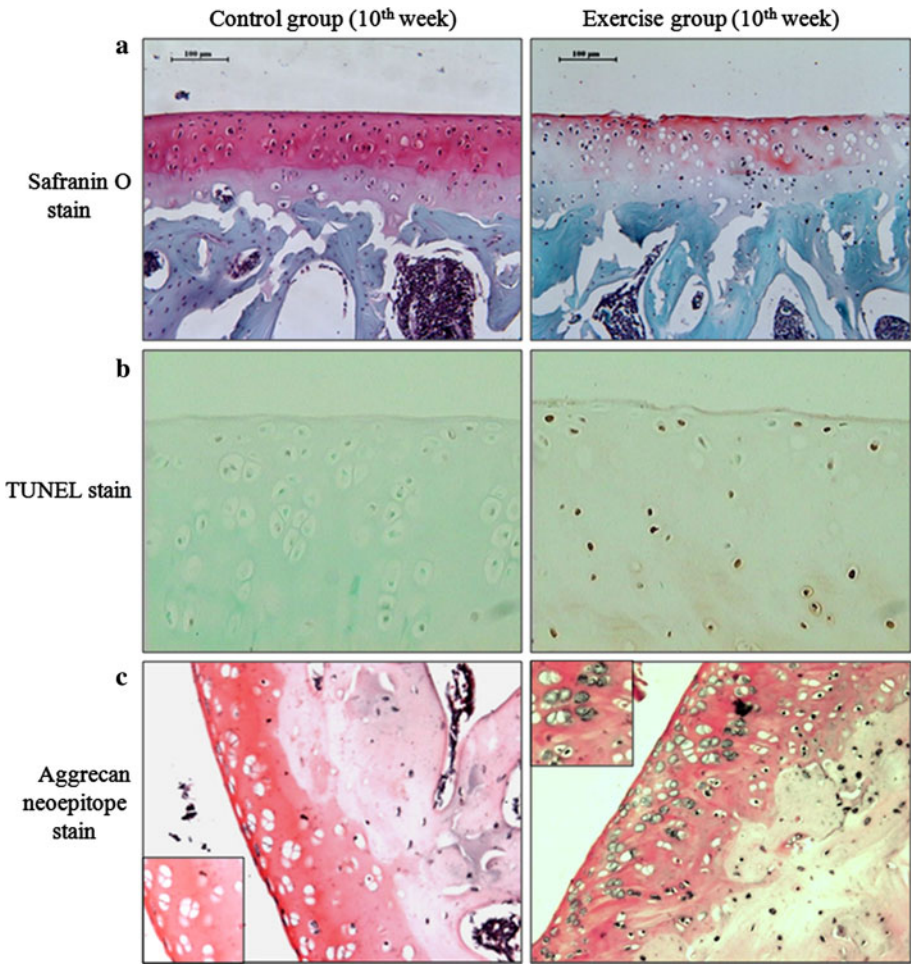

Fig. 1 Pathologic photographs of the representative cartilage tissues in the control and exercise groups at 10 weeks. Cartilage sections stained with safranin-O (**a**, ×200) showed superficial fibrillation, disorientation of chondron columns, empty chondrocytic lacunae, proliferation of chondrocytes, and matrix depletion in the deep zone in an exercised rat. TUNEL staining (**b**, ×400) displayed elevated numbers of TUNEL-positive chondrocytes. In aggrecan neoepitope-stained slides (**c**, ×200), exercise group had more numbers of immunostained chondrocytes than control group. The *insert* shows aggrecan neoepitope-stained chondrocytes with black cytoplasm

aggrecan neoepitope-positive cells in the exercise group; some sections of controls showed weak positivity. Especially, after 10 weeks, this difference between the control and exercise groups was obvious (Fig. 1c).

Semi-quantitative evaluations in the control and exercise groups

On semi-quantitative analysis using MMS, subtotal MMS scores were significantly higher in the exercise group than in controls at 6 ($P = 0.038$ by the Mann–Whitney test for femoral cartilage), 8 ($P = 0.026$ for femoral cartilage; $P = 0.041$ for tibial cartilage), and 10 weeks ($P = 0.005$ for femoral cartilage; $P = 0.001$ for tibial cartilage) (Figs. 2a, b). In addition, the exercise group had significantly higher total MMS scores than control group at 6 ($P = 0.01$), 8 ($P = 0.004$), and 10 weeks ($P = 0.001$) (Fig. 2c). Furthermore, in the exercise group, the distributions of subtotal ($P = 0.036$ for femoral cartilage by the Kruskal–Wallis test; $P = 0.001$ for tibial cartilage) and total MMS ($P = 0.001$) scores were significantly different at the 3 time points. Moreover, femoral subtotal ($P = 0.0075$, Spearman's coefficient $\rho = 0.554$), tibial subtotal ($P = 0.9 \times 10^{-5}$, $\rho = 0.797$), total ($P = 1.5 \times 10^{-5}$, $\rho = 0.786$) MMS scores were found to be significantly correlated with exercise duration.

