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Establishment of a reliable and reproducible murine osteoarthritis model

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SUMMARY

Objective: Many osteoarthritis (OA) models have been developed in mice to understand OA progression and evaluate new OA therapies. However, the individual variation of the joint lesions remains a critical problem in most of the current OA models. We established an OA model in C57BL/6 mice that is more reproducible and amenable to therapeutic intervention by controlling their movement.
Design: OA was induced in 9-week-old C57BL/6 mice by destabilizing the medial meniscus. The mice were then raised in the standard cage for free movement or in a confined cage customized to restrict movement. Mice in the confined cage were subjected to no exercise or exercise of 400, 800, and 1200 m/day.
Results: OA lesions of mice in the confined cage were more severe in the exercise group and showed much less variation. However, the patterns of OA lesions over time were quite different depending on the amount of daily exercise; the patterns increased linearly until 8 weeks in 400 m/day exercise group, but showed plateauing after 4 weeks in 800 m/day and 1200 m/day groups. The validity of our novel OA model with movement control was proven by successfully discriminating the therapeutic effect of hyaluronic acid (HA) in histological scores, while the OA model using standard caging showed a statistically insignificant difference.
Conclusion: The mouse OA model using the confine cage and enforced periodic exercise of mice is more reproducible and reliable than standard caging methods.

Introduction

Osteoarthritis (OA) is a degenerative joint disorder that is prevalent in many people over 60-years of age and often causes severe physical disability[1]. Symptoms of OA are mainly characterized by cartilage degradation, subchondral bone sclerosis, and osteophyte formation. However, its pathogenesis highly depends on other, often unknown, biomechanical, immunological or genetic problems. OA development is difficult to study in humans. In addition, the time course of OA onset is slow and its progression is highly subject to environmental factors such as hormonal status, exercise, occupation, lifestyle, and body mass index[2]. Therefore, the exact cause and mechanism of OA progression is largely unknown, and there are no efficient treatments to inhibit OA progression and/or repair injured cartilages.

Animal models of OA are an important surrogate that provide a means to study OA pathophysiology, and development of therapeutic agents and biomarkers for diagnosing and treating OA[3]. While valuable information on OA pathogenesis and treatments has been gained using animal models of OA, the lack of good OA models also might be a cause of our poor understanding of OA

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pathogenesis. Many different methods have been used to produce OA models in a variety of animal species, and the pros and cons of these models have been reviewed[4,5].

Glasson *et al.* developed a mouse OA model using surgically induced Destabilization of the Medial Meniscus (DMM). The DMM model was more reliable than other protocols, such as the anterior cruciate ligament transection (ACLT) model of OA. The murine ACLT model has been presumed to be too severe to represent human OA, which has complicated the evaluation of OA regression and cartilage repair to test many disease modifying OA drugs. In contrast, the DMM model provided high reproducibility and a slow progression of OA[6]. It is the preferred method of choice for evaluating genetically modified mouse OA models[7,8]. It is also widely used for target validation and efficacy evaluation of many OA therapies based on specific genes and molecular mechanisms[4]. However, the DMM model of OA has some individual variations that cannot be controlled under the current protocol.

It is difficult to develop an ideal animal model that perfectly reproduces the symptoms of OA. But the challenge is worth confronting. A reproducible animal model will offer the opportunity to understand OA pathophysiology as well as aid in the development of therapeutic agents[9] and biological markers for diagnosing the disease and in prognosis. More reliable and stable OA models are necessary to precisely evaluate the effect of candidate drugs and successfully develop OA therapies. In this study, we focused on how to reduce variation of OA phenotypes and increase reproducibility of the mouse DMM model.

Influential factors for the development of OA in humans include age, obesity, repetitive physical activity, and joint injury. Some of these factors are commonly fixed in animal models of OA; these include strain, genotype, sex, age, weight, food, and supply company. Sex hormones play a critical role in the progression of OA in the murine DMM surgical model, where males showed more severe OA than females[10]. However, each approach has limitations that make it difficult to evaluate the physiological relevance of the experimental finding. This study examined the role of standardized joint loading in cartilage destruction and compared the findings to the effects of physiological loading in the OA animal model.

