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CARTILAGE

Intra-articular low-dose parathyroid hormone (1-34) improves mobility and articular cartilage quality in a preclinical age-related knee osteoarthritis model

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Aims

Osteoarthritis (OA) is prevalent among the elderly and incurable. Intra-articular parathyroid hormone (PTH) ameliorated OA in papain-induced and anterior cruciate ligament transection-induced OA models; therefore, we hypothesized that PTH improved OA in a preclinical age-related OA model.

Methods

Guinea pigs aged between six and seven months of age were randomized into control or treatment groups. Three- or four-month-old guinea pigs served as the young control group. The knees were administered 40 µl intra-articular injections of 10 nM PTH or vehicle once a week for three months. Their endurance as determined from time on the treadmill was evaluated before kill. Their tibial plateaus were analyzed using microcalculated tomography (µCT) and histological studies.

Results

PTH increased the endurance on the treadmill test, preserved glycosaminoglycans, and reduced Osteoarthritis Research Society International score and chondrocyte apoptosis rate. No difference was observed in the subchondral plate bone density or metaphyseal trabecular bone volume and bone morphogenetic 2 protein staining.

Conclusion

Subchondral bone is crucial in the initiation and progression of OA. Although previous studies have shown that subcutaneous PTH alleviates knee OA by improving subchondral and metaphyseal bone mass, we demonstrated that intra-articular PTH injections improved spontaneous OA by directly affecting the cartilage rather than the subchondral or metaphyseal bone in a preclinical age-related OA model.

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Keywords: Age-related osteoarthritis, Apoptosis, Parathyroid hormone

Article focus

- The treatment effects of parathyroid hormone (PTH) in a preclinical age-related osteoarthritis (OA) model.
- The effects of PTH on subchondral and metaphyseal bone.

Key messages

- Intra-articular PTH ameliorated knee OA in a preclinical age-related OA model.
- PTH improved spontaneous OA by directly affecting the cartilage by preserving glycosaminoglycan and reducing apoptosis of chondrocytes rather than the subchondral or metaphyseal bone in this preclinical age-related OA model.

Strengths and limitations

- This the first study to confirm the treatment effect of intra-articular PTH in a preclinical age-related OA model.

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- The treatment has a direct intra-articular effect on chondrocytes and matrix rather than on the subchondral or metaphyseal bone.
- The molecular mechanisms were not further investigated due to limited antibody in guinea pigs.

Introduction

Osteoarthritis (OA) is a crucial disease in the field of orthopaedics and is the most prevalent joint disease worldwide,[1] affecting nearly 27 million adults in the USA.[2] Age is a prominent risk factor for OA.[3,4] With medical advances, people live longer; thus, the elderly population has increased drastically. OA has no cure, and current treatments target symptomatic relief of pain, but ultimately, the only option is arthroplasty and reduced quality of life.

The main pathogenesis of OA is characterized by phenotypic changes in the articular chondrocytes in which they experience terminal differentiation and eventually undergo apoptosis in growth plate cartilage.[5,6] This leads to degradation of the cartilage matrix, chondrocyte death, and loss of cartilage integrity.[7,8] Moreover, OA affects the whole joint, including the articular cartilage and subchondral bone.[9-18] Subchondral sclerosis is common in late-stage OA; however, some studies have observed thinning of the subchondral plate with increased porosity and deteriorated subchondral trabeculae with decreased bone density in early-stage OA.[15,19-22] Therefore, the role of subchondral bone in OA remains unclear.

Parathyroid hormone (PTH) is the most important regulator of calcium homeostasis through its direct action on the bones and kidneys.[23] Daily subcutaneous administration of the 1–34 amino acid segment of PTH, a PTH analogue, was approved for treatment of postmenopausal osteoporosis.[24] PTH-related protein (PTHrP) maintains chondrocyte proliferation and inhibits the chondrocyte differentiation that leads to hypertrophy.[25,26] PTH analogues and PTHrP both act through the type I PTH receptor to regulate chondrocyte differentiation.[27]

Dunkin Hartley (DH) guinea pigs are widely used as a preclinical OA model because they experience age-related changes in joint pathology similar to those seen in human OA.[28] A high dose of daily subcutaneous PTH (1-34) improved subchondral bone integrity and thus improved OA.[29] We previously found that PTH increased the messenger RNA (mRNA) expression of type II collagen (Col II) and reduced the mRNA expression of type X collagen (Col X) in chondrocytes in vitro.[30] We also determined that a low dose of intra-articular PTH (1-34) prevented chondrocyte apoptosis and increased the Safranin O-stained area in rats with papain-induced OA.[30] Sampson et al[31] found that systemic recombinant human PTH (1–34) (teriparatide) can alleviate OA in meniscal/ligamentous injury-induced OA model in mice. Moreover, PTH (1-34) ameliorated knee OA through autophagy after anterior cruciate ligament transection (ACLT)[32] and reduced dexamethasone-induced terminal differentiation in human articular chondrocytes.[33] Although systemic PTH can enhance bone formation, a low-dose intra-articular PTH (1-34) may not reach therapeutic concentrations for osteoporosis.

Therefore, we investigated whether intra-articular PTH (1-34) alleviates OA progression in a preclinical age-related OA guinea pig model. Specifically, we investigated the following hypotheses: 1) PTH improves knee function, as determined by a treadmill test; 2) PTH attenuates OA progression, as determined through histological studies; and 3) such effects are not achieved through regulation of subchondral bone.

Table I. Treatment protocol.

