Intra-articular Injections of Platelet-Rich Plasma Releasate Reduce Pain and Synovial Inflammation in a Mouse Model of Osteoarthritis

Sohrab Khatab,*† MD, Gerben M. van Buul,* MD, PhD, Nicole Kops,* BSc, Yvonne M. Bastiaansen-Jenniskens,* PhD, P. Koen Bos,* MD, PhD, Jan A. Verhaar,* MD, PhD, and Gerjo J. van Osch,*‡§ PhD
Investigation performed at Erasmus MC, Rotterdam, the Netherlands

Background: Osteoarthritis (OA) is a degenerative joint disease leading to pain and disability for which no curative treatment exists. A promising biological treatment for OA is intra-articular administration of platelet-rich plasma (PRP). PRP injections in OA joints can relieve pain, although the exact working mechanism is unclear.

Purpose: To examine the effects of PRP releasate (PRPr) on pain, cartilage damage, and synovial inflammation in a mouse OA model.

Study Design: Controlled laboratory study.

Methods: OA was induced unilaterally in the knees of male mice (n = 36) by 2 intra-articular injections of collagenase at days –7 and –5. At day 0, pain was measured by registering weight distribution on the hindlimbs, after which mice were randomly divided into 2 groups. Mice received 3 intra-articular injections of PRP or saline in the affected knee. Seven mice per group were euthanized at day 5 for assessment of early synovial inflammation and cartilage damage. Pain in the remaining mice was registered for a total of 3 weeks. These mice were euthanized at day 21 for assessment of cartilage damage and synovial inflammation on histological evaluation. Antibodies against iNOS, CD163, and CD206 were used to identify different subtypes of macrophages in the synovial membrane.

Results: Mice in the PRPr group increased the distribution of weight on the affected joint in 2 consecutive weeks after the start of the treatment ($P < .05$), whereas mice in the saline group did not. At day 21, PRPr-injected knees had a thinner synovial membrane ($P < .05$) and a trend toward less cartilage damage in the lateral joint compartment ($P = .053$) than saline-injected knees. OA knees treated with saline showed less anti-inflammatory (CD206+ and CD163+) cells at day 5 than healthy knees, an observation that was not made in the PRPr-treated group. A higher level of pain at day 7 was associated with a thicker synovial membrane at day 21. The presence of CD206+ cells was negatively associated with synovial membrane thickness.

Conclusion: In a murine OA model, multiple PRPr injections reduced pain and synovial thickness, possibly through modulation of macrophage subtypes.

Clinical Relevance: PRPr injections in early OA or shortly after joint trauma can reduce pain and synovial inflammation and may inhibit OA development in patients.

Keywords: macrophages; osteoarthritis; pain; synovial inflammation; molecular biology

Osteoarthritis (OA) is a degenerative joint disease characterized by loss of cartilage integrity, changes in subchondral bone, formation of osteophytes, and inflammation of the synovial membrane. This process results in pain and disability. Current treatments focus on pain reduction, exercise therapy, and—in end-stage OA—joint replacement. No curative treatment exists for OA. Since joint arthroplasties have a limited lifespan, the need for disease-modifying drugs or therapies is high. Ideally such a therapy would inhibit or repair damage to the joint tissues and reduce pain and disability. A biological therapy for tissue injury that has emerged in recent years is treatment with platelet-rich plasma (PRP). PRP is a plasma product extracted from whole blood that contains at least 1.0×10^6 platelets per microliter.[29] The platelets undergo degranulation, after which they release growth factors and cytokines such as transforming growth factor β and platelet-derived growth factor

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(PDGF),[24,29,37] two important factors in tissue healing. PRP can be activated before use, resulting in PRP releasate (PRPr), a product containing low amounts of leukocytes and high concentrations of growth factors.[9] Our group and others have used PRPr previously and have shown that it has anti-inflammatory and positive effects on matrix synthesis and accumulation. Furthermore, PRPr can induce the chondrogenic properties of chondrocytes.[20,47,52] PRPr has been used in a rabbit model and clinically to successfully induce the reparative effect of degenerated intervertebral disc degeneration.[1,38]