Physical activity of experimental animals is often not regulated in many studies. Running exercise affects cartilage metabolism of beagle dogs and modifies the articular cartilage structure through a mechano-adaptive homeostatic response[11,12]. Mechanical loading affects OA progression in the human knee joint[13–15]. Previous efforts have addressed the effect of running exercise on OA progression in mice. In transgenic Del1 mice, running increased the incidence and severity of degenerative changes in the articular cartilage of knee joints within 15 months[16]. Forced mobilization of rats after ACLT and partial medial meniscectomy led to an increase in OA development[17]. These studies did not use the well-established DMM model and did not control the free movement of mice in the housing cage.

We hypothesized that restriction of mice movement in the housing cage after DMM surgery would reduce individual variation of cartilage degradation and increase the reproducibility of the OA model. In addition, the combination of regular exercise was expected to produce a reliable and reproducible mice OA model showing a linear OA progression with little variation over time.

Material and methods

Animal care

The animal study was approved by the Animal Care and Use Committee of Ajou University, School of Medicine. Male C57BL/6 mice (Orient-bio, Seongnam, Korea) 9-weeks-old and 22–24 g in weight were raised in a conventional standard cage or a confined cage under 55–65% humidity and controlled temperature of 24 ± 3°C[18]. Three to five mice were raised in a standard cage (260L × 200W × 130H mm^3) and were allowed free movement and free access to food and water. The confined cage (75L × 40W × 200H mm^3) was custom-made to house only one mouse per cage and restrict its movement (Fig. 1). Mice in the confined cage had free access to water and feed. Body weight was recorded at regular intervals (Fig. 2).

Surgical construction of OA

Mice were anesthetized with the mixture of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil, 50 mg/kg, Virbac Laboratories, France) and xylazine (Rompun, 10 mg/kg, Bayer, Korea). OA was induced by dissecting the medial meniscus ligament to destabilize the medial meniscus in the right knee joint of hind limb as described previously in the DMM model[6]. Mice in the sham group were surgically treated in the same manner except for not dissecting the medial meniscus ligament. After surgery, mice randomly assigned in either the standard or confined cage group.

Exercise model of OA mice

Mice in the confined cage were assigned randomly into four groups comprising of a control and three exercise groups. Mice in the control group were restricted to move only within the cage without additional exercise. Mice in the exercise groups were subjected to treadmill running once a day for 6 days a week with varying distances on a motorized multi-track treadmill (JD-A-09, JEUNGDO, Seoul, Korea). The exercise program consisted of a warming phase (10 m/min) for 10 min and a running phase (15 m/min) with various time periods for each of groups as indicated in Table I. Three exercise groups were subjected to the running distance of either 400, 800, or 1200 m/day. Mild electric shock was applied at the starting position of each lane to discourage mice from dropping out of the lane. The electric shock grid was set to deliver 0.2 mA, 400 V and 1 Hz which caused an uncomfortable shock but did not physically harm or injure the animals[19].

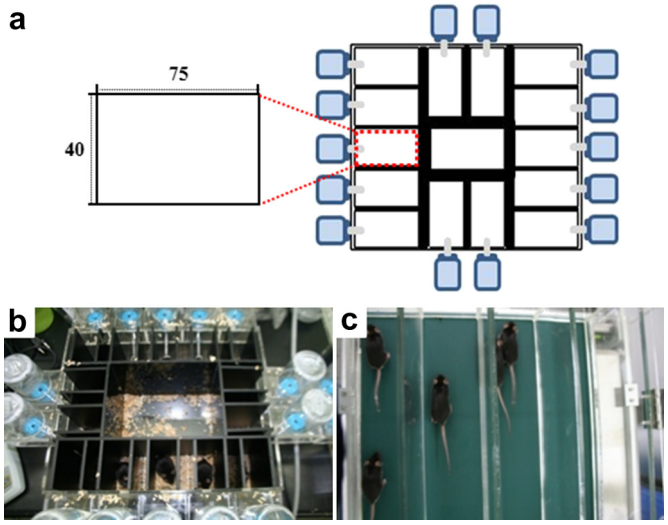

Fig. 1. (a) Schematic drawing of the cages of the confined cage group. The area was (75L × 40W mm^2) for each mouse. (b) Photographic image of the confined cage, (c) treadmill was designed to encourage exercise accordingly.