Variable	Group 1	Group 2
Purchase	3 m/o for young control and 5.5 m/o for Group 1 control and Group 1 treatment	4 m/o for young control and 6.5 m/o for Group 2 control and Group 2 treatment
Treadmill	5.5 m/o for Group 1 control and Group 1 treatment	6.5 m/o for Group 2 control and Group 2 treatment
Treatment start	6 m/o	7 m/o
Treatment course	Once per week	Once per week
Treatment duration	3 mths	3 mths
Treatment end	9 m/o	10 m/o

m/o, months old.

Methods

Experimental animals. The animal experiment in this study was approved by the institutional animal care and use committee of our institute, and follows ARRIVE guidelines.[34] A total of 15 six-week-old male guinea pigs were purchased and housed under standard laboratory conditions. At the age of six months (Group 1), the guinea pigs were randomized by body weight and their ability to endure the treadmill test into a control (Group 1 control; n = 7) or OA with PTH (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). In another batch study (Group 2), 12 × 6.5-month-old male guinea pigs were purchased and were randomized at seven months old into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8) (Table I). After treatment for three months, all the guinea pigs were euthanized by CO_2 overdose.

Drug treatment. The guinea pigs were administered intra-articular injections of PTH (1-34) or vehicle

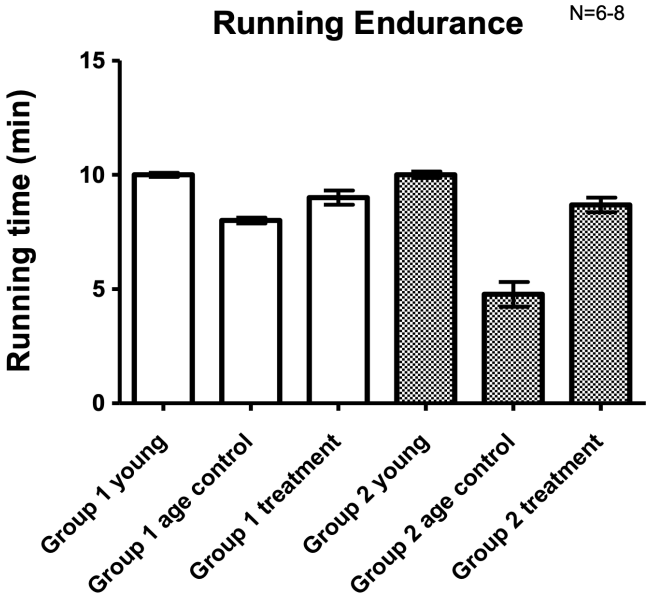

Fig. 1

Parathyroid hormone (PTH) (1-34) improved knee function in treadmill test. At the age of six months (Group 1), guinea pigs were assigned into a control (Group 1 control; n = 7) or osteoarthritis (OA) with PTH (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). Guinea pigs at seven months old (Group 2) were randomized into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8). All guinea pigs received treatment once per week for three months. The guinea pigs in age control group only could endure less time than those in the young group, both in group 1 and group 2 ($p < 0.01$). With treatment of PTH, the guinea pigs could significantly increase the endurance in treadmill test ($p < 0.05$ in group 1 and $p < 0.01$ in group 2) with no significant difference with guinea pigs in young group. **$p < 0.01$ versus Group 1 young group. #$p < 0.05$ versus Group 1 age control group. aa: $p < 0.01$ versus Group 2 age control group. All p-values calculated using one-way analysis of variance.

(phosphate-buffered saline (PBS)) at the age of six months at group 1 and at the age of seven months at group 2. Specifically, 40 µl of PTH (1-34; MilliporeSigma, USA) diluted with PBS to 10 nM (40 ng/ml) or the same volume of the vehicle was injected at a parapatella tendon region into the right knee weekly for three months as in our previous studies.[30,32,35-37]

Treadmill exercise protocol. Before undergoing treatment, the guinea pigs were trained to run on a Columbus Instruments Rodent Treadmill (Columbus, USA) at a speed of 10 to 15 m/min through daily 15-minute sessions for one week.[32,35,37] After training, two treadmill tests were performed each week, and the endurance data were averaged (mean) after treatment. All guinea pigs ran at a speed of 30 m/min. The time at which the running endurance limit was reached was recorded, and the maximal running time was ten minutes. Mild electric shocks (0.2 mA, 400 V, 1 Hz) were applied at the starting position of each lane to discourage the guinea pigs from running outside their lane. Although the shocks were uncomfortable, they did not physically harm the animals.

Bone microarchitecture assessment through microcalculated tomography. All proximal tibiae were scanned and assessed using a microcalculated tomography (µCT) system (SkyScan 1076: SkyScan, Belgium), with voxel size of 18 mm, voltage of 59 kV, exposure time of 440 ms, frame averaging of 1, and beam filtration through a 0.5 mm aluminium filter. After scanning, the knee joint was three-dimensionally reconstructed using SkyScan Nrecon software (SkyScan). For analyzing the subchondral plate, an area of 34 × 17 mm^2 was selected as the region of interest (ROI). Tissue mineral density was calculated using CT-Analyzer software (SkyScan). Morphometric indices of the trabecular bone region were determined from the µCT data through direct 3D morphometry. To analyze the subchondral bone, a trabecular bone cuboid of 1.04 × 1.04 × 0.52 mm^3 beneath the ROI of the subchondral plate was selected. For analysis of the metaphyseal trabecular bone, a cuboid of trabecular bone of 3 × 3 × 3 mm^3 beneath the ROI of the subchondral plate was selected. Percent bone volume was calculated using CTAn software.[38-44]

