the equilibrium between the catabolic and anabolic activities results in catastrophic damage to the articular cartilage, ultimately inducing the pathological condition known as OA.

Prostanoids, including prostaglandin (PG) D2, PGE1, PGE2, PGF2α, prostacyclin (PGI2), and thromboxane A2, are lipid mediators produced in a sequence of cyclooxygenase (COX) -1, -2-catalyzed reactions [8]. The role of PGE2 in the development of OA is controversial. Some reports point to an important role in inflammation [9]. Pro-inflammatory signaling mediators such as IL-1 and TNF-α induce the synthesis of PGE2 by promoting the expression or activities of COX-2 and microsomal PGE synthase-1 [10]. PGE2 then promotes IL-1 expression as part of a positive feedback mechanism, degrades the cartilage ECM [4,10-13], and finally induces apoptosis of chondrocytes [3]. Other reports insist that PGE2 opposes the effect of IL-1 [14] and stimulates the gene expression of type II collagen [3,15]. In addition, PGE2 stimulates the synthesis of proteoglycan and collagen through the expression of an IGF-1-binding protein [16,17]. PGE2 works through four isoforms of the EP receptor, EP1 to EP4. Previously, we considered that the controversy could result from differences in the mode of action and tissue distribution of each receptor [18]. Using an EP2 selective agonist, we showed that EP2 receptor-mediated PGE2 signaling enhances the growth of chondrocytes [18,19] and promotes the regeneration of articular cartilage in rabbits with cartilage defects [19].

In the current study, we investigate the effect of an EP2 agonist on articular cartilage in a rabbit model of traumatic degeneration.

Materials and methods

Materials

Microspheres loaded with a selective EP2 agonist, ONO-8815Ly (lysine salt) [20], were prepared by the emulsion-solvent evaporation method [19,21]. Briefly, ONO-8815Ly and polylactic-co-glycolic acid (PLGA) were mixed to form a water/oil emulsion, and added to the outer water phase containing polyvinyl alcohol under stirring with a turbine-shaped mixer at 5000 rpm to obtain a water/oil/water emulsion. PLGA microspheres that did not contain ONO-8815Ly in its free form were recovered by centrifugation and lyophilized to remove residual organic solvent and water. Then, a gelatin aqueous solution (20%, w/w) was poured into the microsphere suspension to form a gel. For the crosslink reaction, a glutaraldehyde aqueous solution (12.5 mg/ml) was poured into the microsphere suspension. Small cylinder-shaped gelatin hydrogels (4 mm in diameter and 2 mm in thickness) containing ONO-8815Ly (0, 80, or 400 μg of ONO-8815/gel) were obtained by hollowing out the gelatin hydrogel sheet. Diffusion kinetics analyses showed that ONO-8815Ly is gradually released from the microsphere over a period of seven days *in vitro* (Figure 1).

Animal model for traumatic degeneration

Four-month-old female Japanese white rabbits (weighing approximately 3 kg) were used. Traumatic degeneration was induced as described for the anterior cruciate ligament and menisectomy transection (ACLMT) model [22]. Operations were performed under general anesthesia, and a skin incision was made on the medial side of the patella. Soft tissues and articular capsules were cut to expose the knee joints. The anterior cruciate ligament was transected at the attachment to the tibia in the knee-flexed position, and the anterior horn of the medial meniscus was resected. The articular capsule and skin were sutured in layers with 4-0 nylon sutures. After the operation, rabbits were allowed to move freely. Preliminary experiments revealed that osteoarthritic changes were observed in this model at as early as two weeks after operation (data not shown).

Treatments with the EP2-agonist

A total of 64 animals were randomly assigned to five groups: G-S (sham operation), G-C (no further treatment), G-0, G-80, and G-400 (single intra-articular administration of gelatin hydrogel containing 0, 80, and 400 μg of ONO-8815Ly, respectively). Sham-operated rabbits (G-S; n = 4) received no further treatment, and were sacrificed either 2 (n = 2) or 12 weeks (n = 2) after the operation.

The ACLMT surgery was performed on both the knees of each of the remaining 60 rabbits to avoid any unequal bearing of weight due to pain on one side. No further treatment was performed in animals of the

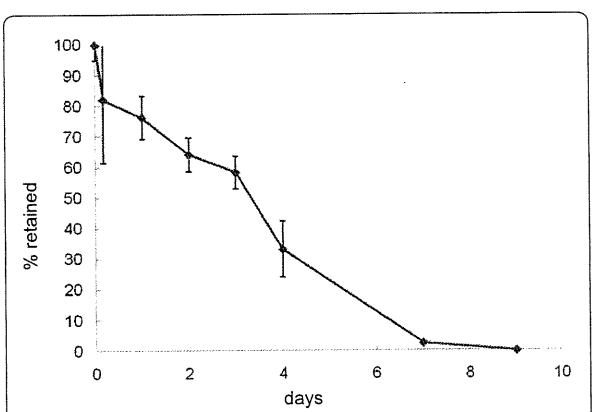

Figure 1 The diffusion kinetics of ONO-8815Ly from microspheres *in vitro*. Microspheres loading ONO-8815Ly were soaked in PBS, and the amount of retained ONO-8815Ly at each time point was measured by high-performance liquid chromatography and calculated as the ratio to the initial amount (n = 5).

control group (G-C; n = 12). In the treatment groups, no further treatment was performed on the right knee, but a gelatin hydrogel cylinder containing ONO-8815Ly (G-0, G-80, and G-400; n = 16 per group) was placed on the fatty pad of the left knee at the time of operation. Rabbits were sacrificed two weeks (G-C, n = 6; G-0, G-80, and G-400, n = 10 per group) or 12 weeks (n = 6 per group) after the operation. All the experiments with animals were approved by the institutional animal research committee, and performed according to the Guidelines for Animal Experiments of Kyoto University.

Histological examination

Rabbits were sacrificed 2 or 12 weeks after surgery, and the distal femur and proximal tibia of the left side of each animal were resected, fixed at 4°C overnight in a 10% formalin solution, and decalcified in formic acid for three days. After neutralization by 10% sodium sulfate for 24 hours, the samples were embedded in paraffin. Serial sections were prepared in the coronal plane through the middle of the femoral and tibia condyles, and one section from each sample was used for each of the histological analyses. In every section, the entire cartilage portion in full depth was evaluated. The specimens were stained with safranin O/Fast Green or H&E using standard procedures. The histological grade of cartilage degeneration was evaluated using the modified Mankin's scoring system [23], which was adopted as the original system [24] for the evaluation of the rabbit model. All the results shown herein represent the combined scoring data of two researchers.

Immunohistochemical analyses

Immunohistochemical examination was performed as follows. In brief, after deparaffinization, sections were incubated with 0.3% hydrogen peroxide for 30 minutes. Then, sections were treated with proteinase K for two minutes (proliferating cell nuclear antigen [PCNA] staining) or with hyaluronidase for 60 minutes (MMP staining), after which they were incubated with the following primary antibodies: mouse anti-human PCNA monoclonal antibody (1:100; Dako, Glostrup, Denmark), mouse anti-human MMP-13 monoclonal antibody (1:20; AnaSpec Inc., San Jose, CA, USA), or mouse anti-rabbit MMP-3 monoclonal antibody (1:50; Daiichi Fine Chemical Co. Toyama, Japan). All antibody dilutions were made in PBS. After an overnight reaction with the primary antibody at 4°C, sections were incubated with horseradish peroxidase-conjugated anti- mouse IgG (Vector Laboratories, Southfield, MI, USA) at room temperature for 30 minutes. Signals were visualized with 3, 3'-diaminobenzidine tetrahydrochloride, and nuclei were counterstained with hematoxylin. The percentage of PCNA-, MMP-13-, and MMP-3-positive cells in the cartilage was calculated by methods similar to those described above. Results of histological and immunohistochemical analyses were evaluated by two observers who were blinded to the identity of each sample.

Primary chondrocyte cultures

Primary culture of chondrocytes was performed using articular cartilage tissues harvested from non-treated rabbits (NRC cells) or ACLMT-operated rabbits (ORC cells). Briefly, thinly sliced cartilage tissues were incubated with collagenase (4 mg/ml; Sigma Aldrich, St. Louis, MO, USA) in DMEM for 12 hours. Cells were then collected by centrifugation, seeded into type I collagen-coated dish (Corning International K.K.,Tokyo, Japan), and cultured with DMEM containing 10% FBS supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2/95% air. Chondrocytes were grown in monolayer cultures, and were passaged when reaching confluence. Cells at the second passage were used for the assay. ONO-AE1-259-01, a selective agonist of EP2, was used to stimulate EP2 signaling in the presence or absence of IL-1β (Sigma Aldrich, St. Louis, MO, USA).

Real-time PCR

Total RNA was extracted from cultured cells using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. All reverse transcription reactions were performed with an RT-PCR kit using 1 μg of total RNA with a Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) for conversion into cDNA. The mRNA expression levels of *MMP-13* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were quantified by real-time PCR using SYBR Green (Applied Biosystems, Foster City, CA, USA) and the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All reactions were run in triplicate, and the amount of PCR product of each gene was calculated using the standard curve method and normalized to *GAPDH* levels, which were used as an internal control. Using the ratio obtained for the untreated sample as a standard (1.0), the relative ratio of the treated samples was presented as the relative expression levels of the *MMP-13* gene. Sequences of primers used in this experiment were as follows: 5'-aggagcatggcgacttctac-3' and 5'-taaaacagctccgcatcaa-3' (*MMP-13*) and 5'-gctctccagaacatcactcctgcc-3' and 5'-cgttgtcataccaggaaatgagct-3' (*GAPDH*).

Statistical analysis

The statistical analyses were performed using the Statcel2 software (The publisher OMS Ltd., Saitama, Japan). The results are shown as the mean ± standard deviation (SD). The Kruskal-Wallis test was performed for screening

purposes, and the Steel-Dwass method for multiple comparisons was used if there was a significant difference between samples. A P value less than 0.05 was considered to be significant.

Results

Therapeutic effect of ONO-8815Ly in the early stages of degeneration

At two weeks after the operation, articular cartilages in medial condyles of G-C (Figure 2a, b) and G-0 (Figure 2a, c) showed severe degenerative findings such as surface irregularity including clefts and reactive changes such as clonal proliferation of chondrocytes. The intensity of safranin O staining was reduced in G-C (Figure 2a, g) and G-0 (Figure 2a, h). The grade of degenerative findings was less prominent in sections of G-S (Figure 2a, a), G-80 (Figure 2a, d) and G-400 (Figure 2a, e) than in those of G-C or G-0. Safranin O staining was stronger in sections of G-80 (Figure 2a, i) and G-400 (Figure 2a, j). Similar findings were observed in sections prepared from lateral femoral condyles. The degenerative changes were less prominent and the safranin O staining was stronger in sections of G-S (Figure 2b a and 2f), G-80 (Figure 2b, d and 2i) and G-400 (Figure 2b, e and 2j) than in those of G-C (Figure 2b, b and 2g) or G-0 (Figure 2b, c and 2h).