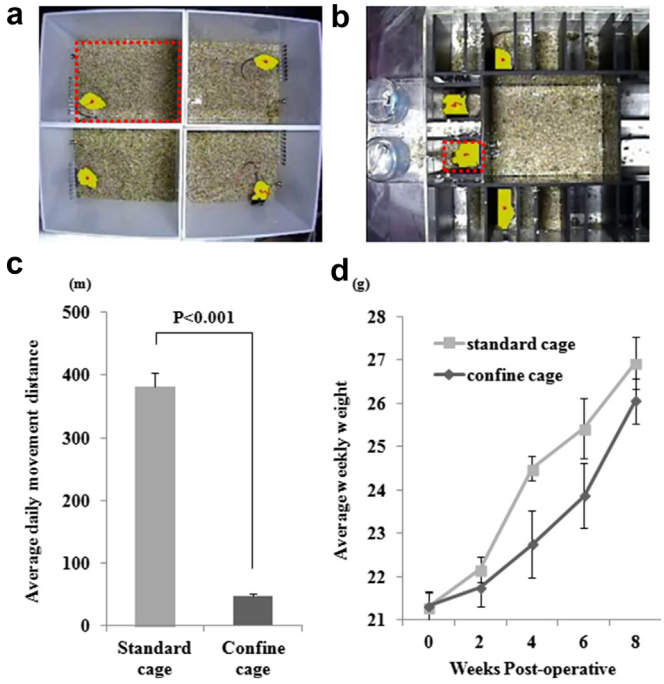

Fig. 2. (a) C57BL/6 mice visualized by Ethovision® XT in the standard cage. The size of red square is 198L × 258W mm^2 per mouse, (b) C57BL/6 mice visualized by Ethovision® XT in the confine cage. The size of red square is 38L × 73W mm^2 per mouse, (c) daily distance of C57BL/6 movement in the confine cage ($n = 4$) compared to the standard cage ($n = 4$) by Ethovision® XT (mean and 95% CIs, $P < 0.001$), (d) weight changes of C57BL/6 in the confine cage ($n = 4$) and standard cage ($n = 4$) (mean and 95% CIs).

Measurement of movement

The movement pattern and distance of mice in the standard and confined cages was measured for 3 days without additional exercise using an Ethovision® XT device (NOLDUS Information Technology, Leesburg, VA, USA). The standard cage customized without an upper lid was used for the experiment. The movement of mice was captured by a CCD camera and video images were analyzed to track the movement patterns and distances using Ethovision tracking software (NOLDUS Information Technology, Leesburg, VA, USA) as described previously[20]. The daily distance of mice movement was presented by mean values with 95% confidence intervals (CIs) obtained from four individual mice during 3 days.

Histology

Mice were euthanized at 2, 4, and 8 weeks after surgery. The right knee joints of mice were separated and fixed in 4% paraformaldehyde for 24 h. Whole joint tissue was decalcified in 5% nitric acid for 10 h at room temperature. The tissue cassette was placed in formalin, dehydrated in a graded series of ethanol and xylene, and embedded in paraffin. Tissue blocks were cut in coronal sections of 4 μm thickness at 100 μm intervals. The sections were stained with hematoxylin and eosin to observe tissue histology and Safranin-O/fast green to examine the amount of sulfated glycosaminoglycans (GAGs)[21]. Coronal sections, from patella to posterior condyles, resulted in seven sections. The Safranin-O/fast green staining images were used to evaluate OA status of samples by an OA scoring system[6]. Scoring was done by two independent researchers blinded to any other information and results were averaged. The maximum score out of the seven sections was taken for the overall score of the knee joint. Histological scoring was performed on the medial tibial plateaus (MTPs) of Safranin-O/fast green staining images, where the OA lesion was commonly addressed in previous studies[22,23].

Hyaluronic acid (HA) injection

HA (LG Life Science, Daejeon, Korea) was injected directly into the synovial cavity at 0, 2, 4, and 6 weeks after OA induction. Ten microliters of 10 mg/ml HA with a molecular weight of 3000 kDa (HA3000) was injected using a 0.5 ml syringe with a 31-gauge needle. The effect of HA was evaluated between the free movement group in the standard cage and the exercise group of 400 m/day in the confined cage. The control group was injected with 10 μl saline.

Statistical analysis

Statistical analysis was performed using the two-tailed unpaired *t* test while analysis for multiple samples was carried out by the one-way analysis of variance (ANOVA) with the Tukey's *post hoc* test using SPSS 12.0.1 (SPSS, Inc.). For the analysis of weight changes depending on the cage sizes, a two-factor repeated-measures ANOVA was used with one factor as the cage size and the other the repeated factor of time. *P* values less than 0.05 were regarded as statistically significant.