Several clinical trials in OA have concluded that multiple PRP injections are safe and have a beneficial effect on OA symptoms, such as pain, for up to 12 months.[6,17,26,33,34,45] Evidence is accumulating from both in vitro and in vivo studies for the potential of PRP in the treatment of OA. From preclinical research we know that PRP promotes the proliferation of cells derived from human synovium and cartilage[39,42] and that PRP-treated chondrocytes repair cartilage better than nontreated chondrocytes.[58] These cells in turn produce more superficial zone protein, which functions as a boundary lubricant that helps to reduce friction and wear.[42,43,48] PRP itself was also shown to reduce friction in bovine articular cartilage explants.[42] The anti-inflammatory effects of PRP have been demonstrated both in a co-culture system of osteoarthritic cartilage and synovium[42] and in human osteoarthritic chondrocytes, where it reduced multiple proinflammatory effects induced by interleukin 1β.[52] Furthermore, in a canine OA model, multiple PRP injections were shown to have beneficial effects on pain and functional impairment but no effect on the severity of radiographic OA.[11]

Although the use of PRP products seems promising for the treatment of OA, the wide variability in outcome parameters evaluated, in models used, and in PRP and PRPr production protocols makes interpretation of results between studies difficult.[13,22,25] This difficulty in comparing the results of PRP research may be one of the reasons why the exact working mechanisms of intra-articularly injected PRP products are not fully understood. Unraveling this mechanism could provide an opportunity to further improve therapeutic PRP efficacy.

In this study we assessed the effect of human PRPr compared with saline in a murine model of collagenase-induced OA (CIOA). We studied the effects of PRPr on several OA-related processes in the joint—pain, cartilage damage, and synovial inflammation—and evaluated correlations between these parameters. We paid particular attention to effects on different macrophage phenotypes in the synovium. Our hypothesis is that multiple intra-articular injections of PRPr will reduce pain, have a protective effect on cartilage, and inhibit synovial inflammation.

METHODS

Platelet-Rich Plasma Releasate Preparation

Human PRP was acquired from the national blood bank (Sanquin, Amsterdam, The Netherlands) with a platelet count of 8.5×10^8/mL. This PRP product is produced by pooling buffy coats of 5 different donors in plasma. After centrifugation, the platelets are filtered out to produce the PRP. The blood samples had identical ABO and Rh(D) compatible blood type and were pathogen free. PRP was activated by adding 10% vol/vol 228 mM $CaCl_2$ and incubated on a rotating device at 37°C.[52] After 1 hour of incubation, a clot was formed and the supernatant was harvested. This supernatant contains the released factors of the activated platelets and hence is called the PRP releasate. Whereas erythrocytes could be detected in the PRP, they were no longer detected after the activation of the PRP by a clinical cytometer (model xn1000; Sysmex Netherlands BV). Leukocytes were not detectable in the PRP or the PRPr. After the PRPr was harvested, the samples were stored at –80°C and used within a week for in vivo experiments. The concentration of PDGF-BB in the cryopreserved samples was 1.2×10^4 pg/mL, measured by enzyme-linked immunosorbent assay, confirming that released products were present in the supernatant.

Animal Model of Injury and Treatment

All animal experiments were performed on 36 male C57/Bl6 mice age 12 weeks (Harlan Netherlands BV, the Netherlands), with approval of the animal ethical committee (#EMC 116-14-03). The mice were housed in groups of 3 or 4 mice per cage, under a 12-hour light-dark cycle at a temperature of 24°C, and had access to water and food ad libitum at the animal testing facilities.