Histological grade was evaluated using a modified Mankin's scoring system [23,24]. The grades of medial condyle in each sample were scored and mean values were compared (Figure 2c). Scores were significantly better for G-80 than for G-0. The effect of ONO-8815Ly was more prominent in lateral condyles, and both G-80 and G-400 showed much better scores than G-C or G-0 (Figure 2d).

Similar findings were observed in medial (Figure 3a) and lateral (Figure 3b) condyles of tibiae. The degenerative changes were less prominent and the safranin O staining was stronger in sections of ONO-8815Ly-treated groups (G-80 and G-400) than in those of non-treated groups (G-C and G-0). The effect of ONO-8815Ly was similar between G-0 and G-80 in medial condyles (Figure 3c), whereas G-80 and G-400 showed better values than G-C or G-0 in lateral condyles (Figure 3d). These results suggested that ONO-8815Ly prevents degenerative change in articular cartilages during the early stages.

Therapeutic effect of ONO-8815Ly in the late stages of degeneration

Similar analyses were performed using sections prepared at 12 weeks after surgery. In the case of femoral condyles, no improvements of cartilage degeneration were observed in sections of ONO-8815Ly-treated groups (G-80 or G-400) (Figure 4a, d and 4e) and the staining of safranin O also showed no difference (Figure 4a, i and 4j). Similar results were obtained in lateral condyles of femora (Figure 4b). In agreement, there was no significant difference in Mankin's score in the analyses of medial (Figure 4c) or lateral (Figure 4d) condyles of femora.

Similar results were obtained in the tibiae. Neither medial nor lateral condyles showed better histological features by the treatment with ONO-8815Ly, and the Mankin's score showed no improvements (data not shown).

These results suggested that the effect of ONO-8815Ly failed to last, at least when using this drug delivery system.

Growth promoting effect of ONO-8815Ly

The proliferating activity of chondrocytes was evaluated by PCNA staining (Figure 5). The proportion of PCNA-positive cells in femoral (Figures 5a and 5b) and in tibial (Figures 5c and 5d) condyles at two weeks after operation were similar among all groups, suggesting that the improvement of cartilage degeneration by the EP2 agonist was not due to the acceleration of cell proliferation.

EP2- selective agonist inhibits the expression of MMP-13 in ACLMT

MMP-3 and MMP-13 are major proteases degrading the ECM. The expression of these enzymes was analyzed by immunohistochemistry using samples prepared at two weeks after the operation. For MMP-3, there were no significant differences in staining intensity or number of positive cells between any of the groups (Figure 6). For MMP-13, however, significant differences were observed (Figure 7). The staining of MMP-13 was much stronger in G-C and G-0 (Figure 7a, b and 7c) than in G-S, G-80, or G-400 (Figure 7a, a, d, and 7e). The proportion of MMP-13-positive cells was significantly lower in sections of G-80 and G-400 than in sections of G-C or G-0 (Figure 7b). Similar results were obtained for the intensity (Figure 7a, f, i, and 7j) and the ratio of MMP-13-positive cells (Figure 7c) in the analyses of lateral condyles.

EP2-selective agonist inhibits IL-1β-induced *MMP-13* mRNA expression

To confirm the effect of EP2 agonist on MMP-13 expression, the expression of the *MMP-13* gene by primary cultured chondrocytes was evaluated by quantitative real-time PCR (Figure 8). The expression levels of *MMP-13* were similar in NRC and ORC cells under basal culture conditions. Similarly, EP2 agonist treatment showed no significant effects on *MMP-13* levels on either cells. When NRC and ORC cells were treated with IL-1β (50 pg/ml), the expression levels of *MMP-13* mRNA were significantly increased in both cells. IL-1β-induced expression of MMP-13 mRNA in ORC cells was reduced by

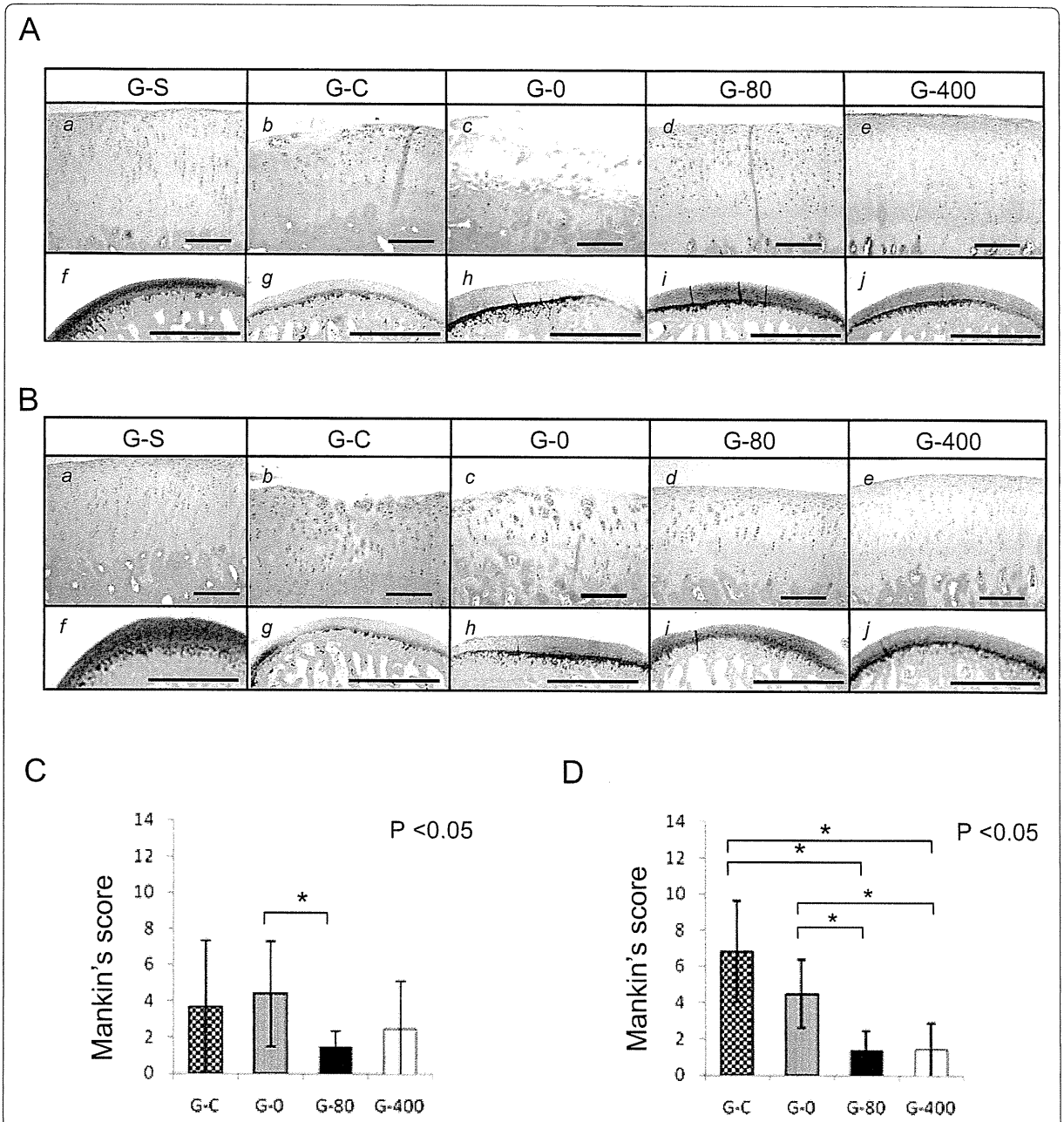

Figure 2 Effect of ONO-8815Ly in the early stages of OA (femur). Histological findings and grade of degeneration, scored using Mankin's system, in (**a**) medial and (**b**) lateral femoral condyles at two weeks after the operation. Sections were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400, and stained with (*a* to *e*) H&E or (*f* to *j*) safranin-O. Magnification, 200× (H&E) and 40× (safranin-O). Scale bar, 200 μm (HE) and 2.0 mm (safranin-O). *$P < 0.05$. Values are the mean ± standard deviation. OA, osteoarthritis.

co-treatment with the EP2 agonist in a dose-dependent manner, and the maximum reduction was 37% at 1 μM of EP2 agonist. In the case of NRC cells, the maximum reduction (27%) was observed at the concentration of 0.1 μM.

Discussion

The effect of PGE2 on the progression of OA is still a matter of debate. In some reports, PGE2 was shown to destroy articular cartilage by degrading cartilage ECM [12,13]. It has also been reported to down-regulate the

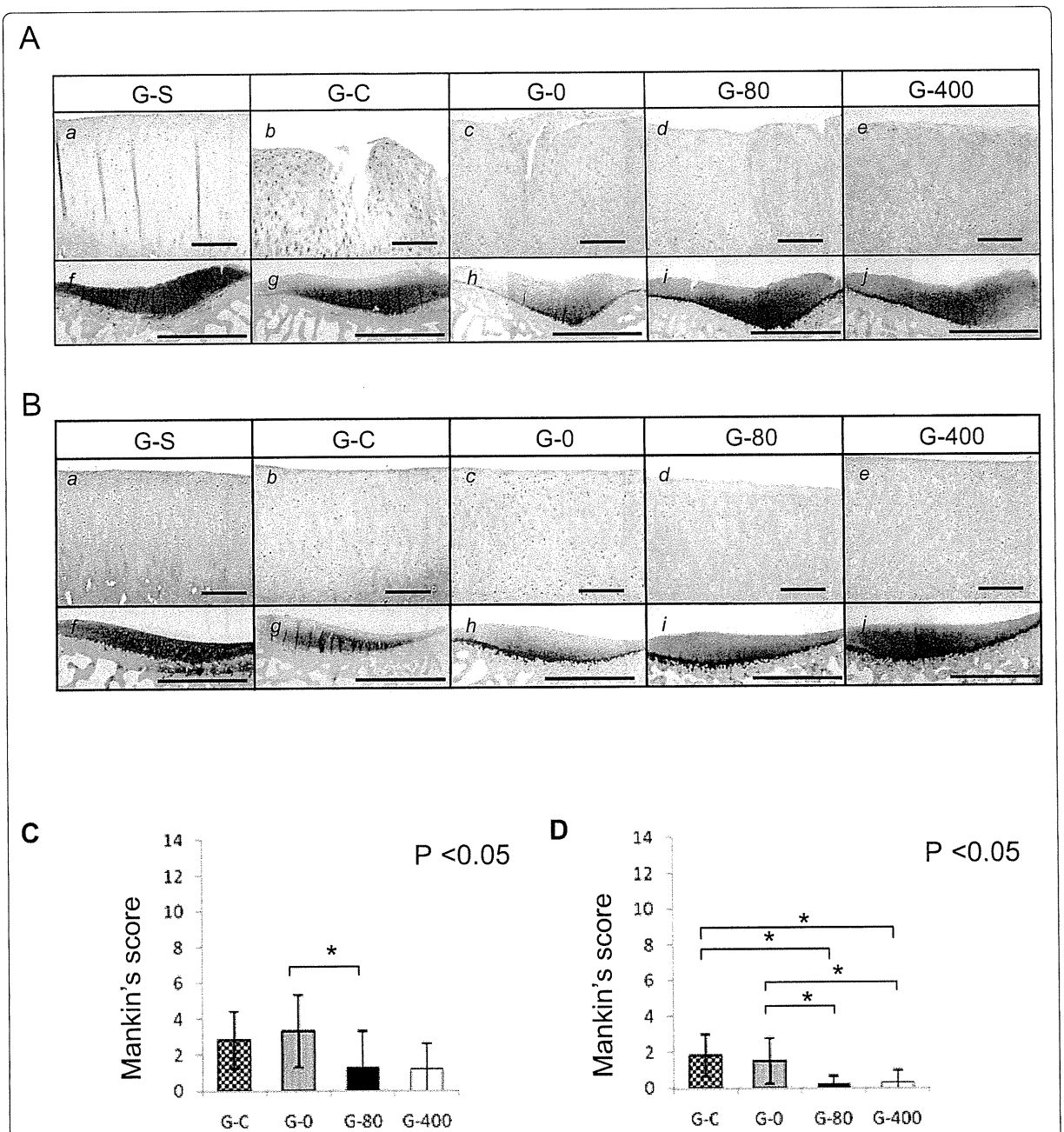

Figure 3 Effect of ONO-8815Ly in the early stages of OA (tibia). Histological findings and grade of degeneration, scored using Mankin's system, in (**a**) medial and (**b**) lateral tibial condyles at two weeks after the operation. Sections were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400, and stained with (*a* to *e*) H&E or (*f* to *j*) safranin-O. Magnification, 200× (H&E) and 40× (safranin-O). Scale bar, 200 μm (H&E) and 2.0 mm (safranin-O). *$P < 0.05$. Values are the mean ± standard deviation. OA, osteoarthritis.