Results

Confined cage significantly reduces mouse movement

The distance of mouse movement for 3 days was compared between the standard and confined cages using an Ethovision® XT device. The daily movement distance was significantly longer in the standard cage (381.4 ± 45.6 m/day, $n = 4$) than in the confined cage (46.9 ± 7.5 m/day, $n = 4$) [$P < 0.001$; Fig. 2(c)]. These results suggest that restriction of movement in the confined cage effectively reduced total movement. Body weight of mice in both cage types ($n = 4$, each group) increased gradually along with time and showed no significant difference between two groups [Fig. 2(d)]. Test of sphericity was met with a $P = 0.724$. Weight gain according to time resulted in a *P* value of <0.001. The comparison between the groups showed a $P = 0.437$.

Fewer but more severe OA lesions in mice in the confined cage

The effect of the movement restriction on the OA progress was examined by comparing the histopathology of OA cartilages from mice maintained in the standard cage ($n = 5$, each week) and confined cage ($n = 5$, each week) at 2, 4, and 8 weeks after OA surgery. In histological sections stained with Safranin-O, structural degeneration of the cartilage was observed predominately in the medial tibial condyles and was more severe in mice raised in the standard cage than in the confined cage. At 8 weeks, significant proteoglycan decrease and diffuse hypercellularity were evident in OA cartilages of mice in the standard cage [Fig. 3(a)]. Semi-quantitative OA scores also increased more sharply and showed larger 95% CIs in mice of the standard cage (95% CIs = 1.74–5.26) than those in the confined cage (95% CIs = 0.89–2.28) at 8 weeks [$P = 0.0157$; Fig. 3(b)]. Mice in the confined cage showed only slight increase of OA scores with little variation with time.

Combination of movement restriction and regular exercise produces stable and linear induction of OA lesions in DMM mice

The results so far supported the view that the addition of regular exercise to movement restricted mice in the confined cages could

Table I
Exercise scheme for OA induced mice in the confine cage

Group	Exercise time	
	Warming (10 m/min)	Running (15 m/min)
0 m/day	0 min	0 min
400 m/day	10 min	20 min
800 m/day	10 min	47 min
1200 m/day	10 min	73 min

accelerate OA progression in a controllable manner depending on the amount of exercise. To test this, OA mice in the confined cage were subjected to treadmill running of 400, 800, and 1200 m/day to control the amount of daily exercise ($n = 15$, each group). As expected, the OA lesion in the exercise groups was more severe than that of the control group and their OA scores increased along with time showing statistically significant differences (Fig. 4). At 4 weeks, the 800 m/day group showed significantly worse OA lesions compared to no exercise group ($P = 0.0122$). At 8 weeks, the standard cage group, the 400 m/day group, and the 800 m/day group each ($P = 0.0018$) showed significantly worse OA lesions compared to no exercise group ($P = 0.0018$). The values of the OA scores were similar overall but were much less variable than those of the standard cages group shown in Fig. 3 by showing statistically significant differences in the standard deviation (SD) particularly at 2 ($P = 0.001$) and 8 weeks ($P = 0.019$) (Table II). Among the different exercise groups, the mice with 400 m/day of exercise displayed OA lesions that increased almost linearly with the analysis time. The OA scores of mice exercised 800 m/day increased rapidly until 4 weeks and were somewhat delayed thereafter. OA scores of mice with 1200 m/day of exercise almost plateaued after 4 weeks. The results suggest that the amount of daily exercise affects OA progression of DMM mice in the confined cage and daily exercise of 400 m/day was optimal to induce linear and stable increase of OA lesion over the time period of the study. Data is presented in histograms as mean and 95% CIs from five individual samples.

Novel OA model with the movement control better represents the therapeutic effect of HA

To assess the validity of the novel OA model with the movement control, the therapeutic efficacy of intra-articular injection of HA was compared by histological observation between mice in the confined cage with 400 m/day of exercise (HA group $n = 18$, saline group $n = 18$) and mice in the standard cage with free movement (HA group $n = 18$, saline group $n = 18$) (Fig. 5). In the standard cage group, HA injection reduced the increase of OA scores but showed no statistical significance due to large deviations. In the experimental group with the movement restriction and regular exercise, HA injection reduced significantly and reliably the OA scores at 2, 4 and 8 weeks with statistical significance ($P = 0.024$, $P = 0.004$, $P < 0.001$), likely due to the stable induction of OA itself.