Before the experiments started mice were allowed to acclimatize for a week. In all mice, OA was induced unilaterally by 2 intra-articular injections of 6 μL of 3 U collagenase type VII (Sigma-Aldrich) at days –7 and –5. CIOA is an established model for instability in mice.[27,44,49,54,55] All intra-articular injections were applied under 2.5% isoflurane anesthesia, with a 50-μL glass syringe (Hamilton Company) using a 30G needle (Becton, Dickinson and Company). The contralateral control knees were kept naïve and were not injected with any substance. Mice were randomly assigned to either the treatment group with 6 μL of PRPr (n = 11 mice) or the control group with 6 μL of saline (n = 11 mice). Both groups received 3 consecutive PRPr or saline injections; the first injection was given 7 days after the first collagenase injection

§Address correspondence to Gerjo J. van Osch, PhD, Department of Orthopaedics and Department of Otorhinolaryngology, Erasmus MC, Room Ee 1655, 's-Gravendijkwal 230, Postbus 2040, 3000 CA Rotterdam, the Netherlands (email: g.vanosch@erasmusmc.nl).

*Department of Orthopaedics, Erasmus MC, University Medical Center, Rotterdam, the Netherlands.

†Department of Radiology & Nuclear Medicine, Erasmus MC, University Medical Center, Rotterdam, the Netherlands.

‡Department of Otorhinolaryngology, Erasmus MC, University Medical Center, Rotterdam, the Netherlands.

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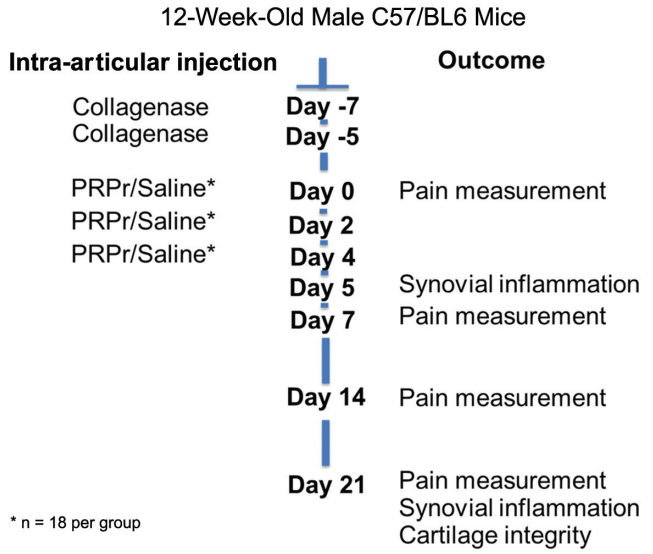

Figure 1. Experimental setup of the in vivo experiment. On the left is a list of the intra-articular injections at different time points. On the right are the outcome measurements. At day 5, a group of 14 mice were euthanized (n = 7 per group) to assess the early effects of PRPr on synovial inflammation. Another group of 22 mice were used to assess the presence of pain up to 3 weeks and to assess synovial inflammation and cartilage damage at end point. PRPr, platelet-rich plasma releasate.

(referred to as day 0) and repeated with consecutive injections at days 2 and 4 (Figure 1). Weight distribution over the left and right hindlimbs was evaluated as an indicator of pain at day 0 and thereafter once weekly for 3 weeks.

During the entire experiment, the animals were able to move around freely and reach the food pellets and drink nozzle on the top of their cage. Animals were euthanized at day 21, and knee joints were harvested for histological analysis.

To assess the early effects of PRPr on synovial inflammation, an additional group of animals was used containing 7 mice in each group. These animals underwent identical OA induction and treatment protocols. They were euthanized at day 5 after the start of PRPr treatment, and knee joints were prepared for histological evaluation.

