


Intra-Articular Injections of Curcumin Monoglucuronide TBPI901 Suppresses Articular Cartilage Damage and Regulates Subchondral Bone Alteration in an Osteoarthritis Rat Model

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Abstract

Objective. Curcumin monoglucuronide (TBPI901) is highly water soluble and can convert to free form curcumin, which has pharmacological effects, on intravenous administration. This study aimed to investigate the effectiveness of TBPI901 intra-articular injections in an osteoarthritis (OA) rat model. **Methods.** Sixty-four male Wistar rats (12 weeks old) who underwent destabilized medial meniscus (DMM) surgery were randomly separated into the TBPI901 injection or saline solution (control) injection group. They were sacrificed at 1, 2, 6, or 10 weeks postoperatively (weeks 1, 2, 6, and 10; $n = 8$ for each group). TBPI901 (30 mg/mL) or saline solution of 50 μ L was injected into the knee joints twice a week during weeks 1 and 2 to investigate the effects in the acute phase of posttraumatic (PT) OA or once a week during weeks 6 and 10 to investigate it in the chronic phase of PTOA. Histology, immunohistochemistry, and micro-computed tomography were performed to evaluate the changes in OA. **Results.** TBPI901 injections significantly reduced synovial inflammation at weeks 1 and 2, and tumor necrosis factor- α expression in the articular cartilage at week 6. The TBPI901 injections also significantly suppressed articular cartilage damage, subchondral bone (SB) plate thickening, SB plate perforation, and osteophyte formation at week 10. **Conclusions:** TBPI901 intra-articular injections suppressed synovial inflammation in the acute phase of PTOA in DMM rats. In the chronic phase, TBPI901 suppresses articular cartilage damage and regulates SB plate changes.

Keywords

TBPI901, curcumin monoglucuronide, osteoarthritis, intra-articular injection, articular cartilage

Introduction

Osteoarthritis (OA) is a progressive joint disease characterized by articular cartilage loss, subchondral bone (SB) alterations, and osteophyte formation. OA progression affects an individual's activities of daily living. Inflammation plays a central role in OA pathogenesis. It contributes to chondrocyte apoptosis, extracellular matrix disruption, and cartilage degeneration through pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF- α).¹⁻⁴

Curcumin, a polyphenol compound present in turmeric (*Curcuma longa*, long used as a spice and herbal medicine), has many positive health benefits, including anti-inflammatory,⁵ antioxidative,⁶ anticancer,⁷ and antidepressant⁸ effects. Oral curcumin ingestion for OA was shown to reduce pain and improve function in clinical studies.⁹⁻¹¹ Animal studies

have shown that curcumin inhibits OA progression by decreasing inflammation by blocking the Toll-like receptor/nuclear factor- κ B (TLR4/NF- κ B) signaling pathway and inhibiting chondrocyte apoptosis through enhanced

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autophagy.¹²⁻¹⁶ Curcumin has few side effects; therefore, its use is expected to be a potential adjunct to pharmaceutical therapies. On the other hand, orally ingested curcumin has low bioavailability because of limited absorption with low hydrophilicity and conjugation in the intestinal tract.¹⁷⁻²⁰ Only a few ingested curcumin molecules are absorbed and present in the conjugated form (mainly curcumin monoglucuronide [CMG]) in the blood.^{21,22} To improve its low absorption, bio-optimization by liposomal delivery systems, chemical modification of curcumin, nanoparticulation of curcumin, and use of polysorbate as an emulsifier have been performed.²³⁻²⁶ However, the low absorbability and hydrophilicity remain, although their efficacy is greater than that of pure curcumin. To overcome the disadvantages of oral curcumin ingestion, intra-articular administration, an ideal drug delivery method commonly used in OA therapies, has been used to deliver a concentrated therapeutic dose of curcumin into the knee joint and has been shown to suppress articular cartilage degeneration and synovial inflammation in an OA rat model.^{24,27} We hypothesized that the intra-articular administration of CMG could overcome the issue of low hydrophilicity due to its high water solubility, could be absorbed into the deep layer of the cartilage and maximize its pharmacological effects. CMG exhibits 10-fold lower antioxidative activity than unconjugated, free form curcumin.²⁸ Therefore, CMG must be metabolized to free form curcumin *in vivo* to execute its antioxidative activity.²⁹ β -Glucuronidase is an important hydrolase for the deconjugation of CMG to free form curcumin. It is released from neutrophils, injured cells at sites of inflammation, and cells in bone.³⁰⁻³³ In addition, β -glucuronidase secretion is enhanced in the articular cartilage, synovium, and synovial fluid with inflammation and OA.³⁴⁻³⁷ Therefore, administered CMG would be deconjugated to free form curcumin in OA joint. To confirm our hypothesis, we utilized the synthesized CMG (TBP1901), which has already been investigated and demonstrated the efficacy of intravenous administration in tumor-bearing mice with colorectal cancer.²⁹ TBP1901, which has high water solubility, may be deconjugated to free form curcumin in OA joint and suitable for intra-articular administration. Although the efficacy of intra-articular administration of TBP1901 is unknown, the pharmaceutical value of curcumin will be enhanced if it is shown to have an anti-osteoarthritic effect in intra-articular administration.

To verify the efficacy of TBP1901, we chose rats with destabilized medial meniscus (DMM) as an OA animal model.^{38,39} Although posttraumatic (PT) OA models include anterior cruciate ligament transection, meniscus resection, combination with ligament transection and meniscectomy, and DMM model,⁴⁰ the DMM model was chosen to be the slower progress model and closer to human OA progression.³⁸ PTOA has an acute phase and a chronic phase for each period from trauma.⁴¹ Although curcumin is effective

for treating chronic inflammation,⁴² it was recently reported as effective for treating acute inflammation.⁴³ Therefore, to comprehensively confirm the efficacy of TBP1901 in DMM rats, we observed its effects from the acute phase to the chronic phase of PTOA.

This study aimed to investigate the effectiveness of intra-articular administration of TBP1901 on inflammation and OA progression in a rat model with DMM.

Methods

Animal Preparation and Surgical Procedures

This study was conducted in accordance with the ARRIVE guidelines.⁴⁴ Sixty-four male Wistar rats (12 weeks old), purchased from SHIMIZU Laboratory Supplies Co. Ltd. (Kyoto, Japan), were placed in a plastic cage with paper bedding on a 12-hour light/dark cycle at a constant temperature. The rats could move freely in cages and had free access to food and water. The rats were randomly separated into TBP1901 and saline solution (control) intra-articular injection groups ($n = 32$ for each group). All rats underwent DMM surgery for the OA model.^{38,39} Briefly, DMM surgery was performed with an anteromedial capsule incision and transection of the medial meniscotibial ligament in the right knee under anesthesia with 1.0 mL/kg pentobarbital sodium (Somnopenyl; Kyoritsu Seiyaku Corp., Tokyo, Japan). The DMM rat model rapidly progresses to moderate OA with severe inflammation by 4 weeks postoperatively and to severe OA by 8 weeks postoperatively, by which time inflammation is milder.^{39,45,46} Therefore, the rats were sacrificed at 1 and 2 weeks postoperatively to confirm the effect of TBP1901 as the acute phase of PTOA and at 6 and 10 weeks postoperatively to confirm the effect as the chronic phase of PTOA ($n = 8$ for each).