Discussion

Development of a reliable OA model is very important in the study of OA pathophysiology and evaluation of disease modifying OA drugs efficacy. In this study, we have established a highly reliable and reproducible mice OA model by fine controlling their movement after DMM surgery. The restriction of everyday movement of mice in a confined cage significantly reduced individual variation and increased reproducibility of OA progression. Daily exercise of 400 m/day in a treadmill ensured linear increase of OA phenotypes over time. The usefulness of the novel OA model was validated by successfully discriminating the therapeutic efficacy of HA with a clear statistical significance. To our knowledge, this is the first report that adopted the confined cage and optimized the amount of daily exercise of mice in combination for producing a more reliable mice OA model than the standard DMM model.

The effects of exercise on cartilage damage have been studied previously in some animal OA models[17,24,25]. In animal OA models, the amount and pattern of animal movement affects overall severity and time frame of OA development. Our results are consistent with the report by Appleton *et al.* that forced mobilization of Sprague Dawley rats after ACLT surgery accelerated OA pathogenesis until 20 weeks[17]. Their result showed, however, a significant increase of OA pathology as early as 2 weeks after surgery in comparison with exercise restricted control, which was probably due to using a more severe OA induction method compared to DMM. We suspect that this previous model may not represent early stages of OA progression and may not be used suitable for evaluating disease modifying OA drugs. Besides, the rotating cylinder used in their study is not a good tool to quantitatively optimize the amount of exercise nor did they provide the result. Lapveteläinen *et al.* reported a lifelong voluntary joint loading increased OA pathology in Del1 mice bearing a mutation in

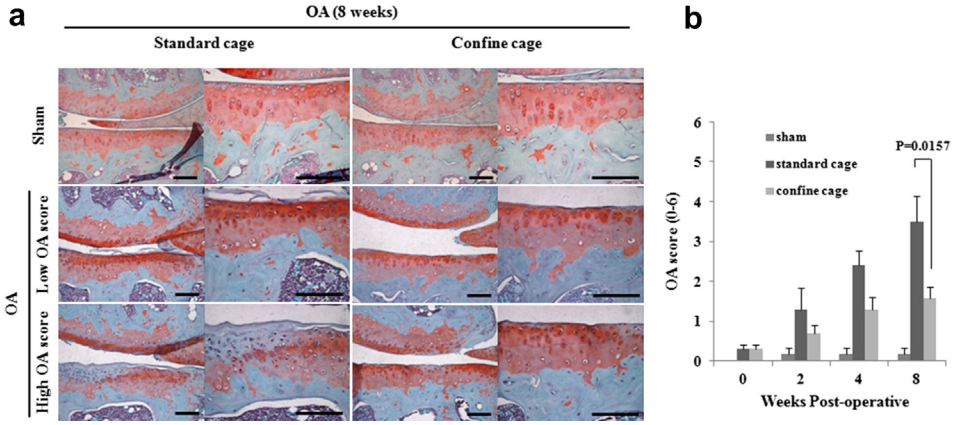

Fig. 3. Histological analysis of OA legions of C57BL/6 mice in the confined cage and standard cage. Histological sections of the medial femoral condyle (MFC) and the MTP were obtained and stained with Safranin-O at 2, 4, and 8 weeks after OA surgery. Mice with sham surgery were used as a control ($n = 3$, each week). (a) Representative images from each group with the highest and lowest OA scores were presented from samples of 8 weeks. Scale bar = 100 μm. (b) Average OA scores in the standard cages ($n = 5$, each week) and confined cages ($n = 5$, each week) are presented as mean and 95% CIs, $P = 0.0157$.