production of IL-6 by IL-1α and IL-1β via EP2/EP4 receptors [25,26]. PGE2 at very low concentrations inhibits the production of IL-1β, TNF-α, and MMP-13 in the articular cartilages of OA patients [27]. In the current study, the production of MMP-13 was decreased by an EP2 agonist (Figures 7 and 8), which is consistent with the *in vitro* data described in a recent report [28]. Continuous administration of non-steroidal anti-inflammatory drugs to patients with OA exacerbates OA [29,30]. These contradictory results

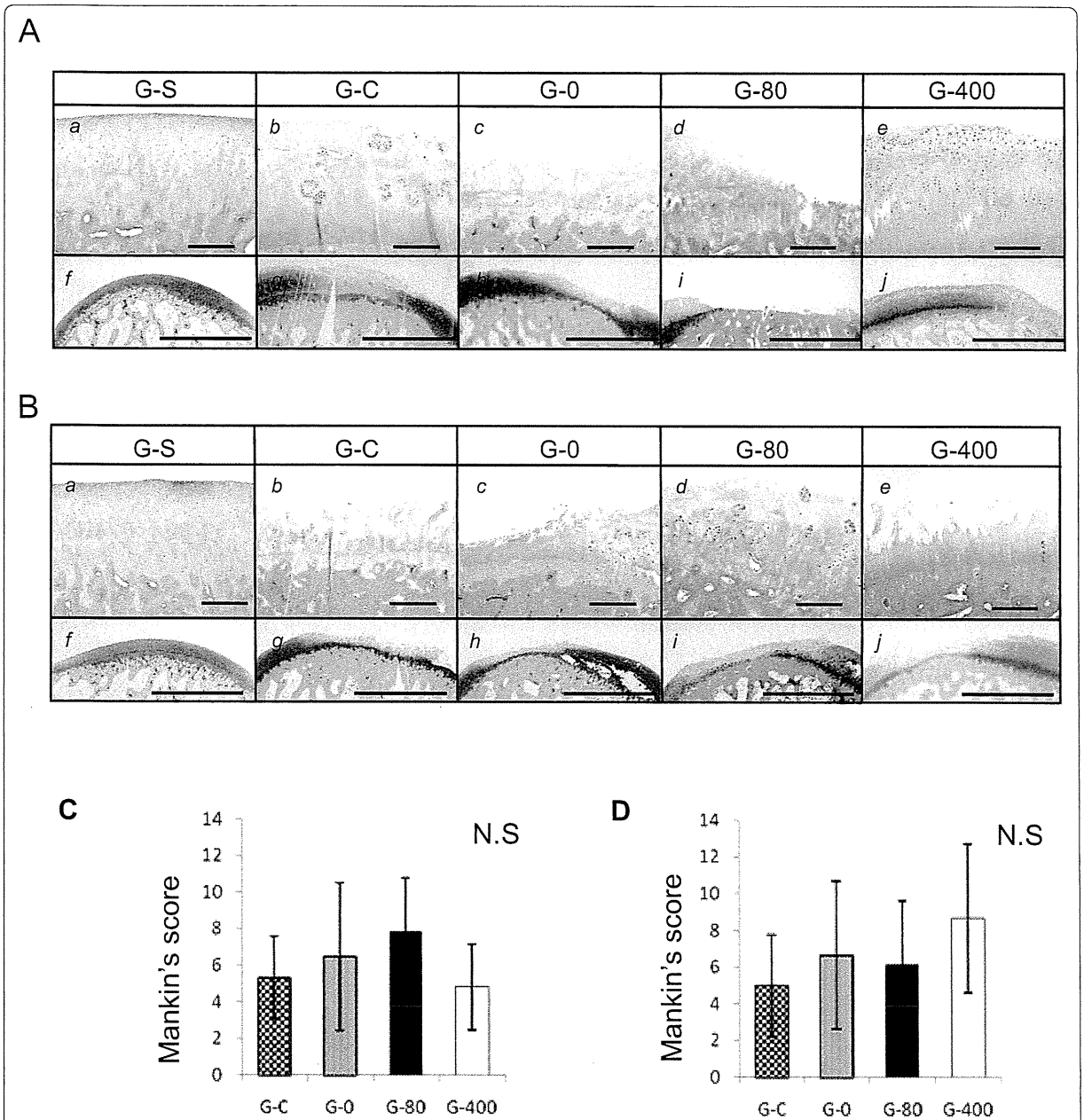

Figure 4 Effect of ONO-8815Ly in the late stages of degeneration (femur). Histological findings of **(a)** medial and **(b)** lateral femoral condyles at 12 weeks after the operation. Sections were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400, and stained with (*a* to *e*) H&E or (*f* to *j*) safranin-O. Magnification, 200× (H&E) and 40× (safranin-O). Scale bar, 200 μm (H&E) and 2.0 mm (safranin-O). Grade of degeneration scored using Mankin's system in **(c)** medial and **(d)** lateral femoral condyles at 12 weeks after the operation. Values are the mean ± standard deviation. N.S, not significant.

may be due to the differences in the experimental dose of PGE2 agonist used, or due to the pleiotropic effects of PGE2 through different types of receptors (EP1 to EP4). Therefore, analyses should be conducted with agonists specific for each type of receptor. IL-1β-induced expression of *MMP-13* mRNA was reduced by EP2 signaling both in NRC and ORC cells *in vitro* (Figure 8). Moreover, IL-1β-induced expression of *MMP-13* mRNA was reduced in ORC cells, but not in NRC cells, in a dose-dependent manner, that is, *MMP-*

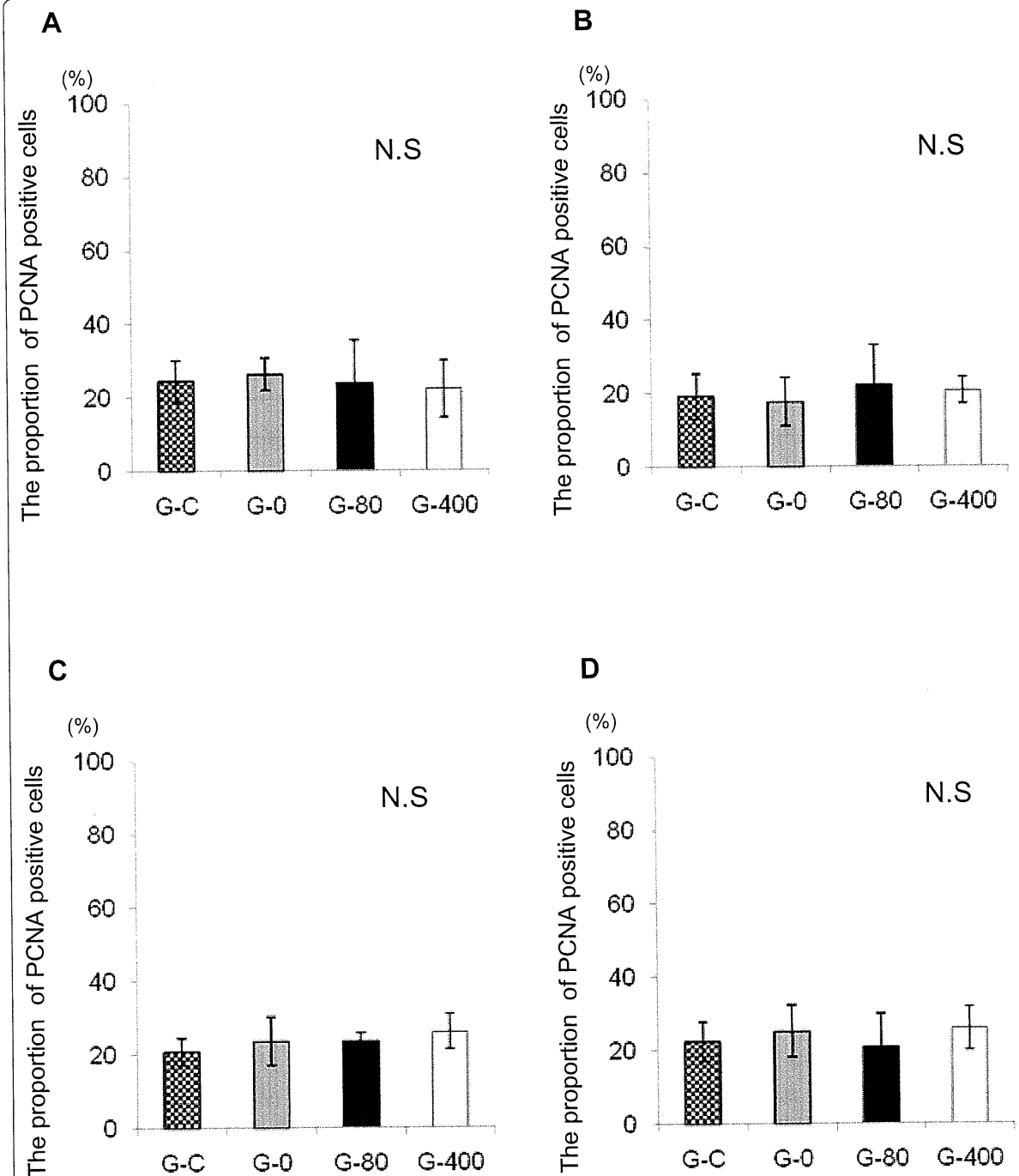

Figure 5 Effect of ONO-8815Ly on PCNA expression. The proportion of PCNA-positive cells in **(a)** medial and **(b)** lateral femoral condyles, and in **(c)** medial and **(d)** lateral tibial condyles. Values are the mean ± standard deviation. N.S, not significant; PCNA, proliferating cell nuclear antigen.