The synthesis of TBP1901 was performed as described previously.²⁹ TBP1901 and saline intra-articular injections of 50 μ L were administered to the right knee joints through the patellar tendon. The concentration of TBP1901 was chosen to be 30 mg/mL (1.5 mg/50 μ L) based on a previous study,²⁹ which showed no abnormalities in mice administered with 125 mg/kg of TBP1901, and a preliminary study (Supplementary Fig. S1). The preliminary study showed no significant effects on TBP1901 injections at 10 mg/mL for 6 weeks in DMM rats. The rats sacrificed at 6 and 10 weeks postoperatively were injected once a week from 1 week postoperatively. All rats were injected an hour before sacrifice to confirm intra-articular curcumin by fluorescence observation. The rats sacrificed at 1 and 2 weeks postoperatively were injected twice a week since the observation periods were short. The rats sacrificed at 1 week were injected on days 3 and 7, and the rats sacrificed at 2 weeks were injected on days 3, 7, 10, and 14. One hour after the final intra-articular injection in each observation period, the rats

were sacrificed by a lethal dose of pentobarbital sodium, and their right knee joints were harvested for micro-computed tomography (micro-CT), histochemical, and immunohistochemical analyses. Body weight was measured weekly for all rats.

Micro-CT Analysis

After fixation of the knees with 4% paraformaldehyde overnight, the tibiofemoral (TF) joints were scanned using a micro-CT system (SMX-100CT, Shimadzu, Kyoto, Japan) at 43 kV and 43 μ A with a scan time of approximately 10 minutes. After scanning, the 3-dimensional (3D) reconstruction and assessment of the TF joint were performed using ImageJ (National Institutes of Health, Bethesda, MD, USA) and Amira (version 5.5, EFI Visualization Science Group, Burlington, MA, USA) software. The SB plate thickness, number of SB plate perforations (holes), diameter of SB plate perforations, bone volume (BV), total volume (TV), and osteophyte volume in the medial tibia SB (above epiphyseal plate) were calculated according to protocols described in previous studies.^{39,47} SB plate thickness was measured at the thickest region around the center of the medial articular surface. The diameter of the SB plate perforation was defined as the largest diameter of the largest perforation in each sample. To assess the bone density of the SB, bone volume was divided by the total volume (BV/TV).

Histological Analysis

After micro-CT, the knee joint samples were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) for 3 to 4 weeks and cut along the mid-sagittal plane at the halfway point. The DMM model causes rapid OA progression in the TF joint and also affects the patellofemoral (PF) joint.⁴⁸ Therefore, the histological evaluation was performed on the TF and PF joints. After decalcification, the samples were paraffin-embedded and cut into 6- μ m sections at 50- μ m intervals. Fluorescence histochemistry was performed using sections with an epifluorescence microscope (MVX10, Olympus Corporation, Tokyo, Japan) to confirm the presence of curcumin in the articular cartilage and synovium. Curcumin is a fluorescent substance with an excitation wavelength of 300 to 550 nm (maximum excitation wavelength: 467 nm) and an emission wavelength of 548 to 600 nm (maximum emission wavelength: 571 nm).⁴⁹ Fluorescence detection is used for curcumin quantification.⁵⁰ Based on these reports, curcumin fluorescence was observed with the mirror unit of U-MYFPHQ/XL (Olympus Corporation, Tokyo, Japan). In addition, other paraffin sections were stained with hematoxylin and eosin (H&E) to evaluate inflammation of the synovium in knee joints and safranin O/fast green to evaluate the severity of cartilage

degeneration. Inflammation was assessed using an inflammation scoring system described in a previous study.⁵¹ Three membrane features (synovial lining cell layer, stroma cell density, and inflammatory infiltrate) were assessed in the whole knee joint as 0 (none), 1 (slight), 2 (moderate), or 3 (strong). The inflammation score was determined as the summed score for all parameters. The values of the parameters were summarized and interpreted as follows: 0-1, no synovitis; 2-4, low-grade synovitis; and 5-9, high-grade synovitis. Cartilage degeneration was assessed using the Osteoarthritis Research Society International (OARSI) score⁵² and the modified Mankin (MM) score.⁵³ The OARSI score consists of 6 grades and 4 stages on a scale from 0 (intact) to 24 (severe damage). The MM score consists of 3 features (pericellular matrix staining, spatial arrangement of chondrocytes, and interterritorial matrix staining) on a scale from 0 (intact) to 8 (severe). Cartilage degeneration was evaluated in the medial TF and PF joints. The maximum score was used for all scoring systems and samples.

Immunohistochemical Analysis

Immunohistochemistry of type I collagen, type II collagen, IL-1 β , IL-6, and TNF- α was performed to determine the principal collagen expression and inflammation in the articular cartilage. Antigen retrieval was performed for 20 minutes by heating with HistoVT One (Nacalai Tesque, Inc., Kyoto, Japan). Blocking was performed using 0.3% H₂O₂ for 15 minutes and 5% goat serum for 20 minutes at room temperature. The sections were then incubated at 4 °C overnight with primary antibodies against type I collagen (AB755P, diluted 1:1000; Merck KGaA, Darmstadt, Germany), type II collagen (diluted 1:100; Kyowa Pharma Chemical Co., Ltd., Toyama, Japan), IL-1 β (ab9722, diluted 1:100; Abcam Co., Cambridge, UK), IL-6 (ab9324, diluted 1:250; Abcam Co., Cambridge, UK), and TNF- α (ab6671, diluted 1:100; Abcam Co., Cambridge, UK). Detection was performed using the streptavidin-biotin-peroxidase complex technique with an Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was visualized by incubation with a diaminobenzidine solution (Vector Laboratories, Burlingame, CA, USA) followed by counterstaining with hematoxylin. The primary antibody was omitted from negative control slides. The expression of type I and type II collagen in the medial tibia and patella cartilage was analyzed by measuring the minimum and mean pixel intensity values using TIFF images (magnification \times 40) taken by microscopy and ImageJ, respectively, on a scale of 0 (minimum; dark) to 255 (maximum; no staining), according to a previous study.⁵⁴ The expression of IL-1 β , IL-6, and TNF- α in the medial tibia and patella cartilage was analyzed by measuring the percentage of positive chondrocytes to detect the severity of cartilage inflammation within the middle region of the medial tibia with an anteroposterior

width of 0.5 mm or the central region of the patella with a superoinferior width of 0.5 mm.