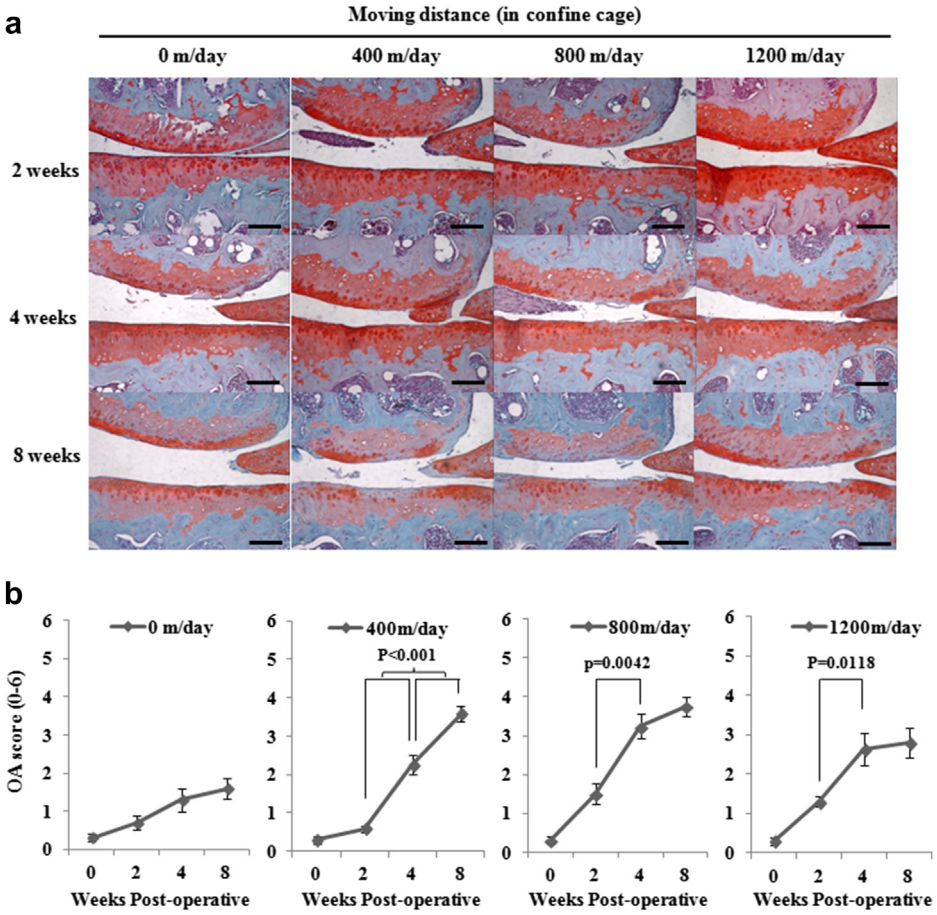

Fig. 4. Effect of increasing the amount of treadmill exercise on the induction of OA in mice maintained in confined cage. (a) C57Bl/6 mice in the confined cage were subjected to treadmill exercise of 0 ($n = 5$, each week), 400 ($n = 5$, each week), 800 ($n = 5$, each week), and 1200 m/day ($n = 5$, each week) at 2, 4, and 8 weeks after OA surgery. Histological sections of MTP at 2, 4, and 8 weeks after OA surgery were presented after Safranin-O staining. Scale bar = 100 μm. (b) In the graphs, OA scores of samples are presented as mean and 95% CIs.

type II procollagen[16]. However, that study involved a genetic OA model and allowed voluntary exercise of mice, which made the model unpredictable and uncontrollable. In an another study published recently, the adjustable treadmill exercise was used to induce OA in mice for 10 weeks[26]. This model can be used for producing early stage OA and needs long period of time to obtain moderate or late stage OA. It must also suffer from individual variations. The key strength of our model compared to previous OA models is that the severity and variation of OA can be controlled by applying different loads of exercise with the merits of DMM model still retained. The confined cage reduces the individual variation and severity of OA lesions as well, while the regular exercise on the treadmill increases the OA severity depending on the amount of exercise without sacrificing the reduced variation. We also found that regular exercise of 400 m/day in standard cage showed OA severity and variation almost similarly to those with no exercise at 2 and 4 weeks, which confirm again that movement restriction in the confined cage is essential in reducing individual variation and controlling OA severity. With regard to the amount of exercise and resultant severity of OA, our data shows a plateauing effect of OA severity over the increase in the amount of exercise. Exercise of 400 m/day resulted in moderate and linear induction of OA until 8 weeks. In contrast, exercise of 800 m/day and 1200 m/day showed rapid onset and linear increase of OA until 4 weeks, but it did not further aggravate OA and plateaued thereafter. Similar results were obtained with moderate exercise in a couple of previous reports that showed increase in collagen II amount with no induction of intra-articular MMP expression[24,27]. Other possible mechanisms for the plateauing effect include resolution of joint inflammation, protective effect of late stage joint fibrosis, and reductions in body weight with 8 weeks of exercise treatment. We think that these changes in pathophysiology could be also associated with changes in mechanical loading. The protective effect of a large amount of exercise could have resulted from a reduction in joint inflammation. Joint fibrosis, often apparent on end stage OA, is also known to provide stabilizing effects on the joint. It is not clear, however, if the joint fibrosis has really occurred at 4 weeks in the 800 and 1200 m/day groups. As for the body weight, there was no significant change in the constrained group. In spite of these unexpected effects of severe exercise, our results demonstrate an ideal amount of mechanical loading can induce linear progression of OA, thereby providing a consistent OA model.