Measurement of Hindlimb Weight Distribution

Hindlimb weight distribution was registered with an incapacitance tester[53] (Linton Instrumentation) as an indicator of pain. It is a static method to measure pain, that is validated in mice, rats, and other animal models.[3,11,14,15,19,53] Mice were positioned on the incapacitance meter with each hindlimb resting on a separate force plate. Measurements were performed at day 0, just before therapy administration, and thereafter at days 7, 14, and 21. The observer was blinded to the pressures measured during the test. Afterward, measurements with a registration of below 3 g (<10% of total body weight) per hindlimb or less than 10 g (<30% of total body weight) in total over both hindlimbs were excluded. On average, 15 measurements per time point per animal were available. For each time point per mouse, the average of these measurements was used to calculate the percentage of weight on the affected limb as an indication of pain in the affected limb. A single value per measurement time point was used for statistical analyses.

Histological Analyses

Knees were fixed in formalin 4% (vol/vol) for 1 week, decalcified in 10% EDTA for 2 weeks, and embedded in paraffin. Coronal sections of 6 μm were cut for analysis of synovial inflammation and cartilage damage.

Structural Integrity. Cartilage damage was evaluated on thionin-stained sections by 2 observers who were blinded to the treatment groups and who used a scoring system described by Glasson et al.[16] This score ranges from 0 for normal cartilage to 6 for cartilage with clefts and erosion to the calcified cartilage in more than 75% of articular surface. For each knee, the cartilage quality in the lateral and medial compartment of the knee was scored on 3 sections with 180-μm intervals between the sections. The maximum and sum scores of the 3 sections for each compartment were used for analyses.[16] Data from the lateral compartment are shown since we used a mild OA model and this was the area where most damage was observed. Thionin stain was used instead of Safranin O because of previously described limitations of the latter, especially regarding sensitivity of glycosaminoglycan (GAG) depletion.[5,7] As an internal check, all knees of the animals terminated at the early time point were stained and scored by use of both thionin and Safranin O stain, which showed a very high accordance between both methods (data not shown).

Synovial Inflammation. For synovial inflammation assessment, sections were stained with hematoxylin and eosin. Images were acquired by use of the NanoZoomer digital pathology program (Hamamatsu Photonics). Synovial thickness was measured from the capsule to the superficial layer of the synovial membrane in the parapatellar recesses at the medial and lateral sides at 3 positions per section, based on a previously described method.[49] These measurements were done on 3 sections per knee, with 180 μm between the sections. In total, we obtained 18 synovium thickness measurements per knee, which were averaged to obtain a single value per knee joint.

Macrophage Staining. To evaluate the macrophage subtypes in the synovial membrane, inducible nitric oxide (iNOS) was used as a marker for proinflammatory macrophages, cluster of differentiation 163 (CD163) as a marker for anti-inflammatory macrophages, and CD206 as a marker for tissue repair macrophages. For this purpose, sections were deparaffinized and washed, and heat-mediated antigen retrieval was performed for CD163 and CD206 by placing the slides in 95°C citrate buffer (pH 6) for 20 minutes. Antigen retrieval for iNOS was performed by placing the slides in 95°C Tris-EDTA buffer (pH 9) for 20 minutes. Blocking of aspecific binding was done with 10% goat serum (Southern Biotech) for 30 minutes. Thereafter, sections were incubated with the primary antibodies

iNOS (2.0 μg/mL; #15323; Abcam), CD163 (0.34 μg/mL; #182422; Abcam), and CD206 (2.5 μg/mL; #64693; Abcam) for 1 hour, followed by 30 minutes of incubation with a biotinylated anti-rabbit Ig link (HK-326-UR; Biogenex) diluted 1:50 in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA). Thereafter, sections were incubated with an alkaline phosphatase-conjugated streptavidin (HK-321-UK; Biogenex) label diluted 1:50 in PBS/1%BSA. To reduce background, endogenous alkaline phosphatase activity was inhibited with levamisole (Sigma-Aldrich Chemie N.V.). Neu Fuchsin (Fisher Scientific) and Napthol AS-MX phosphate (Sigma-Aldrich Chemie N.V.) substrate was used for color development and counterstained with hematoxylin. As a negative control, rabbit IgG antibody (Dako Cytomation) was used. The sections were ranked from weakest to strongest staining for either iNOS, CD163, or CD206 by 2 observers blinded to the treatment group. The maximum rank was based on the total number of joints scored for the individual staining. When multiple sections had similar strength of staining, the mean of the rank numbers was given to each section. Per section, the mean rank of both observers was used for analyses.