Histology and immunohistochemistry. After kill, the tibial plateaus and articular cartilage were fixed in 10% neutral buffered formalin and then decalcified in 10% formic acid. Subsequently, 5 µm microsections of the coronary plane were prepared. Glycosaminoglycan (GAG) was stained with Safranin O/Fast Green (1% Safranin O counterstained with 0.75% haematoxylin and then 1% Fast Green; MilliporeSigma) and quantified using Image-Pro Plus 5.0 software (Media Cybernetics, USA). The density of the red-stained area (red area/total area) in each group was calculated.[6,30,32,45] The histological severity of OA was semiquantified through microscopic scoring, as recommended by Osteoarthritis Research Society International (OARSI).[46]

Terminal deoxynucleotidyl transferase dUTP nick end labeling staining. Apoptotic cells in each section were detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining using an in situ Cell Death Detection Kit (Roche, Germany). Sections were incubated with pepsin (0.25%) for 30 minutes at 37°C.[30,32] They were also stained with 4',6-diamidino-2-phenylindole (DAPI) to locate the cells. The DAPI-stained cells (mean 150 (standard deviation (SD) 40)) were counted in the central area of the cartilage in the tibial plateau. The apoptosis rate of chondrocytes was defined as the proportion of red-stained cells (apoptotic cells) among all cells (total red- and blue-stained cells).[32,47]

Immunohistochemistry. The tibiae were processed for indirect immune detection using rabbit polyclonal anti-bone morphogenic protein (BMP)-2 (Abcam, UK) and mouse- and rabbit-specific horseradish peroxidase/diaminobenzidine detection immunohistochemistry (IHC) kits (Abcam), according to the manufacturer's protocol. The sections were then counterstained with haematoxylin to visualize cell nuclei. BMP-2 was stained brown. The sections were then counterstained with haematoxylin to visualize cell nuclei. Under high power magnification, the