13 expression was higher in the presence of 1 μM of ONO-AE-259-01 than in the presence of 0.1 μM of ONO-AE-259-01 (Figure 8). An EP2 agonist acts as an anti-inflammatory drug at low doses, but if the concentration exceeds 1 μM, the anti-inflammatory effect may become weak (Figure 8). In fact, some authors have reported that excess EP2 agonists may act rather as inflammatory-inductive drugs.

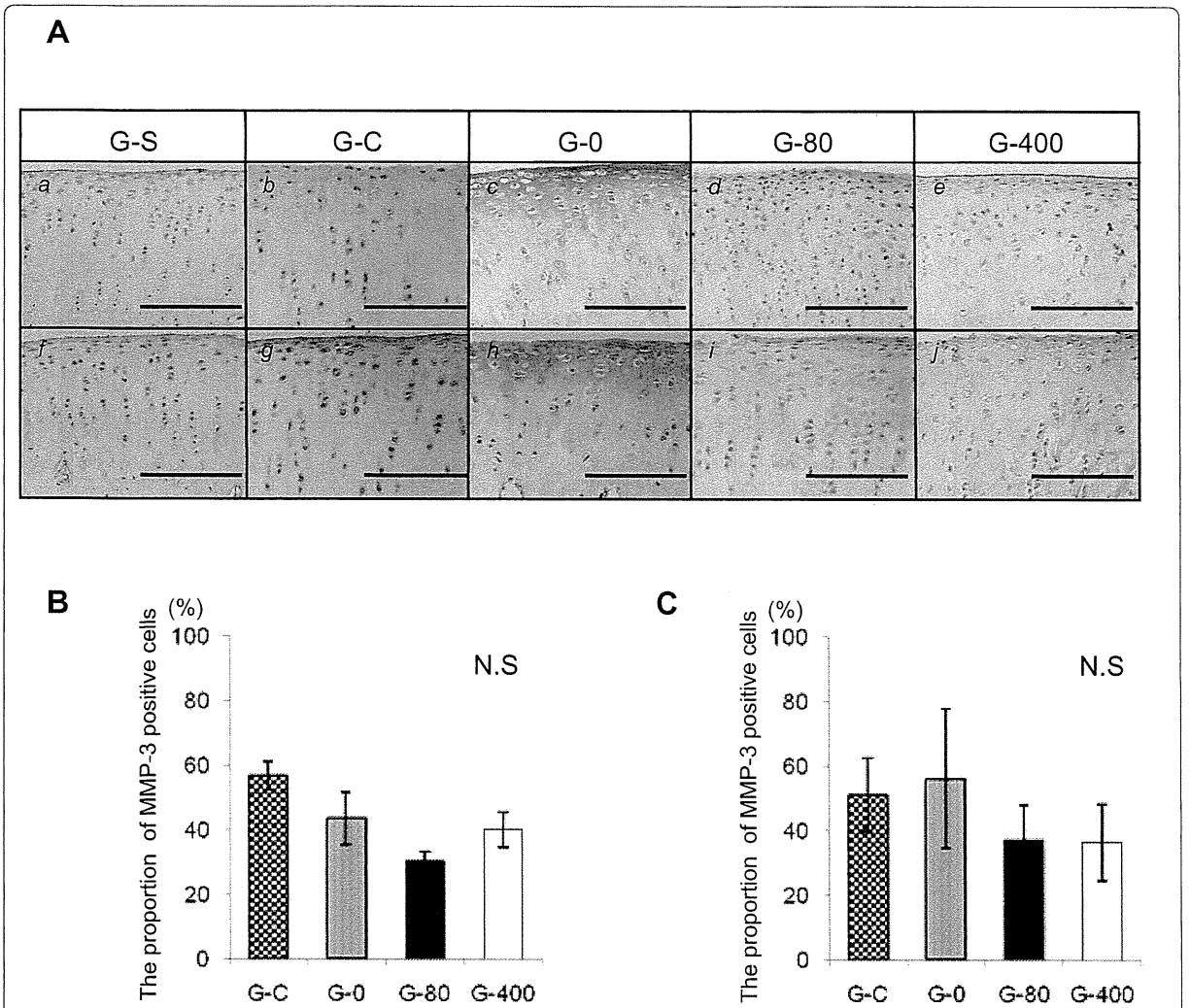

Figure 6 Effect of ONO-8815Ly on the expression of MMP-3 in femoral condyles. **(a)** Immunostaining for MMP-3 at two weeks after the operation. Sections of (*a* to *e*) medial and (*f* to *j*) lateral femoral condyles were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400. Magnification, 400×. Scale bar, 200 μm. Proportion of MMP-3-positive cells in **(b)** medial and **(c)** lateral femoral condyles at two weeks after the operation. Values are the mean ± standard deviation. *$P < 0.05$. MMP-3, matrix metalloproteinase-3; N.S, not significant.

Previously, we showed that EP2 signaling enhances the growth of chondrocytes [18,19] and promotes the regeneration of articular cartilage in rabbits with cartilage defects by an EP2-selective agonist [19]. However, in the current study, EP2 signaling failed to promote chondrocyte proliferation (Figure 5). The differences may result from differences in the animal models. In the previous study, the effect of EP2 signaling on articular cartilage was evaluated using the chondral and osteochondral defect models. In that model, cartilage defects are present before initiation of the treatment with an EP2 agonist. Thus, EP2 signaling may promote cartilage regeneration by inducing proliferation of cartilage chondrocytes and, consequently, contributing to ECM reconstruction. On the other hand, in the present study, the articular chondrocytes appeared normal immediately after the ACLMT operation, and EP2 signaling reduced cartilage degeneration caused by traumatic instability of the knee joint. These differences in models might be the cause of difference in the results.

In the present study, the abnormal stress on cartilage tissues induced by joint instability was the main cause of degeneration. The degeneration was more remarkable in the lateral (Figures 2d and 3d) than in the medial components (Figures 2c and 3c), wherein partial meniscectomy was performed. We have no clear explanation for this

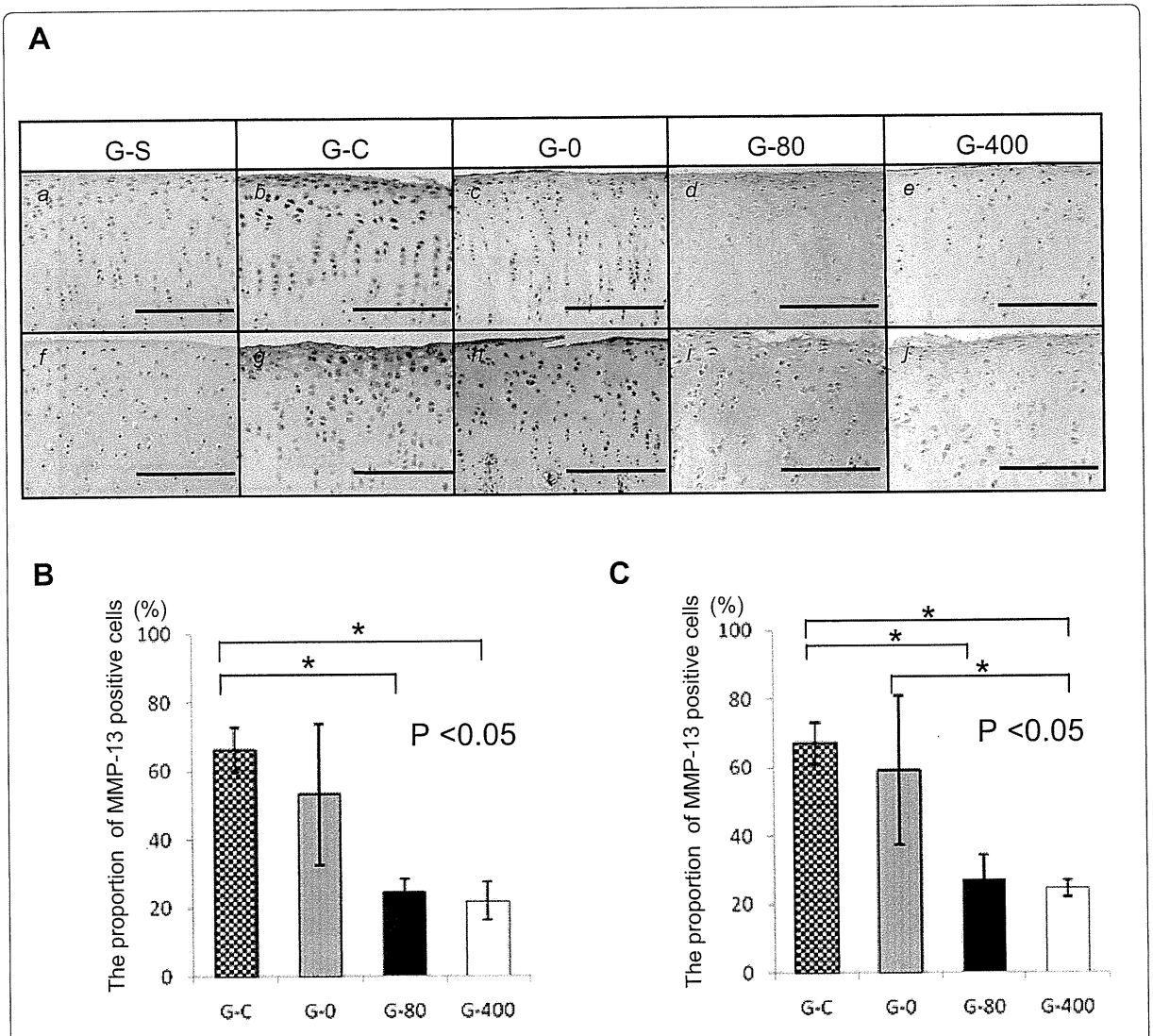

Figure 7 Effect of ONO-8815Ly on the expression of MMP-13 in femoral condyles. **(a)** Immunostaining for MMP-13 at two weeks after the operation. Sections of (*a* to *e*) medial and (*f* to *j*) lateral femoral condyles were prepared from samples of (*a* and *f*) G-S, (*b* and *g*) G-C, (*c* and *h*) G-0, (*d* and *i*) G-80, and (*e* and *j*) G-400. Magnification, 400×. Scale bar, 200 μm. Proportion of MMP-13-positive cells in **(b)** medial and **(c)** lateral femoral condyles at two weeks after the operation. Values are the mean ± standard deviation. *$P < 0.05$. MMP-13, matrix metalloproteinase-13; N. S, not significant.

result. A study has shown that the lateral components of the rabbit knees were more susceptible to degeneration than the medial components in the ACLMT model [31]. The rabbit knee joints are physiologically in the valgus position, causing excess load on the lateral side, which might explain the susceptibility.

The grade of degeneration at 12 weeks was less prominent than we expected (Figure 4). In this injury model, cartilage degeneration will be induced by abnormal stress due to joint instability. Such abnormal stress takes place during weight-bearing movements of the knee joints. Therefore, to enhance such stress, Park et al. forced the rabbits to move in a confined space (5 m × 5 m) for one hour twice a day, from three days after ACLMT onward [22], which increased the Mankin's score up to 12 points at eight weeks after the operation. Restriction in a small cage in the knee-flexed position, as in our study, may minimize such stresses. In addition, both knees were operated on, which may further decrease the activities of the rabbits. These may cause almost no progression of the disease after two weeks.