Statistical Analyses

The median and interquartile range (IQR) of the inflammation score, OARSI score, and MM score (nonparametric data) were calculated for each group. The Wilcoxon test was used to compare differences between the 2 groups. In addition, the mean and 95% confidence intervals (CIs) of body weight, immunohistochemical, and micro-CT analysis data (parametric data) were calculated, and Welch's *t* test was used to compare differences between the groups. The normality of the data was assessed using normal quantile–quantile plots and the Shapiro-Wilk test. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using JMP Pro 14 software (SAS Institute Inc., Cary, NC, USA).

Results

Body weight is shown in Supplementary Figure S2. Body weight in the TBP1901 group was significantly lower than that in the saline control group at week 1 (mean [95% CIs]: 276.59 g [272.65–280.54]; TBP1901, 282.31 g [278.40–286.23]; control, $P = 0.040$), but the difference was small. There were no significant differences between the groups at weeks 2, 6, and 10. No other adverse events were observed.

Histology

The fluorescence expression of curcumin was confirmed on the tibial articular cartilage and was found on the synovium in the TBP1901 group but not in the saline control group (Fig. 1). Severe synovial inflammation was observed at weeks 1 and 2 as an enlargement of the synovial lining cell layer, increased cellularity, and situated lymphocytes or plasma cells, especially in the saline control (Fig. 2). TBP1901 Intra-articular injections significantly reduced the synovial inflammation at week 1 ($P = 0.007$) and week 2 ($P < 0.001$). However, no significant effects were observed after 6 weeks with mild inflammation.

Articular cartilage damage of the medial tibia was found as fissures and matrix staining depletion at week 1 in both groups. Cartilage damage did not change much until week 6 in both groups. In week 10, the cartilage damage in most of the TBP1901 did not progress, but it progressed and eroded in most of the saline control (Fig. 3A). The OARSI and MM scores in the TBP1901 group were significantly lower than those in the saline control group at week 10 ($P = 0.014$ and $P < 0.001$, respectively). In the patella, the cartilage in the saline control group showed fibrillation and matrix staining depletion at all observation periods (Fig. 3B). However, the

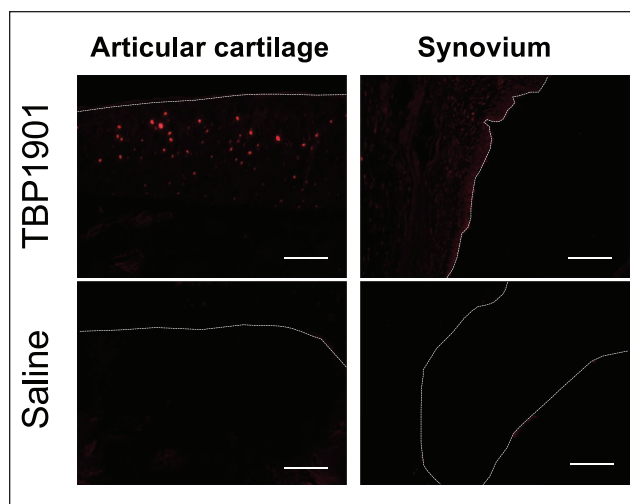


Figure 1. Fluorescence microscopy images of the articular cartilage and synovium of knee joints. Representative histological images. Curcumin fluorescence (red) was confirmed in the articular cartilage and slightly in the synovium of rats injected with TBP1901 intra-articularly. The dotted line indicates the surface line of the articular cartilage and synovium. Magnification: $\times 125$. Scale bar: 100 μm .

cartilage damage in the TBP1901 group at weeks 1 and 10 was lower than that in the saline control group. At week 1, the MM score was significantly lower than that in the saline control group ($P = 0.025$), and at week 10, the OARSI score was significantly lower than that in the saline control group ($P = 0.008$).

Immunohistochemistry

Tibiofemoral Joint. The expression of type I collagen was confirmed on the surface of the articular cartilage in the medial tibia during all observation periods. There were no significant differences between the groups (Fig. 4A). The expression of type II collagen in both groups at weeks 1 and 2 was strong in the surface layer of the cartilage, while it was weakly expressed in the other layers. There were no significant differences in mean intensity at weeks 1, 2, and 6 between the groups. However, at week 10, TBP1901 intra-articular injections significantly increased type II collagen expression ($P = 0.003$).

The expression of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α was confirmed in the articular cartilage (Fig. 5). The percentage of their positive chondrocytes increased from week 1 to 10 in both groups. There were no significant differences between groups at weeks 1, 2, and 10, but TBP1901 intra-articular injections significantly reduced the TNF- α expression at week 6 ($P = 0.020$). In addition, TBP1901 intra-articular injections showed a tendency to reduce IL-6 expression at week 6.

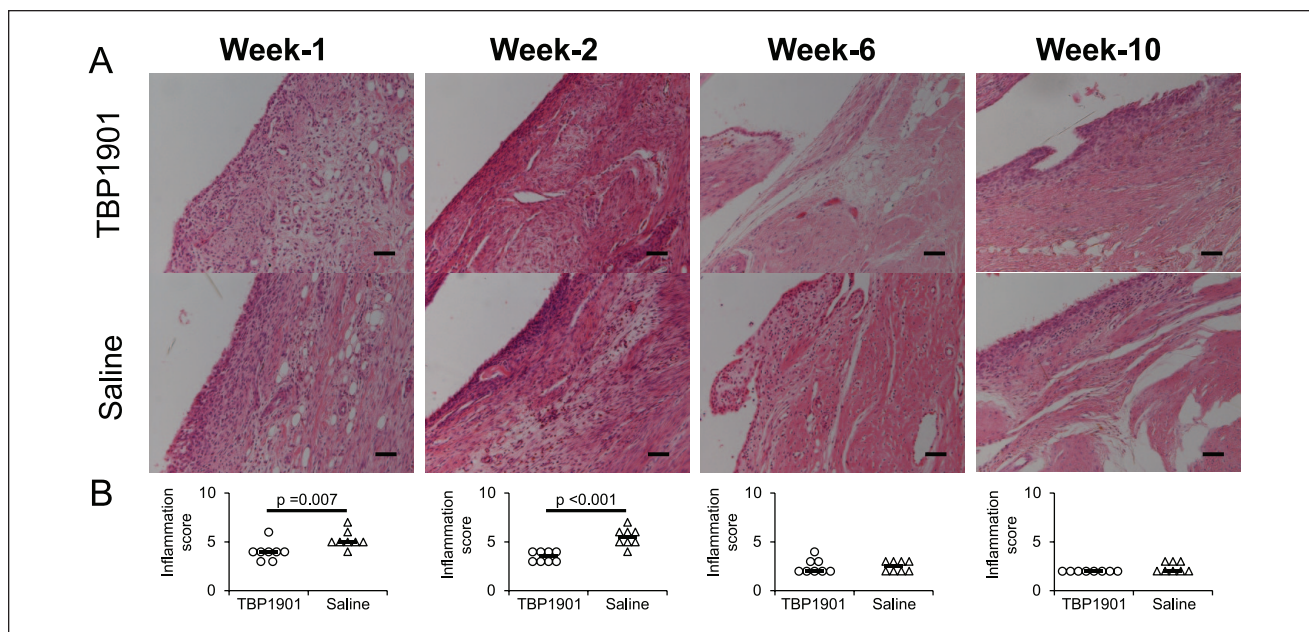


Figure 2. Histological images stained with hematoxylin and eosin and synovial inflammation scores in knee joints. **(A)** Representative histological images of the knee synovium are shown. Severe synovial inflammation was observed at weeks 1 and 2 as enlargement of the synovial lining cell layer, increased cellularity, and situated lymphocytes or plasma cells, especially in the saline control group. Magnification: $\times 200$. Scale bar: 100 μm . **(B)** TBP1901 intra-articular injections significantly reduced the inflammation scores at weeks 1 and 2 compared with the saline control group. Values are the medians in the TBP1901 and the saline control group at several time points ($n = 8$). P values were calculated using the Wilcoxon test.