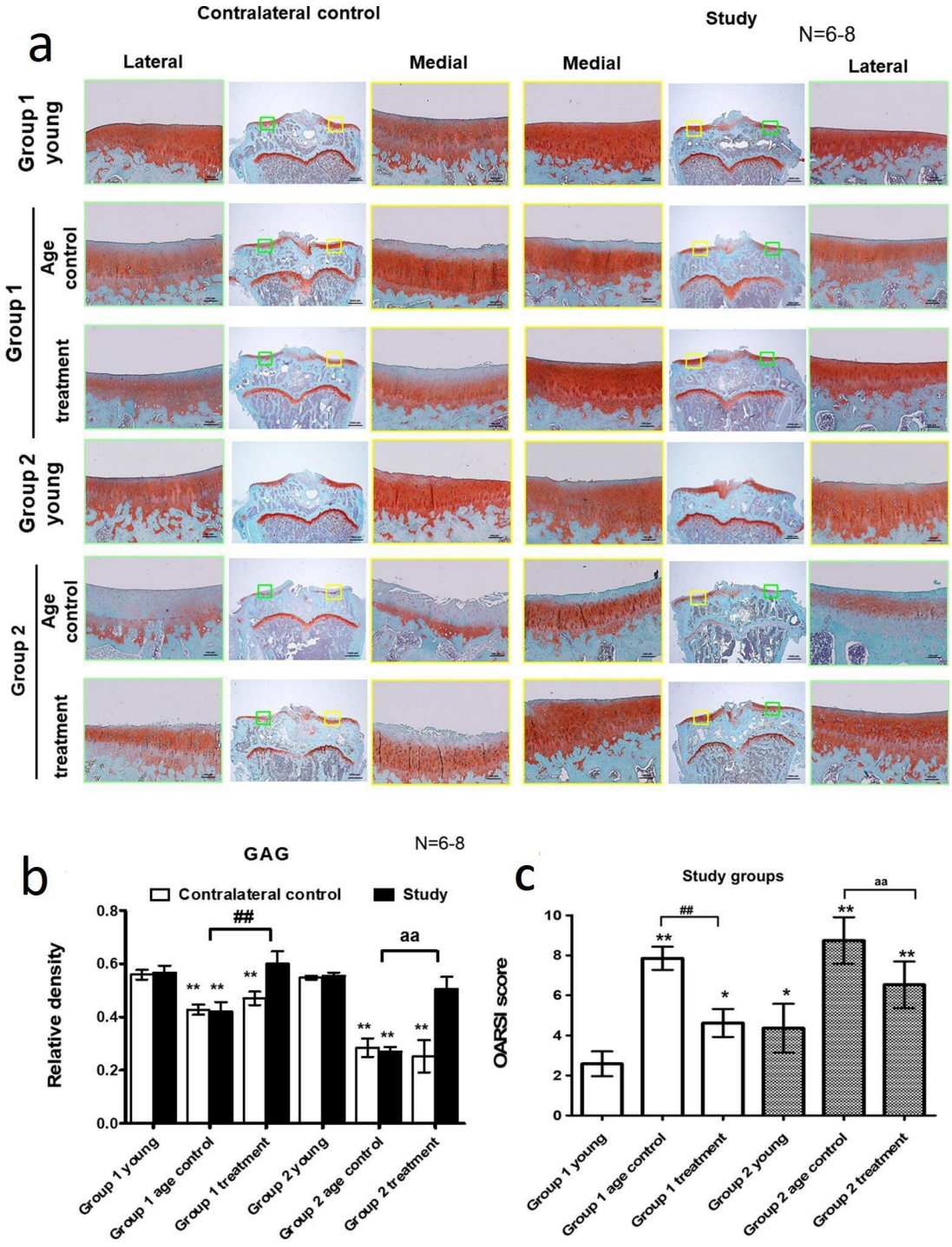

Fig. 2

Effect of parathyroid hormone (PTH) (1-34) on histological appearance and glycosaminoglycan (GAG) level in articular cartilage. a) Representative micrographs of the Safranin-O-stained articular cartilages. At the age of six months (Group 1), guinea pigs were assigned into a control (Group 1 control; n = 7) or osteoarthritis (OA) with PTH (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). Guinea pigs at seven months old (Group 2) were randomized into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8). All guinea pigs received treatment once per week for three months. Representative micrographs of the Safranin-O-stained articular cartilages of the proximal tibia from the study joints in all groups are shown (×1.25 and ×10). b) The relative density of Safranin-O-stained area. The ratio of Safranin-O-stained area to total area (red/total) was measured and compared among groups. Each bar represents the mean and standard error of the mean (SEM) of samples in each group. **p < 0.01 versus Group 1 young group. ##p < 0.01 versus Group 1 age control group. aa: p < 0.01 versus Group 2 age control group. c) The Osteoarthritis Research Society International (OARSI) scores were measured and compared among groups. Each bar represents the mean and SEM of samples in each group. *p < 0.05 versus Group 1 young group. **p < 0.01 versus Group 1 young group. ##p < 0.01 versus Group 1 age control group. aa: p < 0.01 versus Group 2 age control group.

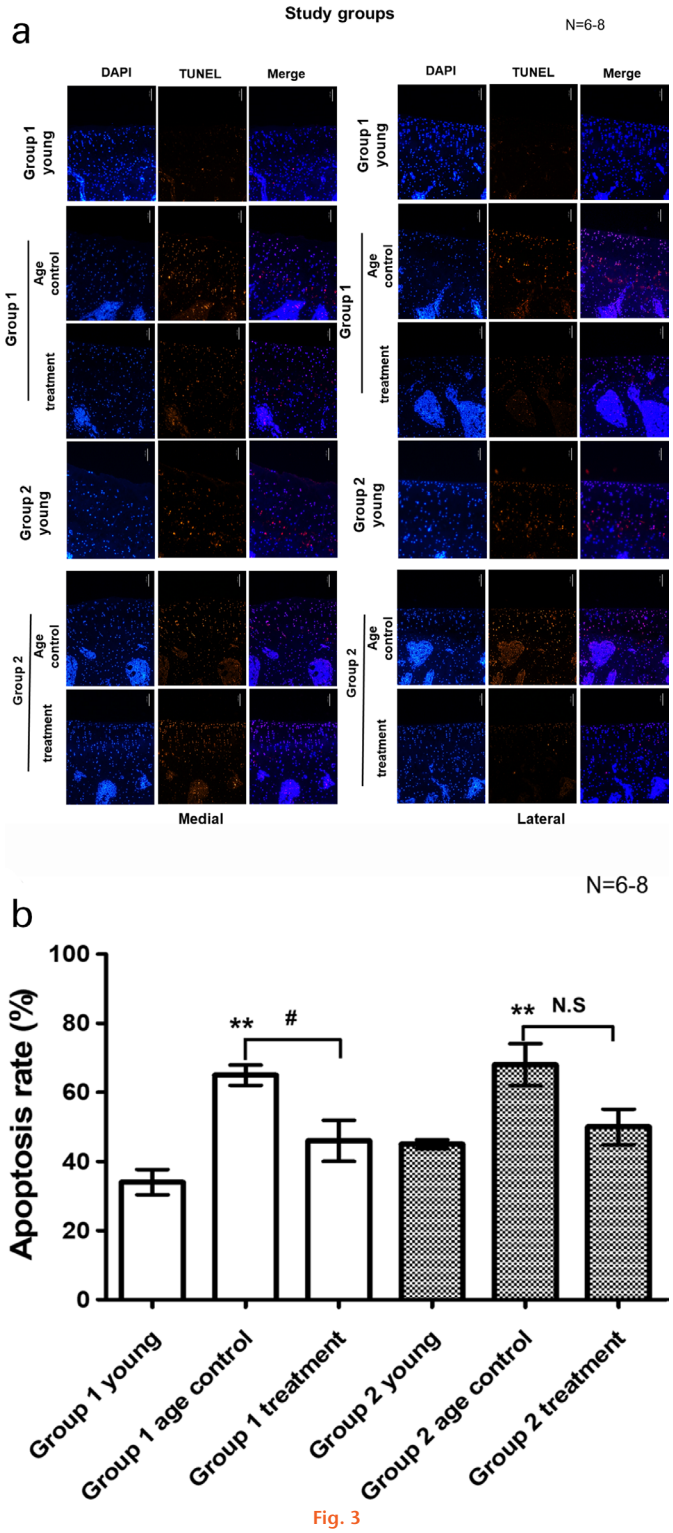

Fig. 3

Effect of parathyroid hormone (PTH) (1-34) on chondrocyte apoptosis in articular cartilage. At the age of six months (Group 1), guinea pigs were assigned into a control (Group 1 control; n = 7) or osteoarthritis (OA) with PTH (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). Guinea pigs at seven months of age (Group 2) were randomized into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8). All guinea pigs received treatment once per week for three months. a) Representative micrographs (×10) of the 4′,6-diamidino-2-phenylindole (DAPI)- and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-stained articular cartilages of the proximal tibia from the study joints of all groups are shown. b) The quantifications of apoptotic rate are shown. Each bar represents the mean and standard error of the mean of samples in each group. **p < 0.01 versus Group 1 young group. #p < 0.05 versus Group 1 age control group. N.S., non-significant.