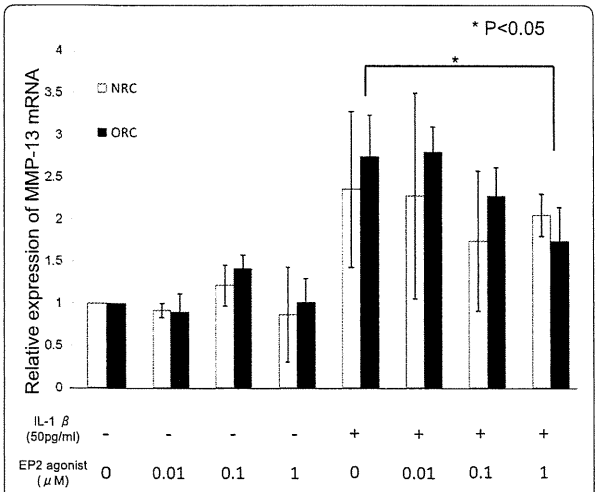

Figure 8 Reduction of IL-1β-induced *MMP-13* mRNA expression by an EP2 agonist on rabbit normal or OA chondrocyte primary culture cells *in vitro*. Expression levels of *MMP-13* mRNA in NRC and ORC at six hours after treatment with IL-1β (50 pg/ml), ONO-AE-259-01, selective EP2 agonist (each at a concentration of 0, 0.01, 0.1, and 1 μM), or a combination of IL-1β and ONO-AE-259-01. GAPDH was used as internal control. Using the ratio obtained for the untreated sample as a standard (1.0), the relative ratio of the treated samples was presented as the relative expression levels of the *MMP-13* gene. Values are the mean ± standard deviation. NRC (n = 4) and ORC (n = 5). *$P < 0.05$. EP2, prostaglandin E2 receptor type 2; IL-1β, interleukin-1β; MMP-13, matrix metalloproteinase-13; NRC, non-treated rabbit chondroctes; ORC, ACLMT-operated rabbit chondrocytes.

Generally, cartilage degeneration in OA is due to the induction of MMP expression. MMP-13 is a product of chondrocytes that reside in cartilage and has a stronger effect that MMP-1 on type II collagen [32]. Some insisted that PGE2 exerts direct inhibitory effects on the expression of MMP-1 [33,34] and MMP-13 [28,33,34] in arthritic chondrocytes, and Sato et al. demonstrated that EP2 signaling was responsible for the down-regulation of MMP-13 *in vitro*, although they used a different agonist [28]. Taken together, EP2 signaling regulates MMP-13 production. In agreement, we showed that production of MMP-13 in articular chondrocytes was reduced when treated with an EP2 agonist *in vivo* (Figure 7) and *in vitro* (Figure 8). Controversially others studies show that PGE2 plays a crucial role in the induction of MMP-13 and MMP-3 in chondrocytes in response to IL-1β in microsomal prostaglandin E synthase-deficient mice [35] or that of PGE2 inhibits chondrocyte maturation [36]. In the current study model, EP2 signaling was shown to inhibit the expression of *MMP-13* mRNA, suggesting that EP2 signaling protects the articular cartilage from degeneration.

MMP-3 is a protease expressed in OA specimens at an early stage [37,38]. MMP-3 cleaves a variety of ECM components such as proteoglycans, collagens, and procollagens [39]. In the current study, ONO-8815Ly had no effect on the production of MMP-3 (Figure 6). Although there is still much to be done, the current study suggested that an EP2 agonist may exert a protective effect on articular cartilage by inhibiting MMP-13.

It is important to clarify whether an EP2 agonist caused inflammation either systemically or locally. PGs are pro-inflammatory lipid mediators whose levels increase in the synovial membrane and synovial fluid of patients with OA. We previously reported that intra-articular administration of an EP2 agonist did not affect the mRNA expression of the *MMP-3*, *TIMP-3*, and *IL-1β* genes in the synovium, or the amounts of TNF-α and C-reactive protein (CRP) in joint fluids. As in our previous study, we found no severe inflammatory changes in the synovium, and no change in the levels of CRP (data not shown), suggesting that this EP2 agonist caused no inflammation either systemically or locally.

The effect of an EP2 agonist did not last long (Figure 4), yet this may be rectified by developing a suitable drug-delivery system. Continuous administration of an EP2 agonist using such a newly developed system could provide a novel therapeutic modality to treat OA.

Conclusions

Stimulation of PGE2 via EP2 prevents degeneration of the articular cartilage during the early stages. The current study suggests that EP2 agonists may exert a protective effect on articular cartilage by inhibiting MMP-13. With a long-term delivery system, the EP2 agonist could be a new therapeutic tool for OA.

Abbreviations

ACLMT: anterior cruciate ligament and menisectomy transaction; COX: cyclo-oxygenase; CRP: C-reactive protein; DMEM: Dulbecco's modified Eagle's medium; ECM: extracellular matrix; EP2: prostaglandin E2 receptor type 2; FBS: fetal bovine serum; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; H&E: hematoxylin & eosin; IGF: insulin-like growth factor; IL: interleukin; MMP: matrix metalloproteinase; OA: osteoarthritis; PBS: phosphate-buffered saline; PCNA: proliferating cell nuclear antigen; PG: prostaglandin; PLGA: polylactic-co-glycolic acid; SD: standard deviation; TNF: tumor necrosis factor.

Acknowledgements

We are grateful to Tomohisa Kato, Akira Nasu, Takashi Kasahara, Kazuo Hayakawa, Kyosuke Kobayashi, Michiko Ueda, Sakura Tamaki, and Yukiko Kobayashi for their assistance. This work was partially supported by Grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science, from the Ministry of Education, Culture, Sports, Science, and Technology, and from the Ministry of Health, Labor, and Welfare.

Author details

[1]Department of Tissue Regeneration, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. [2]Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. [3]Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan. [4]Department of Physical Therapy, Human Health Sciences, Graduate School of Medicine, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. [5]Ono

Pharmaceutical Co. Ltd.,3-1-1 Sakurai, Shimamoto-cho, Mishima-gun, Osaka 618-8585, Japan. [6]Center for iPS Cell Research and Application, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

Authors' contributions

HM performed animal experiments, carried out analysis and interpretation of the data, and drafted the manuscript. TA conceived this study, designed the study, carried out analysis and interpretation of the data, and drafted the manuscript. MF and JY performed animal experiments and carried out analysis of the data. KI performed animal experiments. TM was the chief investigator in the development of materials, and conceived this study. TK designed and performed animal experiments. SF performed animal experiments and obtained samples from animals. HS was responsible for providing materials. NA was responsible for the development of drug delivery system. TO carried out administrative and financial support and helped to draft the manuscript. TN carried out administrative and financial support and helped to draft the manuscript. JT conceived this study, provided financial support, designed experiments, interpreted the data, and drafted the manuscript. All authors have read and approaved the manuscript for publication.

Competing interests

Takayuki Maruyama, Toshiya Kanaji, Shinsei Fujimura, Hikaru Sugihara, and Akio Nishiura are employees of Ono Pharmaceutical Co. Ltd. All other authors have no conflicts of interest.

Received: 19 January 2011 Revised: 28 June 2011
Accepted: 14 September 2011 Published: 14 September 2011