Patellofemoral Joint. The expression of type I collagen was almost undetectable in the patellar articular cartilage at weeks 1 and 2. At week 6, it was confirmed in the perichondrocytes and the middle layer of the cartilage at week 6 in the TBP1901 group. At week 10, it was confirmed on the cartilage surface (**Fig. 4B**). There were no significant differences between the groups at any time point. Type II collagen in the patella was stably expressed throughout the cartilage, unlike that in the tibia. There were no differences between the groups at weeks 1, 2, and 6. However, at week 10, TBP1901 intra-articular injections significantly increased type II collagen expression ($P = 0.016$).

The pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α were more highly expressed in the articular cartilage of the patella than in the tibia (**Fig. 6**). The percentage of positive chondrocytes decreased slightly from week 1 to 10. There were no significant differences between the groups at weeks 1, 2, and 10, but TBP1901 intra-articular injections significantly increased IL-6 and IL-1 β expression at week 6 ($P = 0.001$ each). However, TNF- α expression in the TBP1901 group showed a tendency to decrease at week 6.

Micro-CT Analysis. DMM surgery with saline or TBP1901 injections induced SB changes and osteophyte formation (**Fig. 7A and B**). There were significant differences in the SB plate thickness at week 2 ($P = 0.002$), week 6 ($P = 0.029$), and week 10 ($P = 0.002$) between the groups (**Fig. 7B**). In

particular, TBP1901 intra-articular injections reduced the thickness at week 10 compared with the saline control. SB plate perforations were observed in both groups, especially after 6 weeks (**Fig. 7A**). There were no differences in the number and diameter of SB plate perforations at week 6 between the groups. However, at week 10, TBP1901 intra-articular injections significantly reduced them compared to the control group ($P < 0.001$ and $P = 0.001$, respectively; **Fig. 7B**).

The BV/TV tended to increase from week 1 to week 10 in both groups (**Fig. 7B**). However, TBP1901 injections delayed the timing of the increase and decrease in BV/TV. In the TBP1901 group, the BV/TV was significantly decreased at week 2 ($P = 0.013$) and significantly increased at week 6 ($P = 0.004$) compared with the control group. There was no significant difference at week 10 between the groups.

The osteophytes had formed from week 2 (quantitative data not shown). In the TBP1901 group, the osteophyte volume tended to be lower than that in the control group at week 6. In week 10, that in the TBP1901 group was significantly lower than that in the control group ($P < 0.001$).

Discussion

In this study, we performed an intra-articular injection of TBP1901 for 1, 2, 6, or 10 weeks in DMM rats to investigate