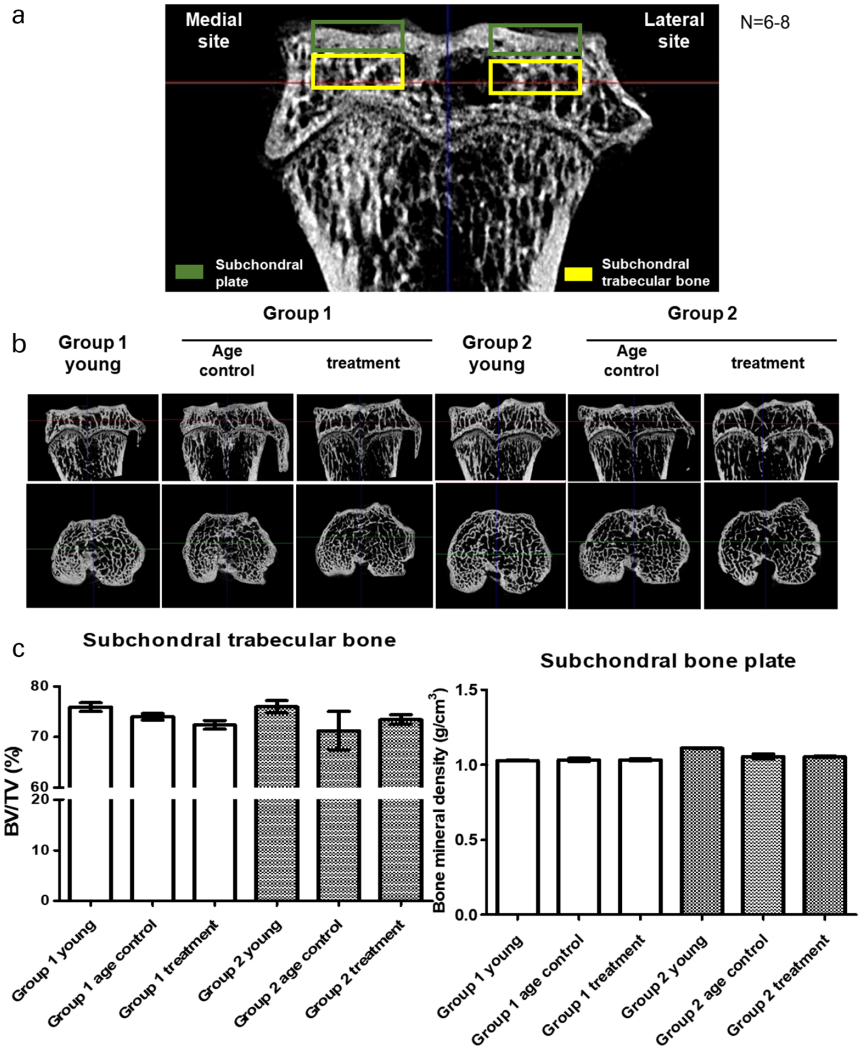

Fig. 4

Effect of parathryroid hormone (PTH) (1-34) on subchondral bone microarchitecture in microcalculated tomography (μCT). At the age of six months (Group 1), guinea pigs were assigned into a control (Group 1 control; n = 7) or osteoarthritis (OA) with PTH (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). Guinea pigs at seven months old (Group 2) were randomized into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8). All guinea pigs received treatment once per week for three months. a) Definition of bone microarchitecture measurement. b) The 3D reconstruction showed the same subchondral bone plate and trabecular bone volume in all groups. c) The quantifications of subchondral trabecular bone volume are shown. There was no significant difference between groups. The quantifications of bone volume (BV)/total volume (TV) in subchondral trabecular bone and bone mineral density in subchondral bone plate volume are also shown, with no significant difference between groups.

BMP-2 stain in the callus area was measured and quantified. [38,40,48,49]

Statistical analysis. All data are presented as means and standard errors (SE). Data were compared using one-way analysis of variance (ANOVA), and multiple comparisons were conducted using the Scheffe post hoc test in SPSS (version 17.1 for Windows; SPSS, USA). Statistical significance was set at $p < 0.05$.

Results

Treadmill test. Greater endurance in the treadmill tests indicated better knee function. In Group 1, the animals in the young and control groups ran for a mean 9.81 (SE 0.10) and 7.97 minutes (SE 0.12), respectively. In Group 2, the young and control groups endured 10.00 (SE 0.14) and 4.77 minutes (SE 0.54) of running, respectively. Notably, the treatment groups of both Groups 1 and 2 exhibited significantly increased endurance levels in the treadmill test, at a mean of 8.97 (SE 0.31) and 8.68 minutes (SE 0.32), respectively, and no significant difference was found between the endurance levels of the treatment and young groups (Figure 1).

Histological and histomorphometric studies and OARSI scores. The representative histological appearances of the Safranin O-stained articular cartilage are depicted in Figure 2a. Overall, the relative densities in the control