References

1. Peat G, McCarney R, Croft P: **Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care.** *Ann Rheum Dis* 2001, **60**:91-97.
2. Buckwalter JA, Saltzman C, Brown T: **The impact of osteoarthritis: implications for research.** *Clin Orthop Relat Res* 2004, **427 Suppl**:S6-15.
3. Goldring SR, Goldring MB: **The role of cytokines in cartilage matrix degeneration in osteoarthritis.** *Clin Orthop Relat Res* 2004, **427 Suppl**: S27-36.
4. Goldring MB, Goldring SR: **Osteoarthritis.** *J Cell Physiol* 2007, **213**:626-634.
5. Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, Mitchell P, Hambor J, Diekmann O, Tschesche H, Chen J, Van Wart H, Poole AR: **Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage.** *J Clin Invest* 1997, **99**:1534-1545.
6. Neuhold LA, Killar L, Zhao W, Sung ML, Warner L, Kulik J, Turner J, Wu W, Billinghurst C, Meijers T, Poole AR, Babij P, DeGennaro LJ: **Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice.** *J Clin Invest* 2001, **107**:35-44.
7. Hu Y, Xiang JS, DiGrandi MJ, Du X, Ipek M, Laakso LM, Li J, Li W, Rush TS, Schmid J, Skotnicki JS, Tam S, Thomason JR, Wang Q, Levin JI: **Potent, selective, and orally bioavailable matrix metalloproteinase-13 inhibitors for the treatment of osteoarthritis.** *Bioorg Med Chem* 2005, **13**:6629-6644.
8. Narumiya S, Sugimoto Y, Ushikubi F: **Prostanoid receptors: structures, properties, and functions.** *Physiol Rev* 1999, **79**:1193-1226.
9. Laufer S: **Role of eicosanoids in structural degradation in osteoarthritis.** *Curr Opin Rheumatol* 2003, **15**:623-627.
10. Martel-Pelletier J, Pelletier JP, Fahmi H: **Cyclooxygenase-2 and prostaglandins in articular tissues.** *Semin Arthritis Rheum* 2003, **33**:155-167.
11. Bunning RA, Russell RG: **The effect of tumor necrosis factor alpha and gamma-interferon on the resorption of human articular cartilage and on the production of prostaglandin E and of caseinase activity by human articular chondrocytes.** *Arthritis Rheum* 1989, **32**:780-784.
12. Lippiello L, Yamamoto K, Robinson D, Mankin HJ: **Involvement of prostaglandins from rheumatoid synovium in inhibition of articular cartilage metabolism.** *Arthritis Rheum* 1978, **21**:909-917.
13. Li X, Ellman M, Muddasani P, Wang JH, Cs-Szabo G, van Wijnen AJ, Im HJ: **Prostaglandin E2 and its cognate EP receptors control human adult articular cartilage homeostasis and are linked to the pathophysiology of osteoarthritis.** *Arthritis Rheum* 2009, **60**:513-523.
14. Riquet FB, Lai WF, Birkhead JR, Suen LF, Karsenty G, Goldring MB: **Suppression of type I collagen gene expression by prostaglandins in fibroblasts is mediated at the transcriptional level.** *Mol Med* 2000, **6**:705-719.
15. Miyamoto M, Ito H, Mukai S, Kobayashi T, Yamamoto H, Kobayashi M, Maruyama T, Akiyama H, Nakamura T: **Simultaneous stimulation of EP2 and EP4 is essential to the effect of prostaglandin E2 in chondrocyte differentiation.** *Osteoarthritis Cartilage* 2003, **11**:644-652.
16. Di Battista JA, Dore S, Morin N, He Y, Pelletier JP, Martel-Pelletier J: **Prostaglandin E2 stimulates insulin-like growth factor binding protein-4 expression and synthesis in cultured human articular chondrocytes: possible mediation by Ca(++)-calmodulin regulated processes.** *J Cell Biochem* 1997, **65**:408-419.
17. Lowe GN, Fu YH, McDougall S, Polendo R, Williams A, Benya PD, Hahn TJ: **Effects of prostaglandins on deoxyribonucleic acid and aggrecan synthesis in the RCJ 3.1C5.18 chondrocyte cell line: role of second messengers.** *Endocrinology* 1996, **137**:2208-2216.
18. Aoyama T, Liang B, Okamoto T, Matsusaki T, Nishijo K, Ishibe T, Yasura K, Nagayama S, Nakayama T, Nakamura T, Toguchida J: **PGE2 signal through EP2 promotes the growth of articular chondrocytes.** *J Bone Miner Res* 2005, **20**:377-389.
19. Otsuka S, Aoyama T, Furu M, Ito K, Jin Y, Nasu A, Fukiage K, Kohno Y, Maruyama T, Kanaji T, Nishiura A, Sugihara H, Fujimura S, Otsuka T, Nakamura T, Toguchida J: **PGE2 signal via EP2 receptors evoked by a selective agonist enhances regeneration of injured articular cartilage.** *Osteoarthritis Cartilage* 2009, **17**:529-538.
20. Tani K, Naganawa A, Ishida A, Egashira H, Sagawa K, Harada H, Ogawa M, Maruyama T, Ohuchida S, Nakai H, Kondo K, Toda M: **Design and synthesis of a highly selective EP2-receptor agonist.** *Bioorg Med Chem Lett* 2001, **11**:2025-2028.
21. Okada H: **One- and three-month release injectable microspheres of the LH-RH superagonist leuprorelin acetate.** *Adv Drug Deliv Rev* 1997, **28**:43-70.
22. Park SR, Park SH, Jang KW, Cho HS, Cui JH, An HJ, Choi MJ, Chung SI, Min BH: **The effect of sonication on simulated osteoarthritis. Part II: alleviation of osteoarthritis pathogenesis by 1 MHz ultrasound with simultaneous hyaluronate injection.** *Ultrasound Med Biol* 2005, **31**:1559-1566.
23. Sakakibara Y, Miura T, Iwata H, Kikuchi T, Yamaguchi T, Yoshimi T, Itoh H: **Effect of high-molecular-weight sodium hyaluronate on immobilized rabbit knee.** *Clin Orthop Relat Res* 1994, 282-292.
24. Mankin HJ, Dorfman H, Lippiello L, Zarins A: **Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data.** *J Bone Joint Surg Am* 1971, **53**:523-537.
25. Noguchi K, Maeda M, Ruwanpura SM, Ishikawa I: **Prostaglandin E2 (PGE2) downregulates interleukin (IL)-1alpha-induced IL-6 production via EP2/EP4 subtypes of PGE2 receptors in human periodontal ligament cells.** *Oral Dis* 2005, **11**:157-162.
26. Noguchi K, Shitashige M, Endo H, Kondo H, Ishikawa I: **Binary regulation of interleukin (IL)-6 production by EP1 and EP2/EP4 subtypes of PGE2 receptors in IL-1beta-stimulated human gingival fibroblasts.** *J Periodontal Res* 2002, **37**:29-36.
27. Tchetina EV, Di Battista JA, Zukor DJ, Antoniou J, Poole AR: **Prostaglandin PGE2 at very low concentrations suppresses collagen cleavage in cultured human osteoarthritic articular cartilage: this involves a decrease in expression of proinflammatory genes, collagenases and COL10A1, a gene linked to chondrocyte hypertrophy.** *Arthritis Res Ther* 2007, **9**:R75.
28. Sato T, Konomi K, Fujii R, Aono H, Aratani S, Yagishita N, Araya N, Yudoh K, Beppu M, Yamano Y, Nishioka K, Nakajima T: **Prostaglandin EP2 receptor signalling inhibits the expression of matrix metalloproteinase 13 in human osteoarthritic chondrocytes.** *Ann Rheum Dis* 2011, **70**:221-226.
29. Rashad S, Low F, Revell P, Hemingway A, Rainsford K, Walker F: **Effect of non-steroidal anti-inflammatory drugs on course of osteoarthritis.** *Lancet* 1989, **2**:1149.
30. Reijman M, Bierma-Zeinstra SM, Pols HA, Koes BW, Stricker BH, Hazes JM: **Is there an association between the use of different types of nonsteroidal antiinflammatory drugs and radiologic progression of osteoarthritis? The Rotterdam Study.** *Arthritis Rheum* 2005, **52**:3137-3142.
31. Shirai T, Kobayashi M, Nishitani K, Satake T, Kuroki H, Nakagawa Y, Nakamura T: **Chondroprotective effect of alendronate in a rabbit model of osteoarthritis.** *J Orthop Res* 2011, **29**:1572-7.
32. Burrage PS, Mix KS, Brinckerhoff CE: **Matrix metalloproteinases: role in arthritis.** *Front Biosci* 2006, **11**:529-543.

33. Nishitani K, Ito H, Hiramitsu T, Tsutsumi R, Tanida S, Kitaori T, Yoshitomi H, Kobayashi M, Nakamura T: **PGE2 inhibits MMP expression by suppressing MKK4-JNK MAP kinase-c-JUN pathway via EP4 in human articular chondrocytes.** *J Cell Biochem* 2010, **109**:425-433.
34. Pillinger MH, Rosenthal PB, Tolani SN, Apsel B, Dinsell V, Greenberg J, Chan ES, Gomez PF, Abramson SB: **Cyclooxygenase-2-derived E prostaglandins down-regulate matrix metalloproteinase-1 expression in fibroblast-like synoviocytes via inhibition of extracellular signal-regulated kinase activation.** *J Immunol* 2003, **171**:6080-6089.
35. Gosset M, Pigenet A, Salvat C, Berenbaum F, Jacques C: **Inhibition of matrix metalloproteinase-3 and -13 synthesis induced by IL-1beta in chondrocytes from mice lacking microsomal prostaglandin E synthase-1.** *J Immunol* 2010, **185**:6244-6252.
36. Clark CA, Li TF, Kim KO, Drissi H, Zuscik MJ, Zhang X, O'Keefe RJ: **Prostaglandin E2 inhibits BMP signaling and delays chondrocyte maturation.** *J Orthop Res* 2009, **27**:785-792.
37. Bau B, Gebhard PM, Haag J, Knorr T, Bartnik E, Aigner T: **Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes in vivo and in vitro.** *Arthritis Rheum* 2002, **46**:2648-2657.
38. Kevorkian L, Young DA, Darrah C, Donell ST, Shepstone L, Porter S, Brockbank SM, Edwards DR, Parker AE, Clark IM: **Expression profiling of metalloproteinases and their inhibitors in cartilage.** *Arthritis Rheum* 2004, **50**:131-141.
39. Klatt AR, Paul-Klausch B, Klinger G, Kuhn G, Renno JH, Banerjee M, Malchau G, Wielckens K: **A critical role for collagen II in cartilage matrix degradation: collagen II induces pro-inflammatory cytokines and MMPs in primary human chondrocytes.** *J Orthop Res* 2009, **27**:65-70.

doi:10.1186/ar3460
Cite this article as: Mitsui *et al.*: Prostaglandin E2 receptor type 2-selective agonist prevents the degeneration of articular cartilage in rabbit knees with traumatic instability. *Arthritis Research & Therapy* 2011 13:R146.

8

Tailor-Made Programs for Preventive Falls that Match the Level of Physical Well-Being in Community-Dwelling Older Adults

Minoru Yamada, Tomoki Aoyama and Hidenori Arai
Kyoto University,
Japan

1. Introduction

Falls are relatively common in the elderly, with approximately 30% of individuals aged 65 and older falling at least once a year and approximately half of them experiencing repeated falls (Tinetti et al., 1988). Falls and fractures have a major impact on elderly individuals, their caregivers, health service providers, and the community. Sherrington et al. reported that up to 42% of falls can be prevented by well-designed exercise programs that target balance and involve a good amount of exercise (Sherrington et al., 2008).

In daily life, locomotion occurs under complicated circumstances, with cognitive attention focused on a particular task, such as watching the traffic or reading street signs, rather than on performing a simple motor task such as walking. A seminal study demonstrating that the characteristic "stops walking when talking" could serve as a predictor of falls introduced a novel method for predicting falls based on dual-task (DT) performance (Lundin-Olsson et al., 1997). Our recent study indicated that different factors may be related to fall incidents depending on the level of frailty of the community-dwelling elderly adults (Yamada et al., 2011a). These findings suggest that fall prevention programs should be tailored to the elderly adult's level of physical well-being. The purpose of this review is to review approaches to fall prevention tailored to an individual's level of physical well-being.

2. What is "Dual-task"?

Recently, several investigators have reported that DT gait is associated with fall incidents in elderly adults. A summary of these DT studies is shown in Table 1.

3. How can we use DT to assess fall risk in the elderly?

3.1 Game-based fall risk assessment

DT performance may be a reliable predictor of falls in elderly adults. The Nintendo Wii Fit program requires the distribution of attention to the motor task and the monitor (cognitive task). Thus, it is assumed that this program includes a constituent of DT. We examined whether the Wii Fit program's Basic Step can be used for fall risk assessment in healthy,

	Primary task	Secondary task	Fall related
Beauchet et al., 2008	Walk	Cognitive task	○
Beauchet et al., 2008	Walk	Cognitive task	○
Kressing RW et al, 2008	Walk	Cognitive task	○
Beauchet et al., 2007	Walk	Cognitive task	○
Faulkner KA et al, 2007	Walk	Cognitive task	○
Toulotte C et al, 2006	Walk	Manual task	○
Springer S et al, 2006	Walk	Cognitive task	×
Bootsma-vander Wiel A et al, 2003	Walk	Cognitive task	×
Verghese et al., 2002	Walk	Cognitive task	○
Stalenhoef PA et al, 2002	Walk	Cognitive task	×
Lundin-Olsson L et al, 1997	Walk	Cognitive task	○

○, related to falls; ×, non-related to falls

Table 1. Effect of dual tasking on falls.

community-dwelling elderly adults (Yamada et al., 2011b). The results suggested that game-based fall risk assessment has a high generality and is very useful for assessing community-dwelling elderly adults (Fig. 1).

This study included a Wii Fit Balance Board, which was placed under the participants' feet. A score of 111 points on Basic Step was used to classify 88.6% of the cases correctly ($p < 0.001$). The horizontal demonstrates the cutoff point.