Statistical Analysis

For the late time point and pain reduction, we considered a decrease in pain of 20% in the therapy groups to be relevant in our study. According to a power calculation (using a standard deviation of 20%), with a statistical power level (1-β) of .8 and significance level (α) of .05, our sample size per group for a 2-tailed hypothesis test was 10 mice. For the short term, we considered a 25% decrease of synovial thickness as relevant for our study. According to a power calculation of the sample size (using a standard deviation of 20%), with a statistical power level (1-β) of .8 and significance level (α) of .05, our sample size per group for a 2-tailed hypothesis test was 6 mice. One additional mouse per group gave us n = 11 mice for the late time point and n = 7 mice for the early time point. This led to 18 mice per treatment group and 36 mice in total.

Data were analyzed with IBM SPSS statistics 21 (SPSS Inc). For the effect on weight distribution, normality was confirmed with a Shapiro-Wilk test. Two-tailed paired *t* tests were conducted to evaluate differences between time points within each group, and 2-tailed unpaired *t* tests were conducted to evaluate differences between treatment groups at each time point. Statistical testing on synovial thickness measurements was conducted by use of a Welch *t* test after normality was confirmed with a Shapiro-Wilk test for knees at both day 5 and day 21. To compare the maximum OA scores between saline and PRPr-treated groups, a Mann-Whitney *U* test was performed. A Kruskal-Wallis test was performed for the ranked macrophage data between the healthy control, saline, and PRPr groups. For all tests, *P* values less than .05 were considered statistically significant.

Correlation analysis was performed by means of Spearman rho test. All data collected were categorized per animal. In this way we were able to make correlations between pain measurements at different time points and the corresponding histological findings at day 21. For the interpretation of the correlation coefficient we used the absolute value of r_s, classifying the correlations as weak (<0.39), moderate (0.40-0.59), strong (0.60-0.79), and very strong (>0.80). Correlations were significant if *P* values were less than .05.

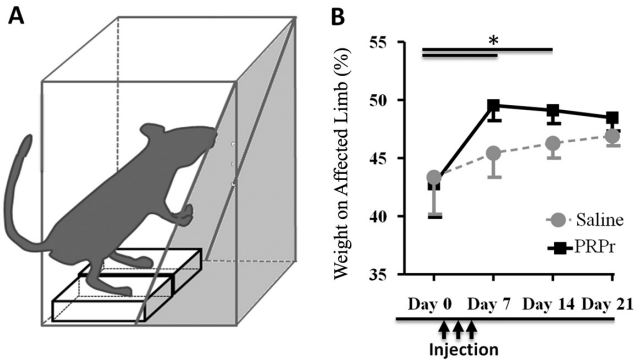

Figure 2. Weight distributed on the affected limb in mice with collagenase-induced osteoarthritis treated with platelet-rich plasma releasate (PRPr). (A) Position of the hindlimbs on the 2 pressure plates of the incapacitance tester. (B) Weight distribution on the affected limb in time: the PRPr group in black versus the saline group in gray (dotted line). Treatment started at day 0, 7 days after the first collagenase injection; the arrows depict the time of injection of PRPr or saline (days 0, 2, and 4). Means and SEMs of 11 mice per group per time point are presented. *$P < .05$.