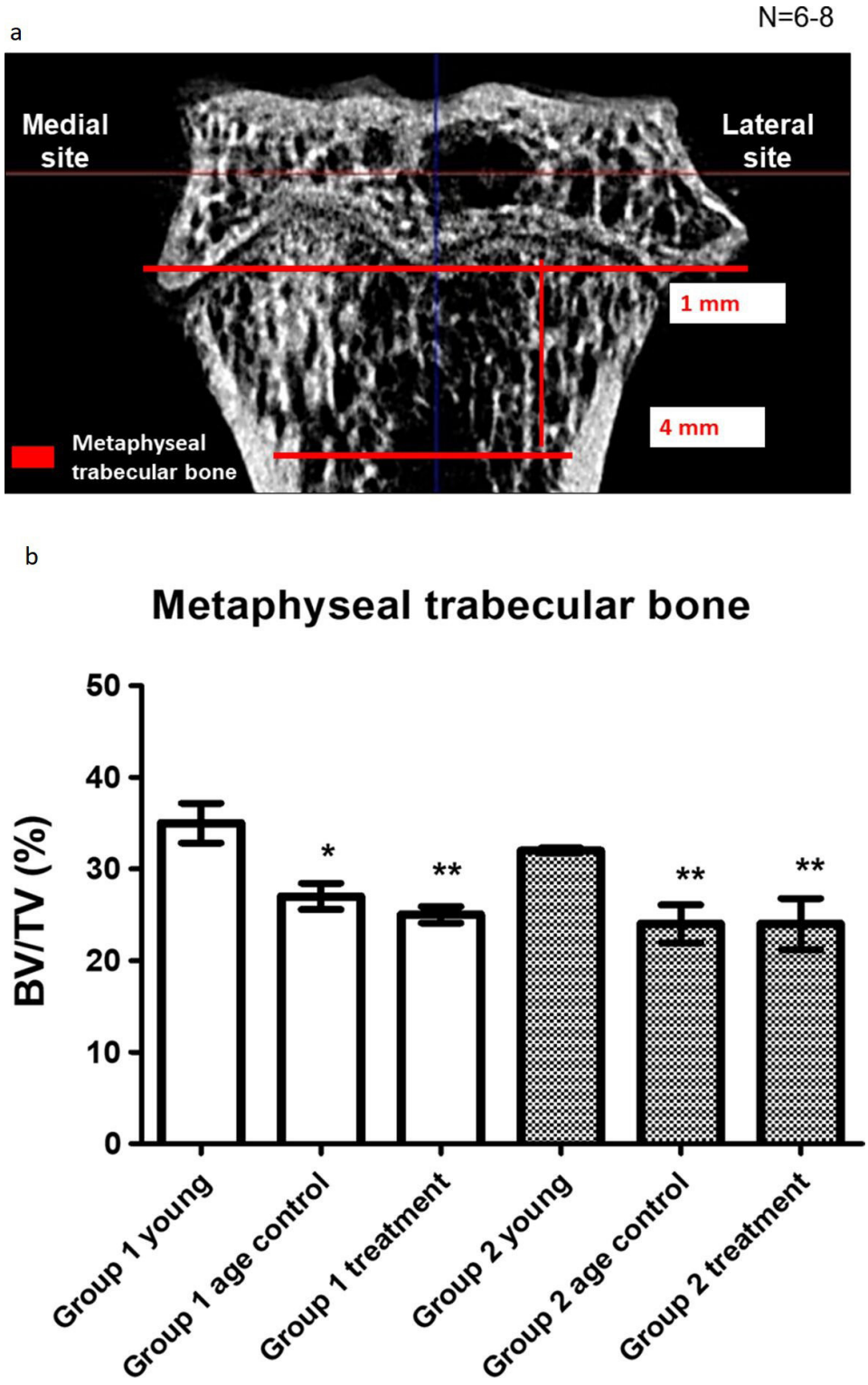

Fig. 5

a) Definition of bone microarchitecture measurement metaphyseal trabecular bone microarchitecture in microcalculated tomography (μCT). At the age of six months (Group 1), guinea pigs were assigned into a control (Group 1 control; n = 7) or osteoarthritis (OA) with parathyroid hormone (PTH) (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). Guinea pigs at seven months old (Group 2) were randomized into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8). All guinea pigs received treatment once per week for three months. b) The quantifications of metaphyseal trabecular volume are shown. Bone volume (BV)/total volume (TV) ratio declined with ageing. There was no significant difference after PTH (1-34) treatment in the same group. Each bar represents the mean and standard error of the mean of samples in each group. *p < 0.05 versus Group 1 young group. **p < 0.01 versus Group 1 young group.