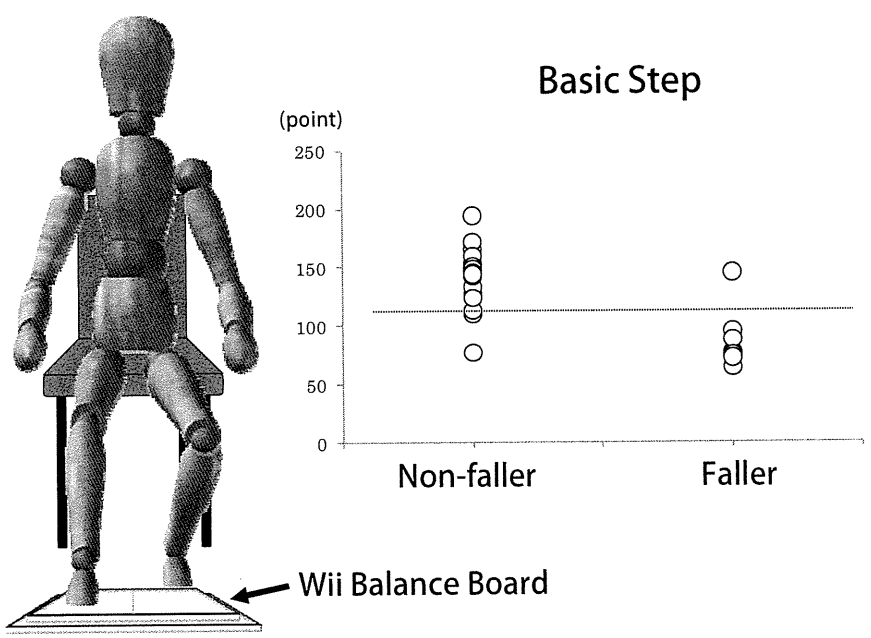

Fig. 1. Schematic diagram of Basic Step as played in a sitting position and scatter chart.

3.2 Smartphone-based fall risk assessment

The Android-based Smartphone can be used to develop applications freely. These applications can then be disseminated across the world via the internet. The use of Android-

based applications is advantageous because they are free to develop, offer flexible design options, and can be easily and rapidly distributed over the internet. We developed an Android application (RollingBall) for the assessment of fall risk (available for download at http://www.kuhp.kyoto-u.ac.jp/~kazuya/RollingBall.apk) in which a small blue ball (1.5 cm in diameter) is moved on a large white circle (4 cm in diameter) by tilting the phone. The angle of the phone is determined by triaxial accelerometers (Fig. 2). The Android application also calculates a score on the basis of the coordinate data of the ball on the circle; higher scores indicate that the blue ball is closer to the center of the circle. The application was based on the "walking while carrying a ball on a tray" task. We previously examined whether the score determined by the Android application for DT-based fall risk assessment was related to falls in a population of community-dwelling elderly people (Yamada et al., 2011c). The results suggested that the Android application is very useful for fall risk assessment in elderly adults (Fig. 2).

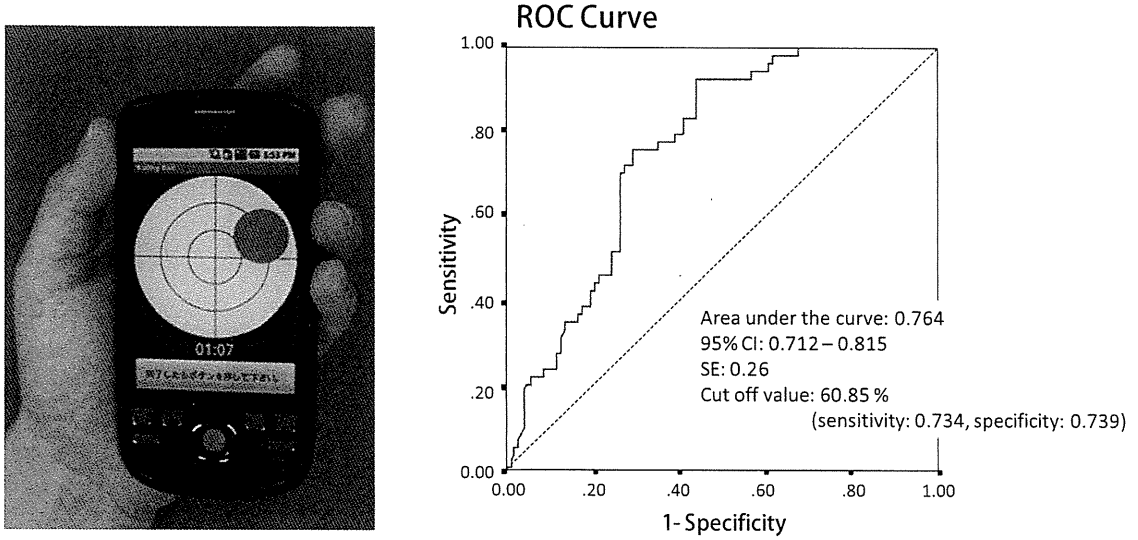

Fig. 2. An Android application allows users to control the position of a small blue circle (1.5 cm in diameter) on a large white circle (4 cm in diameter). Scores are automatically calculated on the basis of coordinate tracking data for the blue circle.

ROC (receiver operating characteristic) curve of the dual tasking (DT) total cost for the classification of fall risk. The area under the curve was 0.764. For the DT total cost, the cut-off value was 60.85% (sensitivity = 73.4%, specificity = 73.9%). CI, confidence interval

3.3 Multitarget Stepping Test (MTST)

We developed a walking test, the multitarget stepping test (MTST) (Yamada et al., 2011d). During the test, stepping and avoidance failures were measured while participants walked along a 10-m walkway and stepped on multiple targets. The MTST was performed on a black elastic mat (10 m long × 1 m wide). Forty-five 10 cm × 10 cm squares were on the mat (see Fig. 3). These squares were arranged into 3 rows (15 cm between each row) and 15 lines (61 cm between each line). Each square was marked with red, blue, or yellow tape. Each line had one of the 3 colored squares in a random order. One square (blue or yellow) was the

footfall target, whereas the others were distracters. The color of the footfall target was counterbalanced among the participants and announced to each participant before he or she began walking.

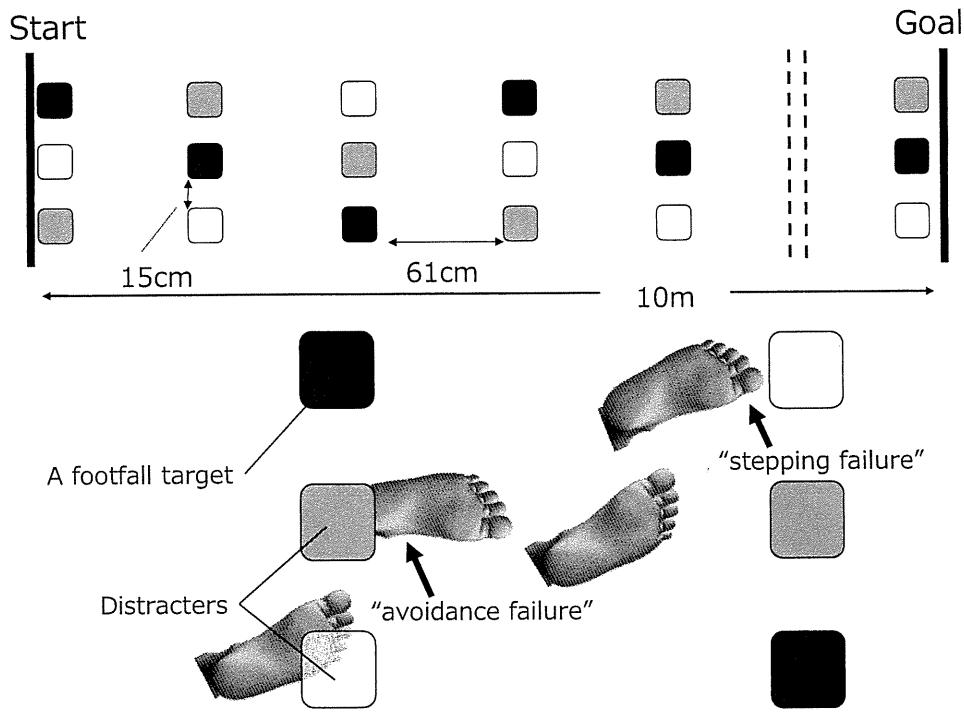

Fig. 3. The 10-m walkway used in the multitarget stepping test (MTST): Each square was made of red, blue, or yellow tape. The MTST measured 2 types of failure. A participant intended to step on footfall targets (displayed in white). Failure to step on the footfall target was regarded as a stepping error. Failure to avoid a distracter was regarded as an avoidance failure. As shown in this figure, avoidance failure was always the result of an accidental step as the participant walked from target to target; it did not occur because of selecting the wrong target out of the 3 squares on the line on which the participant intended to step.

The participants walked on the mat at a self-selected pace while stepping on the target square placed on each line. The participants were instructed (a) to step on a footfall target with either side of the foot and any part of the sole, (b) to take as many steps as necessary while walking between the lines to comfortably walk toward the next footfall target, and (c) to not step on the distracters. The main dependent measures were 2 types of failure indicating less accurate stepping performance: a stepping failure (i.e., failure to step on the footfall target) and an avoidance failure (i.e., failure to avoid distracters).

The results demonstrated that the stepping failure was independently associated with falling (odds ratio [OR] = 19.365, 95% confidence interval [CI] = 3.28–113.95; $p < 0.001$). Hence, measurements of stepping accuracy while performing the MTST, particularly precise stepping failure, could help identify elderly individuals at high risk for a fall.

3.4 Questionnaire-based fall risk assessment

As discussed, DT walking, game-based assessment, Smartphone assessment, and/or MTST can be used to identify elderly adults at high risk of falling. However, more simple and reliable assessment methods are necessary for community-dwelling elderly people. We previously examined whether a newly developed index we had designed to assess complex-task locomotion was related to falls in a robust elderly population (Yamada et al., in press a). The results suggested that a score of more than 1 point on the new index can predict falls in elderly adults (Table 2, Fig. 4).

	Item	0	1
1)	Can you stand up without a support?	Yes	No
2)	Can you turn in the opposite way, while holding an empty glass?	Yes	No
3)	Can you walk without dropping a glass of water?	Yes	No
4)	Have you ever tripped over an obstacle while going to the bathroom or picking up the telephone?	No	Yes

Table 2. Newly developed index

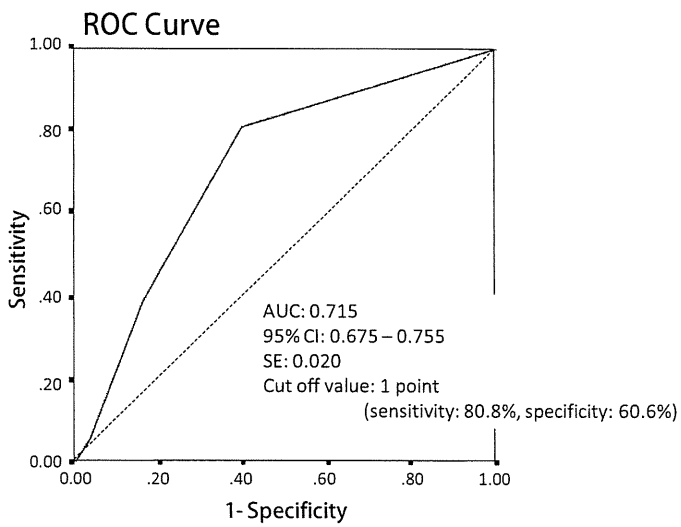

Fig. 4. The ROC (receiver operating characteristic) curve for the total points used for the classification of fall risk: The area under the curve (AUC) was 0.715. Concerning the total points, the cut-off value was determined at 1 point (sensitivity, 80.8%; specificity, 60.6%). CI, confidence interval

4. Different factors related to fall incidents

Our research has indicated that different factors may be related to fall incidents depending on the level of frailty in community-dwelling elderly adults.