Several limitations exist in our study. First, when examined at 8 weeks, the standard cage group showed low levels of individual variation, being quite a similar to those of the confined cage groups [Supplementary Fig. 1(a and b)]. Besides, its OA lesion was not significantly developed and similar to that of the non-exercise group in the confined cage. The cause of this difference in the lateral cartilages is not clear but possibly attributes to the low level of OA induction by itself. The femoral and tibial cartilages showed similar pattern of data, and the activity of the regular exercise in the confined cage was still observed in all four quadrants of the

Table II
Means and SDs, their *P* values, CV, and 95% CIs of histologic scores between the standard cage (free moving) and confined cage with 400 m/day exercise groups and between the confined cage with 0 and 400 m/day exercise groups after OA surgery. The diversity of variance was determined by an *F*-test. The statistical significance of differences between means was verified in two sample *t* tests

OA induced	Group		Mean	Mean *P* value	SD	SD *P* value	CV	95% CIs
2 weeks	S.C	A. free moving ($n = 5$)	1.3	A vs B = 0.327	1.2	A vs B = 0.041	0.92	−0.19 to 2.8
	C.C	B. 0 m/day ($n = 5$)	0.7	B vs C = 0.579	0.44	B vs C = 0.058	0.57	0.14–1.26
		C. 400 m/day ($n = 5$)	0.6	C vs A = 0.181	0.20	C vs A = 0.001	0.33	0.37–0.8
4 weeks	S.C	A. free moving ($n = 5$)	2.4	A vs B = 0.049	0.82	A vs B = 0.352	0.34	1.38–3.42
	C.C	B. 0 m/day ($n = 5$)	1.3	B vs C = 0.051	0.67	B vs C = 0.328	0.52	0.47–2.13
		C. 400 m/day ($n = 5$)	2.25	C vs A = 0.759	0.50	C vs A = 0.220	0.22	1.46–3.05
8 weeks	S.C	A. free moving ($n = 5$)	3.5	A vs B = 0.015	1.41	A vs B = 0.064	0.40	1.74–5.26
	C.C	B. 0 m/day ($n = 5$)	1.6	B vs C = 0.0001	0.66	B vs C = 0.262	0.41	0.88–2.28
		C. 400 m/day ($n = 5$)	3.6	C vs A = 0.894	0.49	C vs A = 0.019	0.13	3.06–4.09

S.C = standard cage, C.C = confined cage.

cartilage. Again, the osteophyte formation showed no significant individual variation but was suppressed in the confined cage at 8 weeks [Supplementary Fig. 1(c)]. Therefore the effect on the confined cage on the individual variation of OA lesion might vary depending on the target tissues and assays examined, while that of the regular exercise was consistently observed. Second, the confined cage floor area may affect the health and behavior of the animals. As Fullwood *et al.* have reported[28], confined cage floor area can significantly alter immune function and increase stress hormone concentrations in the animals even without significant change in body weight. Stress related hormones may also have an impact in the development of OA[29,30]. Plasma corticosterone concentrations were influenced by space allowances. Mice given smaller spaces (5 in^2 [32 cm^2] per mouse) had elevated corticosterone concentrations[28]. However, we did not have any animals showing abnormal, stress related behavior reported previously[31,32] such as weight reduction (>10% of total body weight), overgrooming, nibbling, eating (trichophagia), plucking, pulling, dewhiskering and death. According to earlier studies, we can expect an increase of stress hormones in the confined cage group. While our results show that use of the confined cage can reduce variability and improve reproducibility of the OA model, the influence of stress hormones should also be considered when applying our model in other studies.

Reduced cage floor area also carries an ethical problem. Yet we thought our benefits may outweigh the ethical risks. By reducing the variability in the DMM model, researchers may reduce the total number of animals. Our results also show the effects of controlled exercise, which cannot be obtained from standard caged animals. Running the same exercise protocol on standard caged animals does not reduce the variability in DMM models (Supplementary Fig. 2). Our results suggest a more standardized way to produce OA model animals, which complies with the ARRIVE guidelines[5,33].