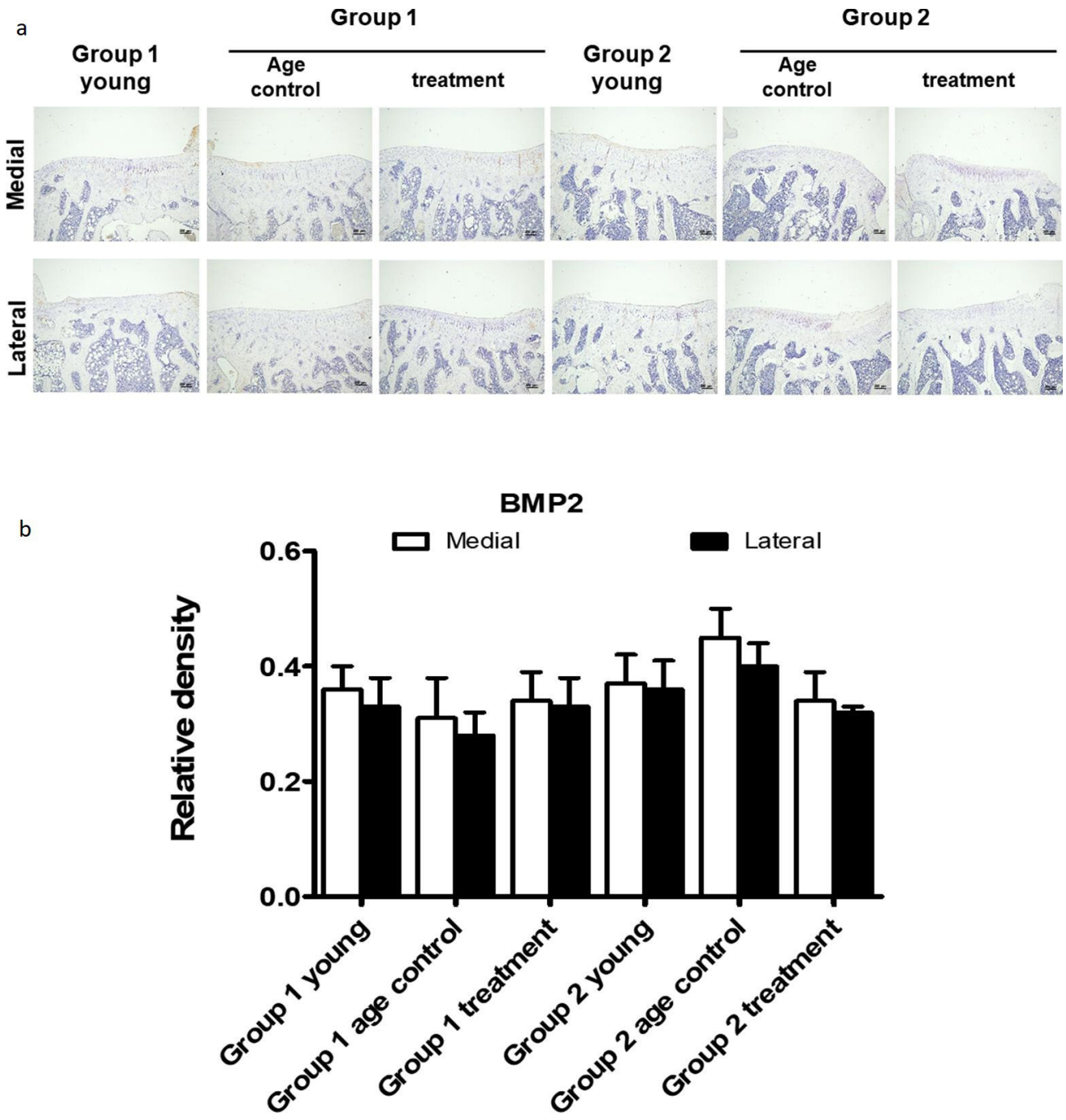

Fig. 6

Effect of parathyroid hormone (PTH) (1-34) on immunolocalized bone morphogenic protein (BMP)-2 in subchondral plate. At the age of six months (Group 1), guinea pigs were assigned into a control (Group 1 control; n = 7) or osteoarthritis (OA) with PTH (1-34) treatment (Group 1 treatment) group (n = 8). Another eight three-month-old guinea pigs were purchased and served as the young control group (Group 1 young; n = 8). Guinea pigs at seven months old (Group 2) were randomized into either the control (Group 2 control; n = 6) or OA with PTH (1-34) treatment (Group 2 treatment) group (n = 6). Another eight four-month-old guinea pigs were purchased and served as the young control group (Group 2 young; n = 8). All guinea pigs received treatment once per week for three months. a) Representative micrographs of immunostained BMP2 on subchondral plate from the study joints of all groups are shown. b) The relative density of BMP2 was measured and compared among groups. Each bar represents the mean and standard error of the mean of samples in each group. There was no significant difference between groups.

groups were significantly lower than those in the young groups in both Groups 1 and 2 ($p < 0.01$, ANOVA; Figure 2). However, the relative density in the Group 1 treatment group was significantly higher than in the control group ($p < 0.01$), with no significant difference between the young and treatment groups. The mean OARSI score of the Group 1 young group (2.58 (SE 0.62)) was lower than that of the corresponding control group

(7.85 (SE 0.58)); however, the score decreased significantly in the treatment group (4.62 (SE 0.70)) but did not reach the level of the young group in Group 1 (Figure 2). Similar results were observed in Group 2.

TUNEL staining. The representative appearances of the TUNEL-stained articular cartilage are depicted in Figure 3a. In Group 1, the apoptosis rate of chondrocytes in the control group (65% (SE 2.95%)) was considerably higher than that in the young group (34% (SE 3.63%); Figure 3b). However, the apoptosis rate of chondrocytes in the treatment group was significantly lower (46% (SE 5.94%)) than that in the control group ($p < 0.05$) and not significantly different from that in the young group (Figure 3b). In Group 2, the apoptosis rate of chondrocytes in the treatment group was lower (50% (SE 5.10%)) than that in the control group (68% (SE 6.09%)); however, this finding did not reach statistical significance ($p = 0.058$).

Bone microarchitecture assessment using μCT. Knee parameters were quantified through μCT. No significant differences were noted among the Group 1 young, control, and treatment groups in the bone densities of the subchondral plate and subchondral trabecular bone. Weekly PTH (1-34) injections did not increase the volume of subchondral bone; no significant difference was observed between the Group 1 control and treatment groups (Figure 4). Similar results were also noted in Group 2.

We also analyzed the volume of metaphyseal trabecular bone in the proximal tibia because it tends to decrease with age. The bone volume of metaphyseal trabecular bone was notably lower in both the Group 1 control and treatment groups compared with the young group. Bone volume was not different between the Group 1 and Group 2 treatment groups (Figure 5).

BMP2 IHC analysis. In the IHC study, immunolocalized BMP2 in the subchondral bone was quantified. There were no significant differences in BMP2 expression after quantification (Figure 6).

Discussion

To the best of our knowledge, this study is the first to determine the effect of intra-articular PTH (1-34) on cartilage degeneration in a preclinical age-related OA model. We demonstrated that weekly intra-articular PTH (1-34) treatment can improve knee function by increasing endurance, preserving GAG, and reducing chondrocyte apoptosis; moreover, it had no effect on the subchondral bone. An intra-articular improvement in cartilage may be the main reason.

Half of all people aged over 65 years are affected by OA.[50] Ageing is the primary risk factor for OA because of age-related loss of normal bone structure and gradual bone microdamage.[4,51,52] Ageing also has profound effects on cellular processes, notably leading to enhanced chondrocyte apoptosis and reduced cellular regeneration.[53] DH guinea pigs often develop cartilage degeneration similar to that seen in human knee OA; therefore, we used it as a preclinical age-related spontaneous OA model.[54,55] DH guinea pigs also demonstrate temporally predictable and spontaneous knee OA similar to human knee OA, and histological and pathological findings include chondrocyte death, articular cartilage surface fibrillation, and osteophyte formation.[56] Histological staining, OARSI scores, and cartilage degeneration at six months demonstrated age-related progressive OA in this DH guinea pig model and progressed through nine months of age, a result similar to a previous study.[28] Intra-articular PTH (1-34) reduced OA progression by preserving GAG and reducing chondrocyte apoptosis.