When OA changes were stratified according to total MMS scores, the exercise group had a significantly higher prevalence of mild OA change at 6 weeks (75% vs. 0%, $P = 0.007$ by the Fishers' exact test) and of moderate OA change at 10 weeks (62.5% vs. 0%, $P = 0.026$) than controls (Table 1). After 8 weeks, the exercise group showed a higher, though non-significant, prevalence of mild OA (100% versus 33.3%; $P = 0.061$).

When OARSI system was applied, tibial OARSI scores were significantly higher at 10 weeks in the exercise group than in control group ($P = 0.038$) and femoral scores showed a tendency to increase in exercise group at 10 weeks ($P = 0.050$; Fig. 3). During the earlier period of exercise, no significant inter-group difference was found for OARSI scores. Both OARSI femoral and tibial scores ($P = 0.034$, $\rho = 0.443$ for femoral cartilage; $P = 0.003$, $\rho = 0.604$ for tibial cartilage) were found to be significantly correlated with exercise duration, but only tibial OARSI scores were significantly different among three time points of 6, 8, and 10 weeks in the exercise group ($P = 0.005$ by

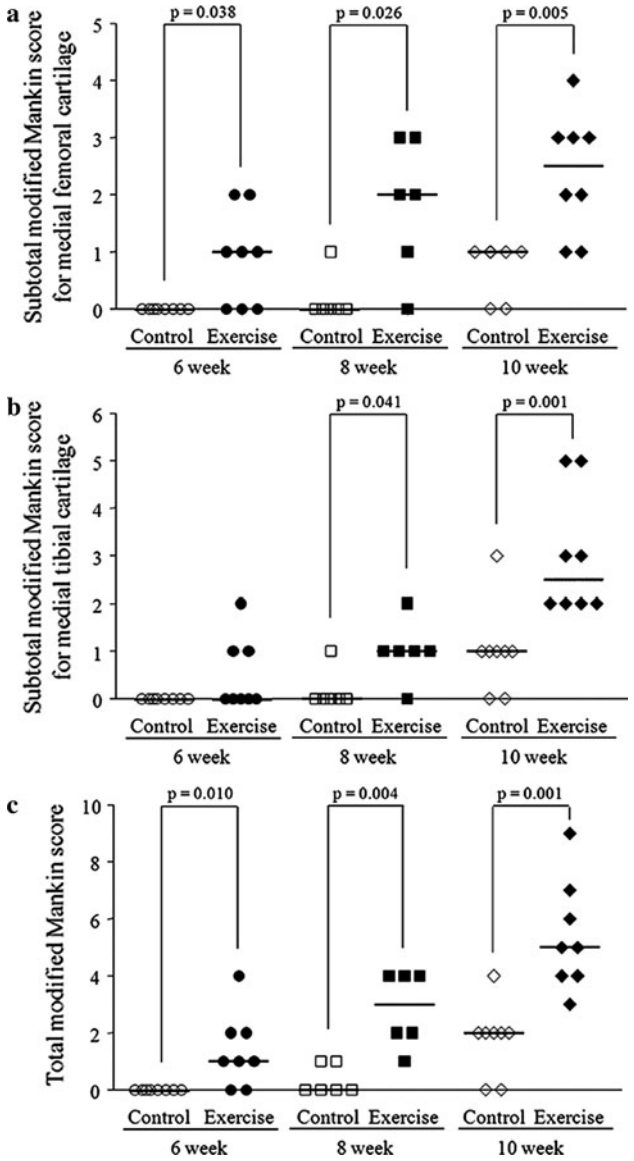

Fig. 2 Histopathological evaluation conducted using the modified Mankin grading system. Femoral (**a**), tibial (**b**), and total (**c**) Mankin scores were significantly higher in the exercise group at all time points (each $P < 0.05$), with the exception of 6-week tibial subtotal scores. *Black symbols* indicate scores in the exercise group and *white symbols* control group scores. *Bars* represent median values

the Kruskal–Wallis test). Femoral and tibial OARSI scores were significantly correlated with subtotal femoral ($P = 9.2 \times 10^{-5}$, $\rho = 0.737$) and tibial ($P = 1.3 \times 10^{-5}$, $\rho = 0.788$) MMS scores, respectively (Fig. 4).