In conclusion, that restriction of mice movement and combination of controlled exercise after DMM surgery reduce individual variation of cartilage degradation and increase the reproducibility of the OA model. These results provide valuable insight for the researcher in producing more reliable OA models for the use in the evaluation of OA pathophysiology and testing disease modifying OA drugs.

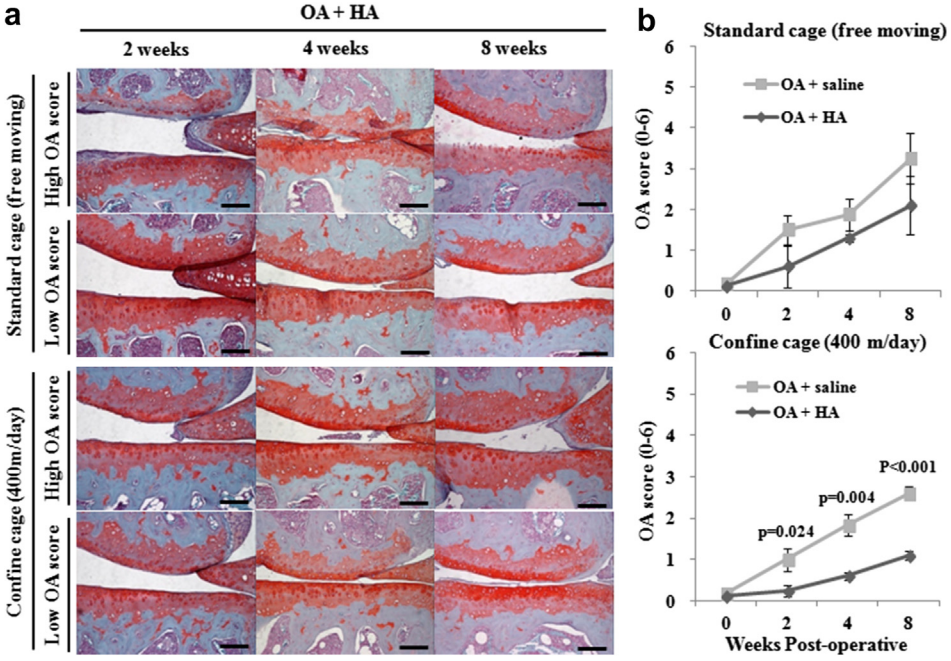

Fig. 5. Effect of intra-articular HA injection on the OA lesions of C57/Bl6 mice depending on the movement condition. (a) Mice were subjected to OA surgery and maintained either in the standard cage with free movement ($n = 36$) or confined cage with an exercise of 400 m/day ($n = 36$). Saline or HA (10 mg/ml) was injected into the synovial cavity of OA knee joints at 0, 2, 4, and 6 weeks after OA surgery. Histological sections of cartilages were obtained at 2, 4, and 8 weeks and stained with Safranin-O. Representative images of the MFC and the MTP showing the highest and lowest OA scores were presented. Scale bar = 100 μm. (b) The graphs show OA scores of samples presented as mean and 95% CIs.

Contributions

Byoung Ju Kim: Conception and design, Collection and assembly of data, Analysis and interpretation of the data, Drafting of the article, Statistical expertise.

Dae-Won Kim: Critical revision of the article for important intellectual content, technical or logistic support.

Se Hoon Kim: Analysis assistant and interpretation of the data, technical support.

Jae Ho Cho: Analysis and interpretation of the data, Provision of study materials or patients.

Hyun Jung Lee: Critical revision of the article for important intellectual content, logistic support.

Do Young Park: Critical revision of the article for important intellectual content, logistic support.

So Ra Park: Conception and design, Critical revision of the article for important intellectual content, Technical or logistic support.

Byung Hyune Choi: Administrative, Scientific, technical and logistic support, Interpretation of data, Critical revision of the article for important intellectual content, Statistical expertise.

Byoung-Hyun Min: Conception and design, Administrative, Technical, and logistic support, Critical revision of the article for important intellectual content, Final approval of the article, Provision of study materials or patients, Obtaining of funding.

Disclosure of potential conflicts of interest

The authors indicate no potential conflicts of interest.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.09.012.

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