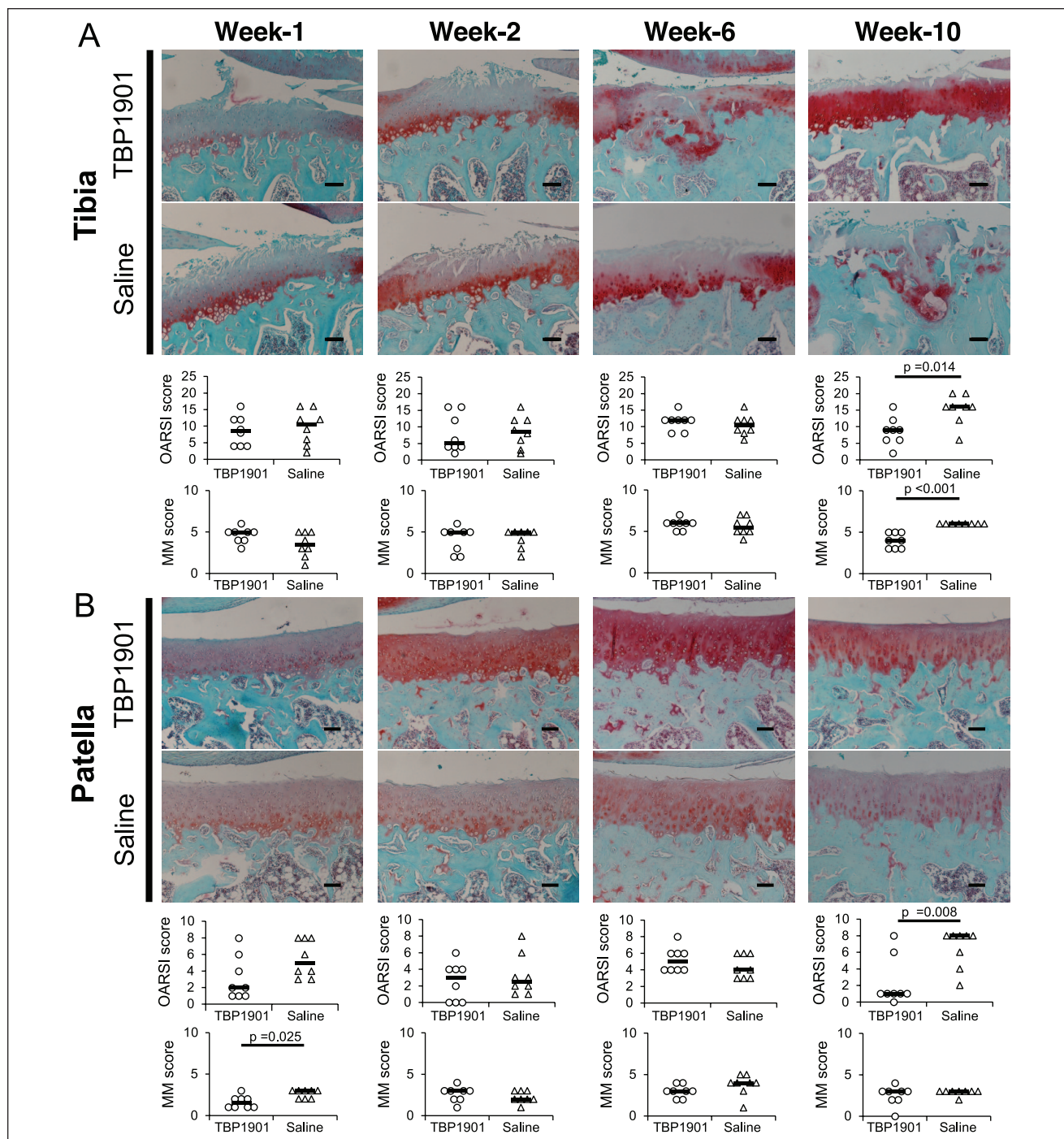


Figure 3. Histological images of safranin O/fast green–stained specimens and osteoarthritis (OA) scores. **(A)** Representative histological images of the articular cartilage and the Osteoarthritis Research Society International (OARSJ) score and the modified Mankin (MM) score on the tibial plateau are shown. At week 10, the cartilage structure in the TBPI901 was relatively preserved. Furthermore, the cartilage matrix staining and the chondrocyte cellularity in the TBPI901 group were less reduced than those in the saline control group. There were no significant intergroup changes in OA scores until week 6. However, TBPI901 intra-articular injections significantly reduced the OARSJ score and the MM score at week 10. **(B)** Representative histological images of the articular cartilage, the OARSJ score, and the MM score on the patella are shown. At week 1, the matrix staining in the TBPI901 group was less reduced than that in the saline control group. TBPI901 intra-articular injections significantly reduced the MM score in the patella at 1 week. Furthermore, at week 10, TBPI901 intra-articular injections reduced the fibrillation of the cartilage surface, and the OARSJ score was significantly lower than that in the saline control. Values are the medians in the TBPI901 and saline control groups at several time points ($n = 8$ each). P values were calculated using the Wilcoxon test. Magnification: $\times 100$. Scale bar: 100 μm .

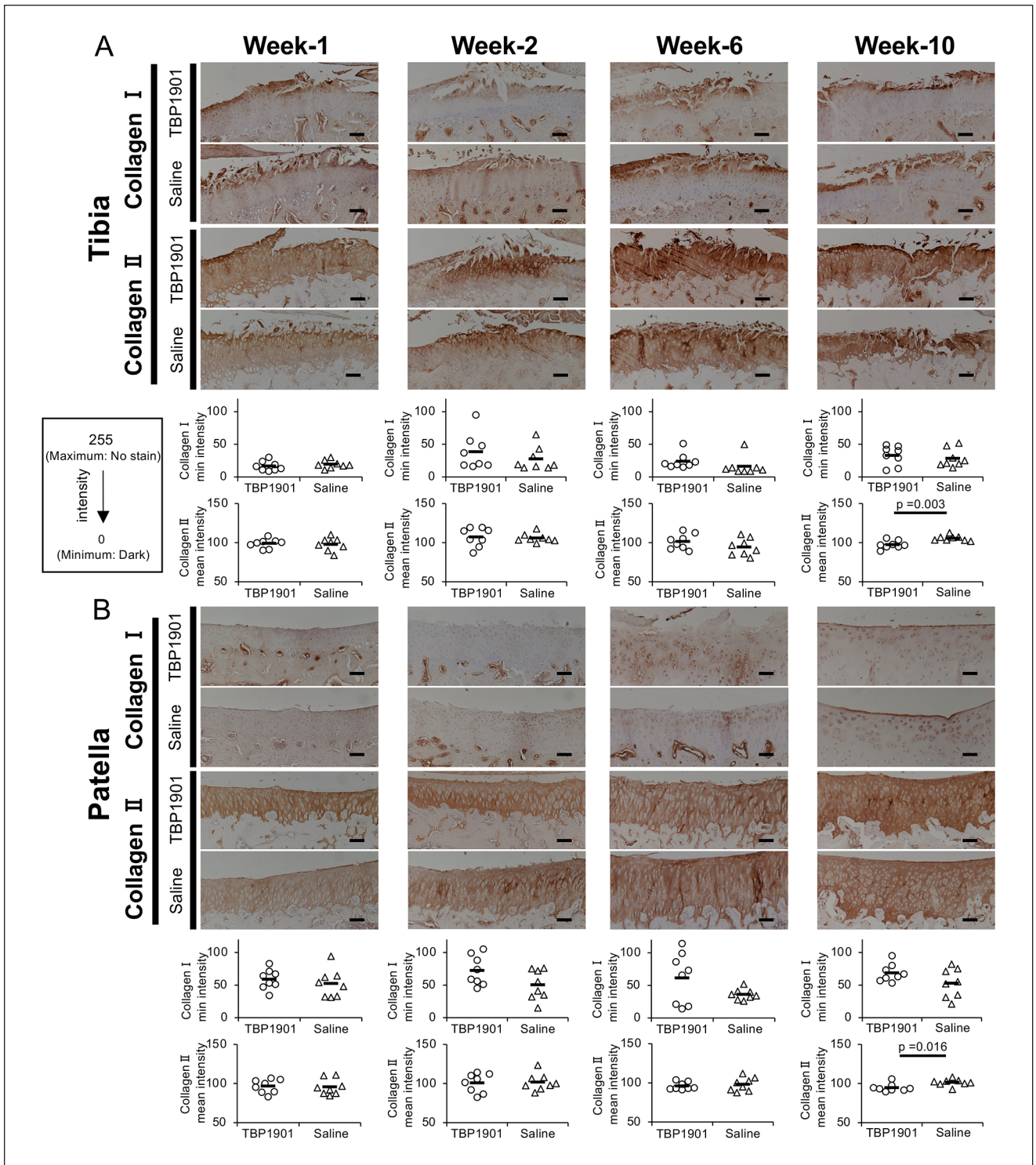


Figure 4. TBP1901 intra-articular injections increased type II collagen expression at 10 weeks. **(A)** Immunohistochemical images with type I and II collagen and quantitative analyses in the tibia of the tibiofemoral (TF) joint. TBP1901 intra-articular injections significantly increased the type II collagen expression at week 10. **(B)** Immunohistochemical images with type I and II collagen and quantitative analyses in the patella of the patellofemoral joint. TBP1901 intra-articular injections significantly increased the type II collagen expression at week-10. The expressions of type I and type II collagen were analyzed by measuring the minimum and mean intensity values using ImageJ, respectively, on a scale from 0 (dark) to 255 (no staining). Values are the means in the TBP1901 and saline control groups at several time points ($n = 8$ each). P values were calculated using Welch's t test. Magnification: $\times 100$. Scale bar: 100 μm .