Discussion

Human primary OA has been considered to be a multifactorial disease with genetic, inflammatory, metabolic, and mechanical causes and it occurs without a definite mutation or history of injury. Exercise is a less potent stimulus than surgical or chemical interventions, and the effect of exercise on cartilage tissue can be affected by other potential factors, such as, weight and quadriceps muscle power. Physical

Table 1 Osteoarthritic changes in Wistar rats subject to the treadmill-running exercise

	No change*	Mild change†	Moderate change¶
6 week			
Control ($n = 8$)	8 (100%)	0	0
Exercise ($n = 8$)	2 (25.0%)	6 (75.0%)**	0
8 week			
Control ($n = 6$)	4 (66.7%)	2 (33.3%)	0
Exercise ($n = 6$)	0	6 (100%)	0
10 week			
Control ($n = 8$)	2 (25.0%)	6 (75.0%)	0
Exercise ($n = 8$)	0	3 (37.5%)	5 (62.5%)††

* Modified Mankin score = 0; † $1 \leq$ modified Mankin score ≤ 4; ¶ $5 \leq$ modified Mankin score ≤ 9; ** $P = 0.007$ by the Fishers' exact test for the exercise versus the control groups at 6 weeks of exercise; †† $P = 0.026$ in the exercise versus the control groups at 10 weeks

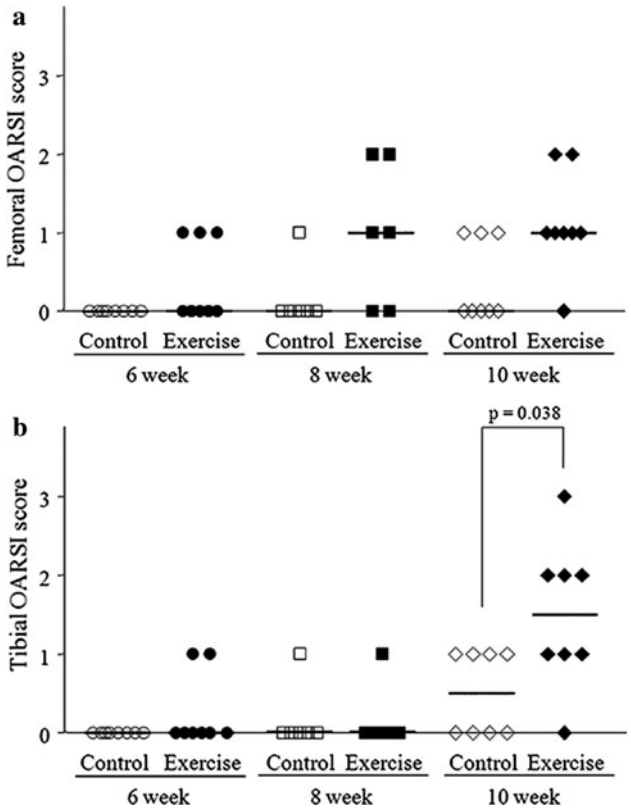

Fig. 3 Histopathological evaluations using the OARSI scoring system. Tibial OARSI scores (**b**) in the exercise group were significantly higher at 10 weeks than control group scores ($P = 0.038$), but femoral OARSI scores were not (**a**). *Black symbols* indicate the scores in exercise group and *white symbols* control group scores. *Bars* represent median values

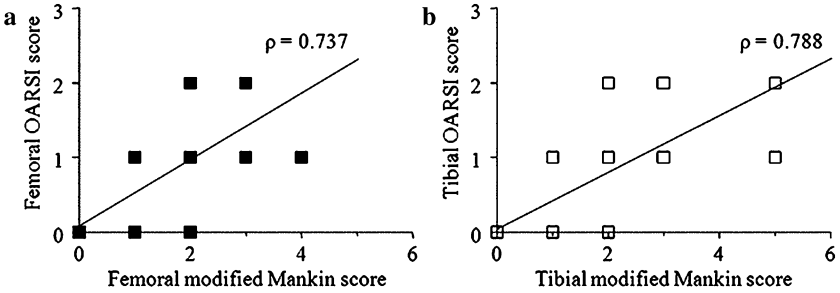

Fig. 4 Correlations between modified Mankin and OARSI scores in the exercise group. Femoral and tibial modified Mankin scores were found to be significantly correlated to femoral (**a**, $P = 9.2 \times 10^{-5}$, Spearman's coefficient $\rho = 0.737$) and tibial (**b**, $P = 1.3 \times 10^{-5}$, $\rho = 0.788$) OARSI scores, respectively

loading has been considered a two-sided sword, that is, it is essential for joint health but excessive loading beyond the normal activities can lead to OA changes [8]. Therefore, exercise-induced animal models can serve as more reliable OA models than surgically or chemically induced models, even though existing evidence on the effect of long-term long-distance running on the development of human OA is not sufficient to draw clear-cut conclusions [9].