RESULTS

Multiple PRPr Injections Reduce Pain

We determined the weight distribution over the hindlimbs as an indicator of pain (Figure 2A). Seven days after induction of OA, 43.1% ± 9.6% of weight was distributed on the affected limb, indicating pain (Figure 2B). Mice in the PRPr group significantly increased the weight on the affected joint on days 7 ($P = .041$) and 14 ($P = .034$) compared with day 0, indicating a reduction in pain. The mice that received saline injections did not significantly change their weight distribution at any of the time points compared with the start of treatment, although a trend for improvement over time was visible. The largest difference between PRPr and saline treated animals was seen at day 7, although this did not reach statistical significance.

Multiple PRPr Injections Have No Effect on Cartilage Damage

Collagenase-injected knees that were treated with saline (control group) had more cartilage damage than the healthy controls at day 5 on sum OA scores and at day 21 for maximum and sum OA scores, confirming development of OA (Figure 3, A and B) ($P < .05$). At day 21,

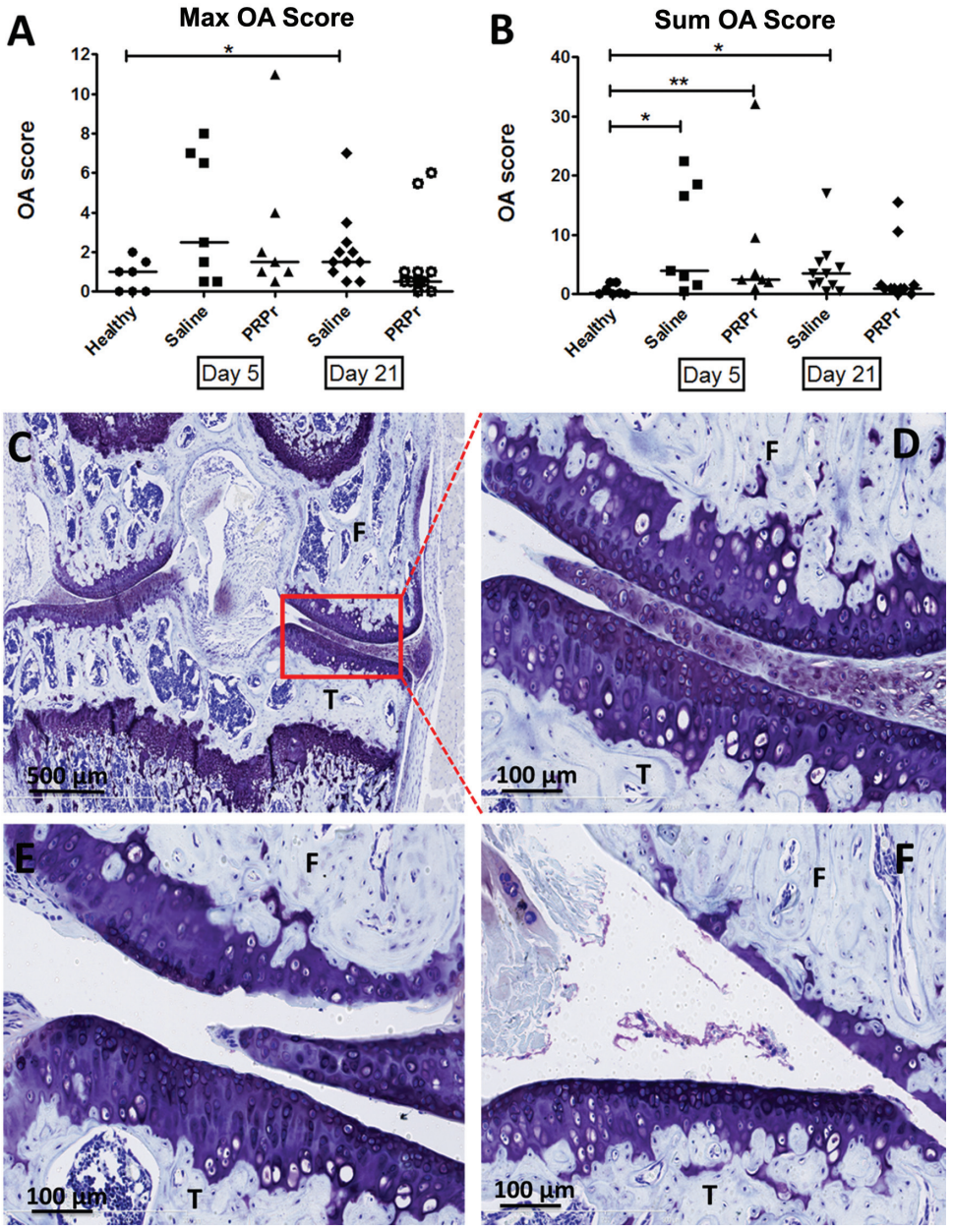