One study population consisted of 1038 elderly Japanese subjects aged 65 years or older living in a community (401 men, 637 women; mean age, 77 ± 8 years). We assessed 6 items of physical functioning: timed up and go (TUG), functional reach, 5-chair stand, single-task (ST) 10-m walking time, and DT (CT [cognitive task], MT [manual task]) 10-m walking time.

In the TUG test, participants were asked to stand up from a standard chair with a seat height of 40 cm, walk a distance of 3 m at a maximum pace, turn, walk back to the chair, and sit down (Podsiadlo et al., 1991). Functional reach was measured using a simple clinical apparatus consisting of a yardstick secured to the wall at right acromion height as previously described (Duncan et al., 1990). In the 5-chair stand, participants were asked to stand up and sit down 5 times as quickly as possible and were timed from the initial sitting position to the final standing position at the end of the fifth stand (Guralnik et al., 1994). In ST walking, the participants were asked to walk as fast as possible along a 10-m straight line, with a 1-m approach at both ends, for a total length of 12 m. The time required was measured. In CT walking, participants walked 15 m at the most comfortable speed while counting numbers aloud in reverse order starting at 100. In MT walking, participants walked 15 m at the most comfortable speed while carrying a ball (7 cm in diameter, 150 g in weight) on a tray (17 cm in diameter, 50 g in weight). The DT cost (CT and MT) was then calculated as follows: DT cost [%] = 100 × (DT walking time - ST walking time)/([ST walking time + DT walking time]/2).

Information on fall incidents over the following year was collected from participants via a monthly telephone interview. A fall was defined as any event that led to unplanned, unexpected contact with a supporting surface during walking.

For analysis, we divided the TUG test results into quartiles (fastest, faster, slower, and slowest). A multivariate analysis by means of logistic regression using a stepwise-forward method was performed to investigate which of the 5 measures of physical functioning (i.e., ST walking time, CT cost, MT cost, functional reach, or 5-chair stand test) was independently associated with falls.

A total of 20% in the fastest group, 18.2% in the faster group, 34.1% in the slower group, and 44.1% in the slowest group experienced falls over the following year. In the fastest group ($n = 230$), the regression analysis indicated that the MT cost (OR = 1.068, 95% CI = 1.04–1.10; $p < 0.001$) was an independent variable that remained in the final step of the regression model. In the faster group ($n = 258$), the regression analysis indicated that the CT cost (OR = 1.03, 95% CI = 1.01–1.04; $p < 0.001$) was an independent variable. In the slower ($n = 264$) and slowest ($n = 286$) groups, the 5-chair stand test (slower group: OR = 1.11, 95% CI = 1.03–1.19; $p < 0.001$; slowest group: OR = 1.05, CI = 1.01–1.09; $p < 0.045$) was found to be a significant and independent variable of falls. A summary of these results is shown in Fig. 5.

5. Fall prevention programs tailored to levels of physical well-being

Fig. 5 shows that different factors may be related to fall incidents depending on one's level of physical well-being. DT walking is associated with falls in the robust elderly population, and thus this population should be given the rhythmic stepping exercise (Yamada et al., 2011e). DT walking and muscle strength are associated with falls in the intermediate elderly population, and thus this population should be given the seated stepping exercise (Yamada et al., 2010a). Muscle strength and DT walking are associated with falls in the pre-frail elderly population (Yamada et al., 2010b) and thus this population should be given the trail walking exercise. Finally, muscle strength is associated with falls in the frail elderly population, and thus this population should be given resistance exercise (Yamada et al., 2011f).

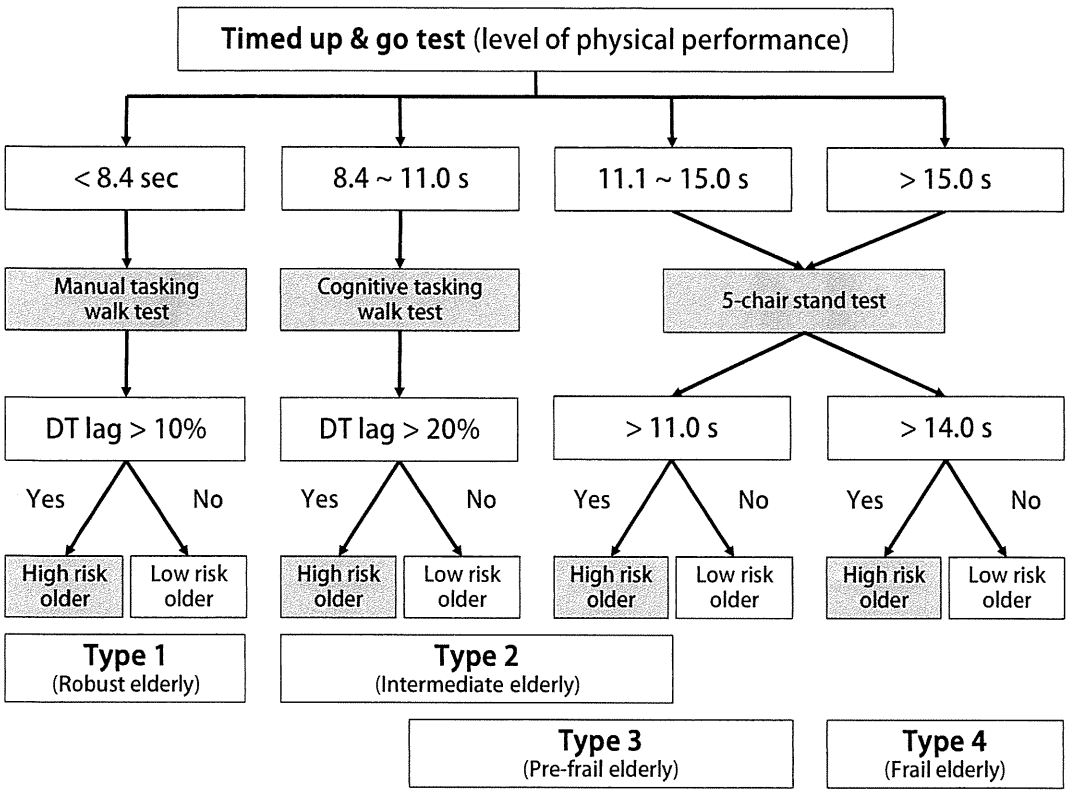

Fig. 5. Flow chart showing that the different factors may be related to fall incidents depending on an individual's level of physical well-being; DT, dual tasking

5.1 Rhythmic stepping exercise

Rhythmic stepping exercises were performed on a thin elastic mat (150 × 150 cm) that was partitioned into 5 squares (50 cm each) to form a cross (Fig. 6). The stepping exercises included forward, backward, and sideways step patterns. The participants were required to step at a tempo of 60–120 beats/min along with the accompanying rhythm sound and to step into the square indicated verbally by the supervisor (e.g., "right," "forward," "back"). Cognitive functioning (reaction, short-term memory, etc.) and motor functioning (stepping in multiple directions) were simultaneously required of the participants. In order to change the level of difficulty, the instruction method transposed not only direction but also color (e.g., "red," "blue") or number (e.g., "3," "7"). The participants completed 5 sets of 1 min per set of stepping exercises between weeks 1 and 8, which was then increased to 3 sets of 3 min per set between weeks 9 and 16 and 3 sets of 5 min per set between weeks 17 and 24. The instructions given at the beginning of each class were as follows: "Please step as correctly as possible, and avoid making mistakes as much as you can."

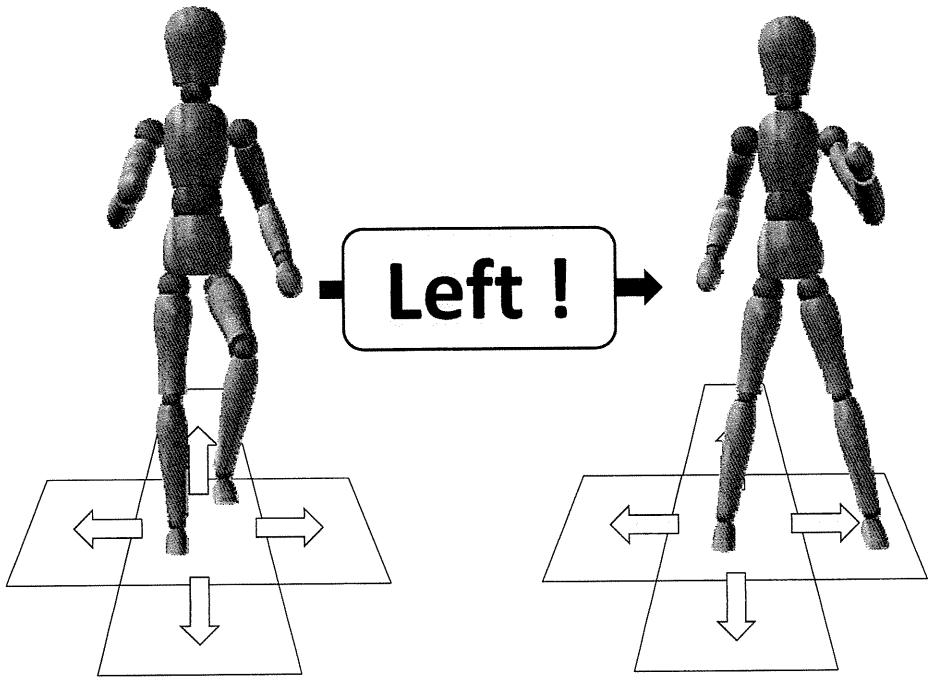

Fig. 6. Schematic representation of the stepping exercises.

The stepping exercises were performed on a thin elastic mat that was partitioned into 5 squares (50 cm each) to form a cross shape. The stepping exercises included forward, backward, and sideways step patterns.

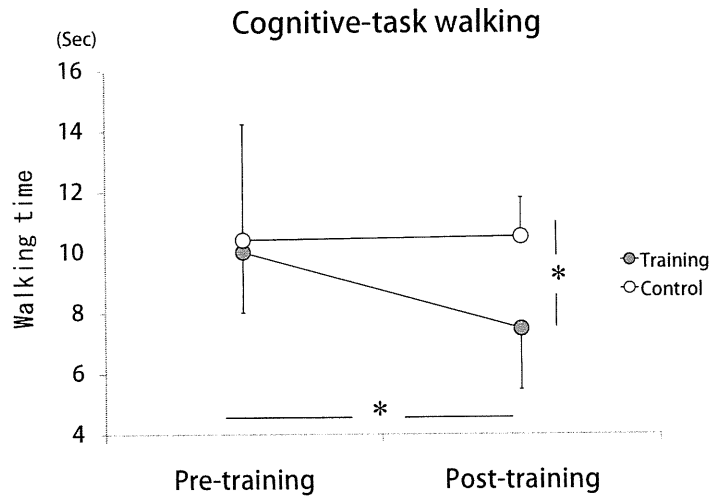

Fig. 7. Cognitive task walk time in training (rhythmic stepping exercise) and control groups during pre- and post-training. Significant differences were observed between the 2 groups ($p < 0.05$).

We evaluated whether a 24-week rhythmic stepping exercise program would effectively improve physical functioning and reduce fear of falling in community-dwelling elderly