In the present study, the treadmill-running exercise induced histopathologic knee OA changes, and degrees of changes were found to be significantly correlated to exercise duration. After 8 weeks of exercise, the exercise group had a median OARSI score of 1.0 [range 0–2] for femoral and of 0.0 [0–1] for tibial cartilage, and the median total MMS score was 3.0 [1–4]. These scores fall into the range of early or mild OA according to the definitions of both histological scoring systems [2]. However, there was no clear-cut evidence of synovitis in the runners. It has previously been reported that synovial inflammation may precede and contribute to cartilage damage in surgically or chemically induced OA, but that non-invasive exercise-induced OA develops synovitis at a later stage [10]. Although the exercise group did not show definitely advanced or severe OA changes in cartilage (median total MMS 5.0 [3–9], femoral OARSI score 1.0 [0–2], and tibial OARSI score 1.5 [0–3]) at 10 weeks, TUNEL and aggrecan neoepitope immunostaining revealed OA-related activation of matrix-degrading enzymes and chondrocyte damage. Chondrocyte apoptosis occurs during the early phase of OA [11], and is associated with cartilage matrix degradation [12]. Aggrecan degradation is also regarded as an early event during OA cartilage damage, and is induced by a disintegrin and metalloproteinase with a thrombospondin type 1 motif (ADAMTS), 4 or 5 [13]. Recently, it was reported that syndecan-4 controls the activation of ADAMTS-5, and that syndecan-4 expression is elevated in early OA cartilage. Our treadmill-running exercise-induced OA model showed strong syndecan-4 immunostaining in the exercise group [14]. These findings suggest that the treadmill-running exercise-induced OA is a suitable experimental animal model of early OA. Previously, Tang et al. [15] investigated the effect of hyaluronan in experimental OA using a treadmill similar to ours. In their study, Wistar rats that ran on the treadmill also showed OA changes, but they did not have non-exercise control group.

Researchers have applied the microscopic histological histochemical grading system designed by Mankin et al. or modified by van der Sluijs et al. [1, 6] to many studies on OA cartilage change in human OA and experimental OA models. However, the results of previous studies have been inconsistent with respect to the intra- and inter-observer variabilities for the assessment of human OA cartilage [3, 6, 16, 17]. Furthermore, this system has rarely been validated for the evaluation of OA cartilage changes in experimental OA, and has been considered to reflect the early phases of OA poorly. Consequently, the OARSI working group proposed a new histopathological assessment method in 2006 [2], which is based on OA lesion depth (6 grades) and OA extent over joint surfaces (4 stages). On the other hand, MMS evaluates grade, but not the extent of OA. Because semiquantitative OARSI scores are calculated by multiplying grade and extent, the OARSI scoring system has a wider range of scores (0–12 of 24 points) for early or mild OA than the MMS system. To date, only one study has evaluated the reliability and reproducibility of the OARSI scoring system using goat OA knee cartilage [3], and it was reported that both MMS and OARSI scoring systems have acceptable reproducibility and variability, and that they are well correlated. However, it was pointed out that the staging component is difficult to determine because the surface extents of OA lesions are not easily estimated in the OARSI scoring system [2].

When we compared these two histological scoring systems, we also found that semiquantitative MMS and OARSI scores were significantly correlated. In addition, OARSI scores as well as MMS scores were significantly positively associated with exercise duration. However, in our rodent model of early OA, the OARSI system was

unexpectedly less sensitive than MMS to reflect the progression of cartilage change. This may have been due to the absence of severe change in OA grades; most exercised rats had grade 1 lesion showing cellular changes (7/8 rats in femoral cartilage and 5/8 rats in tibial cartilage) even at the final 10-week time point. MMS classifies degree of cellular abnormality into 4 grades and matrix depletion into 5 grades, whereas the OARSI system assigns 1 grade to any cellular change and stratifies matrix staining into 3 grades. Therefore, the OARSI system might be insensitive to early OA cartilage lesion progression over a relatively short period in the rodent model.

Our results should be interpreted with caution because of the small sample size and lack of validation data for MMS in rodents OA models. Further study is required to validate the MMS and OARSI scoring system in rodent OA models.

In conclusion, our treadmill-running exercise-induced OA model could be used to study the pathogenesis of early OA, because it induced histopathological and histochemical findings compatible with early OA. Furthermore, the MMS was found to be more useful than OARSI scoring system for evaluating early histological OA changes in our rodent OA model.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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