Figure 3. The (A) maximum and (B) sum osteoarthritis (OA) score per knee of the lateral (femoral condyle and tibial plateau) compartment of each knee. Bars represent the median of each group. n = 7 mice per group for Healthy group and day 5 time point; n = 11 mice per group for day 21 time point. *$P < .05$; **$P < .01$. (C) Overview of thionin staining of the mouse knee joint and typical examples of (D) OA score 1-2, (E) OA score 3-4, and (F) OA score 5-6 of the femoral condyle. OA score ranges from 0 to 12 per compartment. F, femur; PRPr, platelet-rich plasma releasate; T, tibia.

PRPr-injected knees were not significantly different from the healthy group in terms of maximum or sum OA scores. This could suggest a protective effect of PRPr on cartilage, although direct comparison of cartilage damage between saline and PRPr groups at day 21 showed only a trend toward a protective effect of PRPr ($P = .053$). Representative images of healthy, saline, and PRPr treated knees are shown in Appendix Figure A1 (see the Appendix, available in the online version of this article).

Multiple PRPr Injections Reduce Synovial Membrane Thickening

Collagenase-injected knees displayed a significantly thickened synovial membrane compared with healthy knees. Although the synovial membrane thickness was not statistically different between PRPr and saline-injected groups 5 days after start of treatment, synovial membrane thickness at day 21 was significantly less in the PRPr group