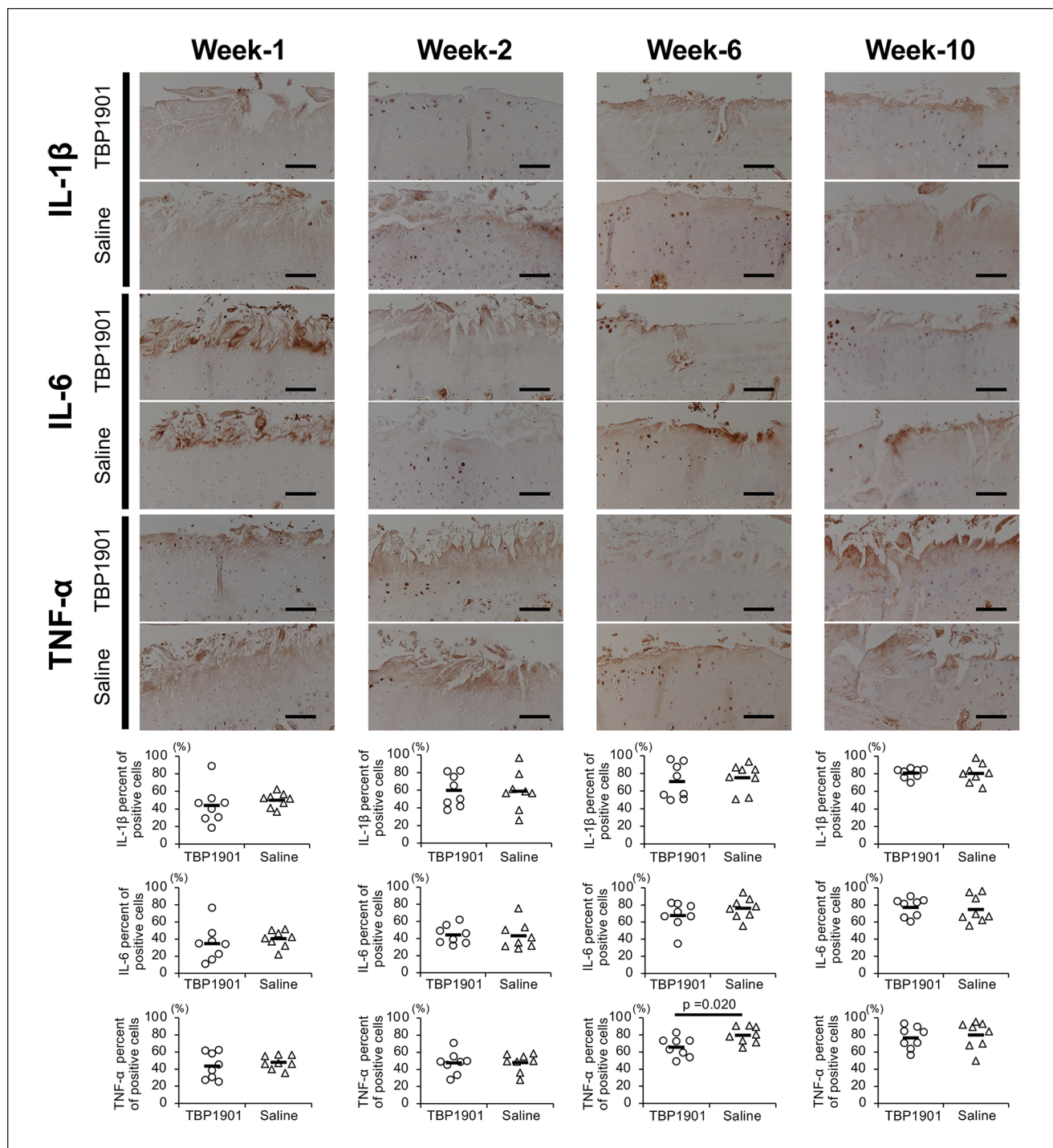


Figure 5. TBP1901 intra-articular injections suppressed the expression of pro-inflammatory cytokines in the articular cartilage of the tibiofemoral joint. Representative immunohistochemical images of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF)-α in the articular cartilage of the tibia are shown in the upper row. The percentages of positive cells are shown in the lower row. The percentage of TNF-α positive cells in the TBP1901 group was significantly reduced at week 6 compared with that in the saline control group. Values are the means of the TBP1901 and saline control groups at several time points (*n* = 8 each). *P* values were calculated using Welch's *t* test. Magnification: ×200. Scale bar: 100 μm.