Subchondral bone plays a crucial role in the initiation and progression of OA.[57] Subchondral bone sclerosis, together with progressive cartilage degradation, is a hallmark of OA.[58] Moreover, subchondral bone deterioration is commonly associated with articular cartilage defects.[59] The microarchitecture of subchondral trabecular bone also changes with age, characterized by decreasing trabecular thickness and bone volume fraction, loss of trabecular connectivity, and increased trabecular separation and bone marrow space volume.[60] A previous study showed that the subchondral bone plate underwent thinning and low and fragile subchondral cancellous bone marrow density decreased (low bone volume/total volume and trabecular thickness) before the onset of cartilage degeneration in DH guinea pigs.[61] Therefore, improving the quality of subchondral bone may be beneficial in the treatment of OA.

PTH (1-34), also known as teriparatide, is an anabolic bone agent that has been used for the treatment of osteoporosis for many years.[24,62-66] It enhances the differentiation, proliferation, and activity of osteoblasts, thereby restoring the synthesis of the bone matrix, increasing the life span of osteoblasts, and inhibiting their apoptosis.[67-69] Yan et al[29] found that daily subcutaneous PTH (1-34) at 15 μg/kg for three and six months from the age of three months reduced the progression of OA in DH guinea pigs. PTH (1-34) treatment increased the bone volume ratio and bone mineral density while slowing the subchondral trabecular bone microarchitectural changes from rod-like to plate-like. PTH (1-34) treatment reduced SOST gene expression in and elevated the osteoprotegerin (OPG)/receptor activator of nuclear factor-kB ligand (RANKL) expression ratio in subchondral trabecular bone. In this study, we found that weekly intra-articular PTH (1-34) alleviated OA progression in DH guinea pigs at a relatively low dose of 1.6 ng/kg and the daily mean dose is 0.23 ng/kg. Weekly application can thus be more convenient if implemented clinically. In one study, PTH (1-34) of > 5 μg/kg induced osteosarcoma in rats in a dose-dependent manner.[70]

The use of teriparatide for osteoporosis treatment is limited to a maximum duration of two years. The Osteosarcoma Surveillance Study was initiated in the USA in 2003 to monitor for a potential association between the osteoporosis treatment teriparatide and osteosarcoma.

The results indicated the incidence of osteosarcoma associated with teriparatide use during the 15-year surveillance period was no different than would be expected based on the background incidence rate of osteosarcoma.[71] This indicated that a low dose of intra-articular PTH (1-34) may be safe.

Though previous studies have found systemic application of teriparatide can ameliorate OA, the dose of teriparatide was not low. The dose was 40 μg/kg/day in Sampson et al,[31] 15 μg/kg/day (five days per week) in Yan et al,[29] and 10 μg/kg/day or 40 μg/kg/day in Shao et al[72] with a minimal treatment period for at least three months. The once and weekly doses are approximately 25,000 and 170,000 times lower than the dose in Sampson et al,[31] and about 9,300 and 46,000 times lower than the dose in Yan et al.[29] Our study makes PTH (1-34) for OA treatment more applicable. In addition, the mechanisms that provided the benefits in the present study differed from those in Yan et al.[29] In this study, preserving GAG and decreasing chondrocyte apoptosis are the main mechanisms through which intra-articular PTH (1-34) mitigated OA progression; the mechanisms were unrelated to improvements in the subchondral and trabecular bone in the proximal tibia. Although PTH (1-34) is a potent anabolic agent that can enhance BMP2 expression in osteogenic cells,[73] we observed no difference in BMP2 expression after PTH treatment in our study. Our results indicated that intra-articular PTH (1-34) did not affect the activity of subchondral osteoblasts. We inferred that with early OA treatment, PTH did not penetrate the subchondral bone and intact tidemark. Furthermore, improvement in knee function was observed, as reflected by endurance in the treadmill test.

In the treatment of osteoporosis, teriparatide should be used intermittently each day rather than continuously to stimulate bone formation.[66,74] Our previous study found the sustained release of PTH/poly(lactic-co-glycolic acid) (PLGA) controlled-release carrier formulation continuous release of PTH (1–34) at 0.1 to 100 nM can suppress the OA progression (decreasing GAG and Col II and increasing Col X) in rat knee cartilage.[36] In this study, the effects of PTH (1–34) in the guinea pigs are similar to those in rats, both in a papain-induced OA model and an ACLT-induced OA model.[30,32,36] Taken together, both intermittent and continuous intra-articular use of PTH (1-34) can attenuate OA progression in different animal models.

In the present study, weekly low-dose (0.4 pmol) intra-articular PTH (1-34) ameliorated cartilage degradation in a preclinical age-related OA model through a direct cartilage effect rather than subchondral or metaphyseal bone effects. In addition, knee mobility and articular cartilage quality can improve with the PTH (1-34) treatment. The treatment for OA would potentially need to be continued for many years, provided that treatment could be started early in the course of OA. It may be preferable to use low-dose PTH rather than high-dose PTH, which is approved for osteoporosis limited to two years, and this study has shown that intra-articular low-dose PTH is effective at least in guinea pigs. The study has also provided evidence for a different mechanism of PTH acting directly on cartilage rather than on subchondral bone.

Supplementary material

An ARRIVE checklist is included to show that the ARRIVE guidelines were adhered to in this study.

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- L. Kang: Conceptualization, Formal analysis, Writing - original draft.
- L-H. Chang: Investigation, Formal analysis, Writing - original draft.
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