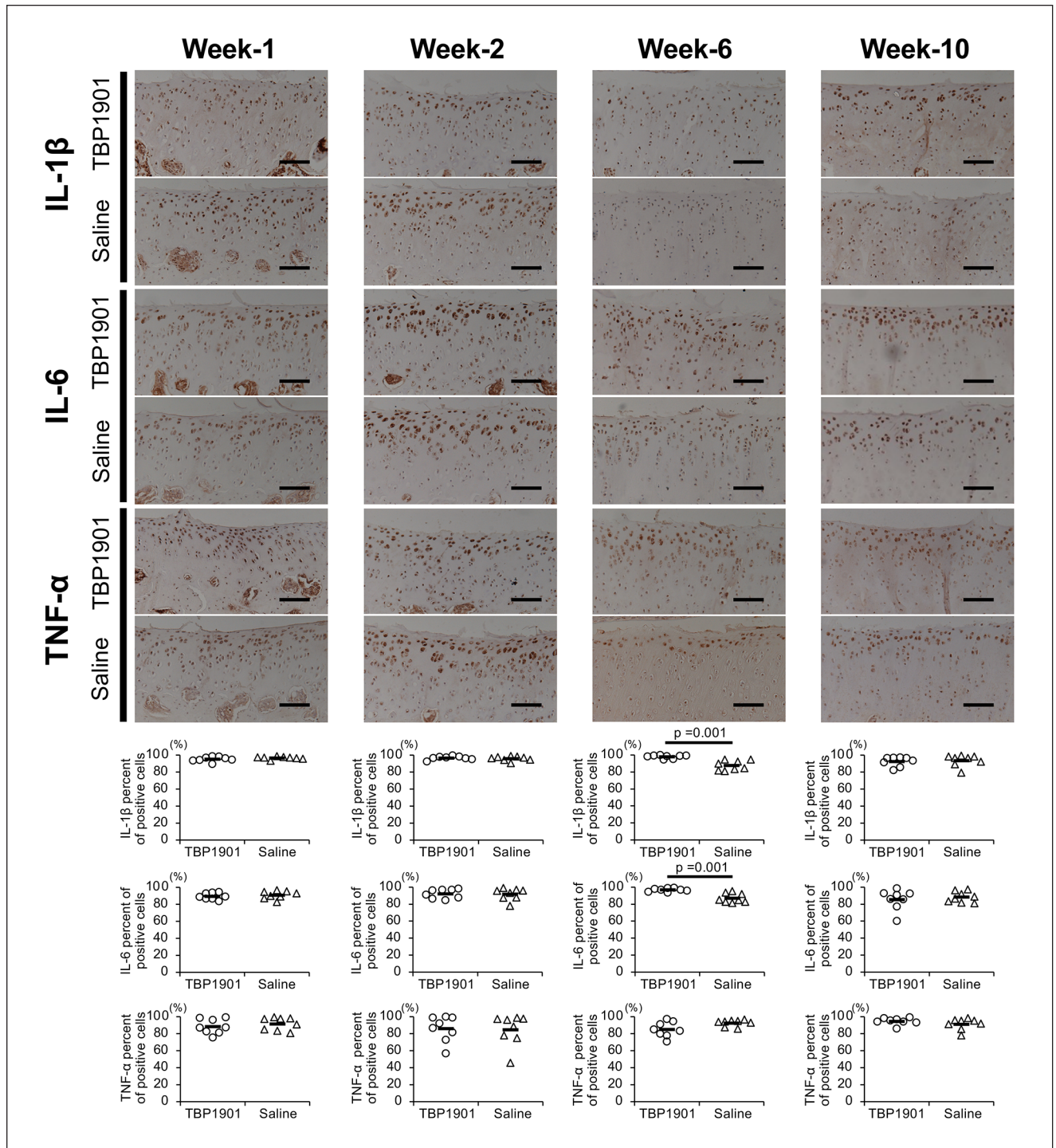


Figure 6. TBP1901 intra-articular injections did not suppress the expression of pro-inflammatory cytokines in the articular cartilage of the patellofemoral joint. Representative immunohistochemical images of interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF)- α in the articular cartilage of the patella are shown in the upper row. The percentages of positive cells are shown in the lower row. The percentages of IL-1 β and IL-6 positive cells were significantly higher at week 6 in the TBP1901 group than in the saline control group. Values are the means of the TBP1901 and saline control groups at several time points ($n = 8$ each). P values were calculated using Welch's t test. Magnification: $\times 200$. Scale bar: 100 μm .

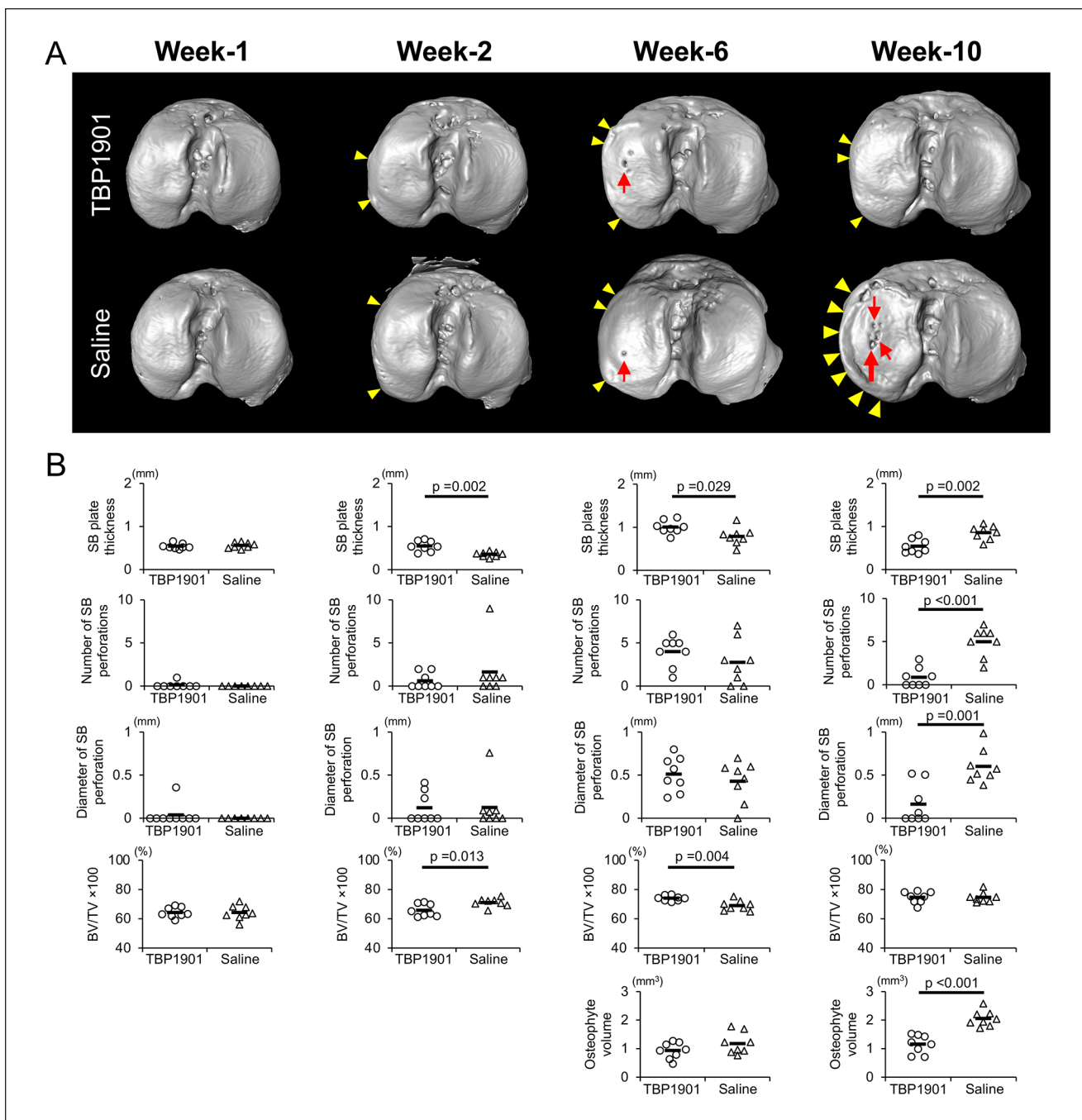


Figure 7. TBP1901 intra-articular injections regulated subchondral bone (SB) plate formation and prevented excessive bone formation. **(A)** Representative 3-dimensional micro-computed tomography images of the SB in the tibial plateau. SB plate perforations (holes; red arrows) were found in the medial tibial plateau, especially at weeks 6 and 10. Osteophytes (yellow arrowheads) were confirmed on the medial margin at weeks 2, 6, and 10. **(B)** TBP1901 intra-articular injections significantly suppressed the SB plate thinning in week 2, reduced SB plate thickness, the number of SB plate perforations, the diameters of the SB plate perforations, and the osteophyte volume at week 10. Values are the means of all measurements at several time points ($n = 8$ each). P values were calculated using Welch's t test for all measurements.

its effects on inflammation and OA progression. First, the fluorescence of curcumin was confirmed in the articular cartilage and synovium of the TBP1901 group. This indicates

that TBP1901 is incorporated into the cartilage and synovium and might induce biological effects. In addition, we found that TBP1901 intra-articular injections suppressed synovial

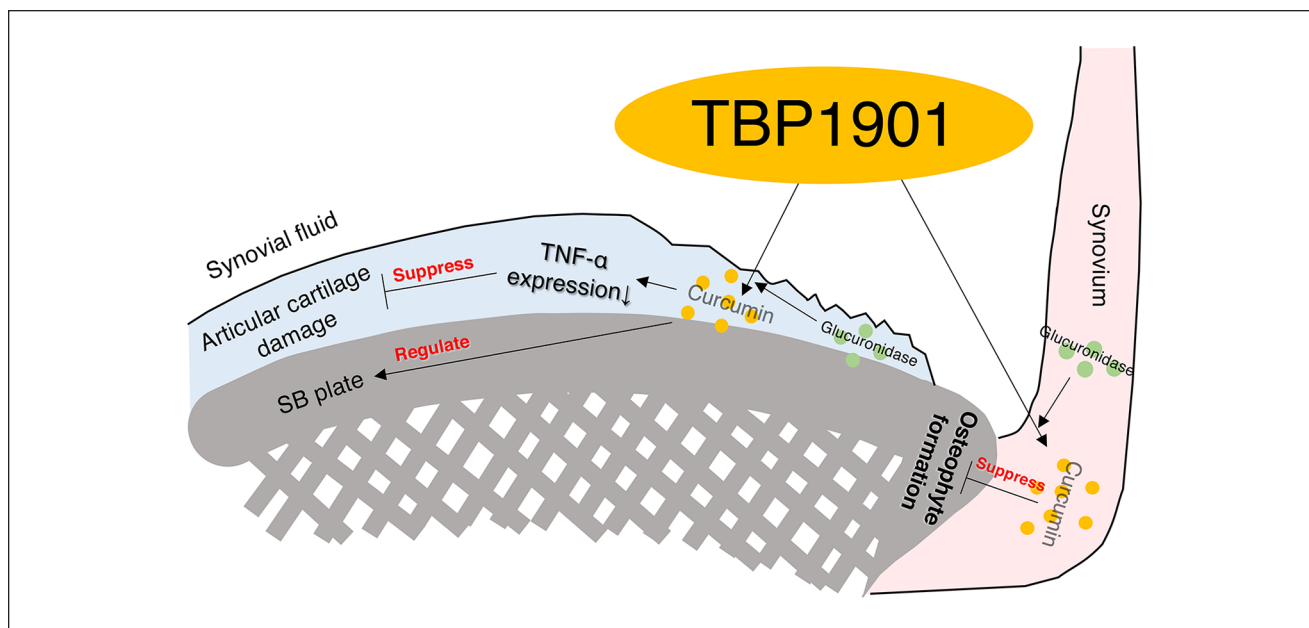


Figure 8. Graphical abstract of the effects of the intra-articular injection of TBP1901 on the suppression of osteoarthritis (OA) development in an OA rat model (destabilized medial meniscus). Curcumin was absorbed in the articular cartilage and synovium by the TBP1901 injection. Curcumin suppressed inflammation, osteophyte formation, articular cartilage, and subchondral bone (SB) pathology.

inflammation in the acute phase and degeneration of cartilage and SB in the chronic phase (Fig. 8).

Suppression of Inflammation and Articular Cartilage Degeneration by TBP1901 Intra-Articular Injections

This study showed the suppression of synovial inflammation in the acute phase of PTOA by TBP1901 intra-articular injections in an OA rat model. The mammalian target of the rapamycin (mTOR) pathway plays an important role in regulating cell growth, proliferation, differentiation, and apoptosis. It has been reported that curcumin inhibits synovial inflammation and hyperplasia via the mTOR pathway in rats with collagen-induced arthritis.⁵⁵ The present study showed the suppression of synovial inflammation in a rat model of PTOA. In contrast, TBP1901 did not suppress cartilage damage during the acute phase. In a previous report, oral administration of curcumin or curcumin-loaded poly lactic-*co*-glycolic acid nanoparticles for 14 days showed a preventative effect on articular cartilage in a monoiodoacetate (MIA) model of osteoarthritis in rats.⁵⁶ MIA inhibits glyceraldehyde-3-phosphate dehydrogenase of the Krebs cycle, leading to the death of chondrocytes, leading to unique pathophysiology that does not correlate with PTOA.⁵⁷ In the present study, the progression of cartilage damage by DMM surgery may have been too rapid for TBP1901 to be sufficiently effective in the acute phase.

In the chronic phase, articular cartilage degeneration is suppressed by TBP1901. Curcumin suppresses inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6. It inhibits the production of pro-inflammatory mediators and matrix-degrading enzymes, metalloproteinase (MMP)-3 and MMP-13.^{58,59} In the present study, TNF- α expression in the cartilage was also reduced by TBP1901 injections. In addition, type II collagen deposition was higher in the TBP1901 group at week-10. It has been reported that curcumin promotes chondrogenic differentiation, proliferation, and migration of mesenchymal stem cells (MSCs).^{60,61} These effects may improve articular cartilage conditions. Furthermore, SB and articular cartilage interact with each other and work as a unit.⁶² The protection of the SB damage by TBP1901 injections may have suppressed cartilage degeneration.

SB Improvement by TBP1901 Intra-Articular Injections

There are few reports on the effects of curcumin on SB. The present study evaluated the effect of TBP1901 on SB in detail using μ -CT. Some reports have shown that curcumin attenuates bone resorption by suppressing osteoclast proliferation⁶³ and osteoblast apoptosis.⁶⁴ The SB plate thins as bone remodeling increases in early OA and thickens as the remodeling balance changes as OA progresses.^{65,66} The attenuation of bone resorption may have suppressed the SB

plate thinning in the acute phase and the SB plate thickening and perforating in the chronic phase in the present study. A previous study demonstrated histologically that the oral administration of curcumin reduced SB plate thickness in the chronic phase,¹² similar to our results.

In addition, TBP1901 injection suppressed osteophyte formation. Osteophytes are formed by synovial cells in a process similar to endochondral ossification and limit range of motion.⁶⁷ Transforming growth factor- β (TGF- β) and macrophages play an important role in osteophyte formation.^{68,69} In a previous report, the administration of oral curcumin suppressed TGF- β activation in the synovium.⁷⁰ TBP1901 injections may have suppressed the osteophyte formation by the inactivation of TGF β on synovium. The remarkable effects on SB in our study may have been due to the presence of rich β -glucuronidase, which hydrolyzes TBP1901 to free form curcumin in the bone and synovium.^{29,32}

Limitations and Conclusion

The present study has some limitations. First, it is still unclear when and how much TBP1901 should be administered, although it was administered at a dose of 30 mg/mL by intra-articular injection from postoperative day 3 or 7 in this study. Additional investigations are necessary to determine the optimal postoperative timing and dose of TBP1901. Second, the working mechanism of TBP1901 in SB and osteophytes is unclear. Additional comprehensive studies focusing on TGF- β and macrophages are required. Third, although TBP1901 suppressed articular cartilage in a rat OA model, its effects on human OA are unclear. Fourth, it has not been determined whether TBP1901 is more effective than traditional curcumin or other curcumin-related products. We plan to conduct comparative studies to demonstrate the efficacy of TBP1901 in the future.

In conclusion, this study showed that intra-articular injections of TBP1901 reduced synovial inflammation at weeks 1 and 2 in the acute phase of PTOA. In the chronic phase of PTOA, TBP1901 reduced TNF- α expression at week 6 and suppressed articular cartilage degeneration, SB plate thickening, SB plate perforation, and osteophyte formation at week 10.

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Declaration of Conflicting Interests


The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this

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Ethical Approval

This study was approved by the animal research committee of Kyoto University (approval number: Med Kyo 19016).

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