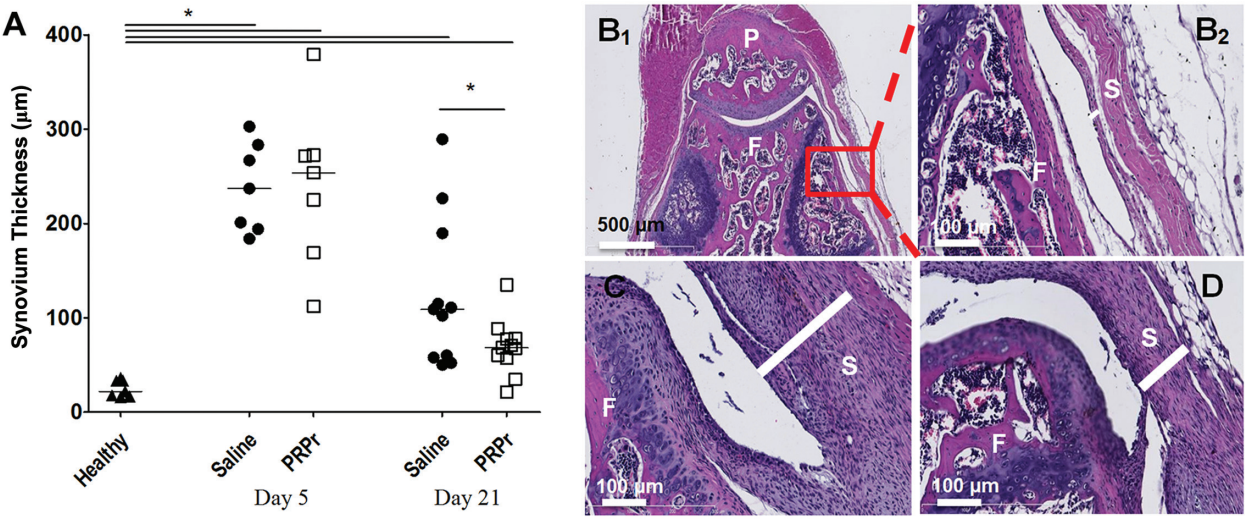

Figure 4. Synovial membrane thickness in healthy controls and in osteoarthritic knees after treatment with platelet-rich plasma releasate (PRPr) or saline. (A) Average synovial thickness at day 5 and day 21 after treatment. Bars represent the means of each group. Day 5, n = 7 mice per group. Day 21, n = 11 mice per group. *$P < .05$. Right panel, overview of the patellofemoral joint: (B1, B2) Representative examples of a healthy knee, (C) example of osteoarthritic knee injected with saline, and (D) osteoarthritic knee injected with PRPr, with different thickness of the synovial membrane at the parapatellar recesses. F, femur; P, patella; S, synovial membrane.

than in the saline control group (Figure 4A) ($P = .041$). Overall, synovial membrane thickness was largely reduced over time between day 21 and day 5 ($P < .001$).

Multiple PRPr Injections Maintain the CD206 and CD163 Positive Macrophages

To further analyze the synovial inflammation process, we assessed the presence of different macrophage subtypes. We examined the presence of iNOS, CD206, and CD163 positive macrophages by ranking healthy, collagenase-injected saline control and collagenase-injected PRPr-treated knees at day 5 and day 21 based on staining intensity (Figure 5). The iNOS positive staining intensity, indicating a proinflammatory response, was higher in collagenase-injected knees than in healthy control knees at both day 5 (saline, $P = .004$; PRPr, $P = .006$) and day 21 (saline, $P = .016$; PRPr, $P = .046$), independent of treatment (Figure 5A). Although no significant differences were observed in the presence of iNOS positive macrophages between treatment groups, PRPr-injected knees showed a trend toward less iNOS positive staining than in the saline-injected knees at day 5 ($P = .109$). We furthermore determined the presence of macrophages related to tissue repair (CD206+) and anti-inflammatory macrophages (CD163+). In the collagenase-injected saline control group, CD163 and CD206 positive staining was significantly lower than in the healthy knees at day 5 ($P = .024$ and $P = .042$, respectively) (Figure 5, B and C). CD206 and CD163 positive staining in the PRPr-treated knees did not differ from that of saline-treated knees at day 5 or day 21. In the saline group at day 21, CD206 but not CD163 staining intensity was significantly increased compared with day 5 ($P = .023$ and $P = .185$, respectively).

Pain Reduction Is Associated With a Thinner Synovial Membrane

Interestingly, reduction of pain measured at day 7 was strongly associated with a thinner synovial membrane at day 21 within the same animals ($P = .002$) (Table 1). No significant correlation was found between pain reduction at day 21 and synovial thickness at day 21, possibly due to the overall pain reduction seen in all animals. Furthermore, the presence of iNOS positive macrophages was moderately associated with lateral OA damage ($P = .02$). No significant associations were found between iNOS positive macrophages and pain or synovial thickness between treatment groups. The presence of repair macrophages (CD206+) was associated with a thinner synovial membrane ($P = .007$) and anti-inflammatory macrophages (CD163+; $P < .001$).

DISCUSSION

The results of this study suggest that multiple intra-articular injections of PRPr in a CIOA mouse model reduce synovial inflammation and might have a protective effect on cartilage while at the same time reducing pain. The strongest effect on pain reduction was seen in the period shortly after the start of treatment. In addition to providing pain reduction, multiple PRPr injections inhibited synovial inflammation, as demonstrated by a thinner synovial membrane compared with the saline control. Furthermore, PRPr injections affected the balance between

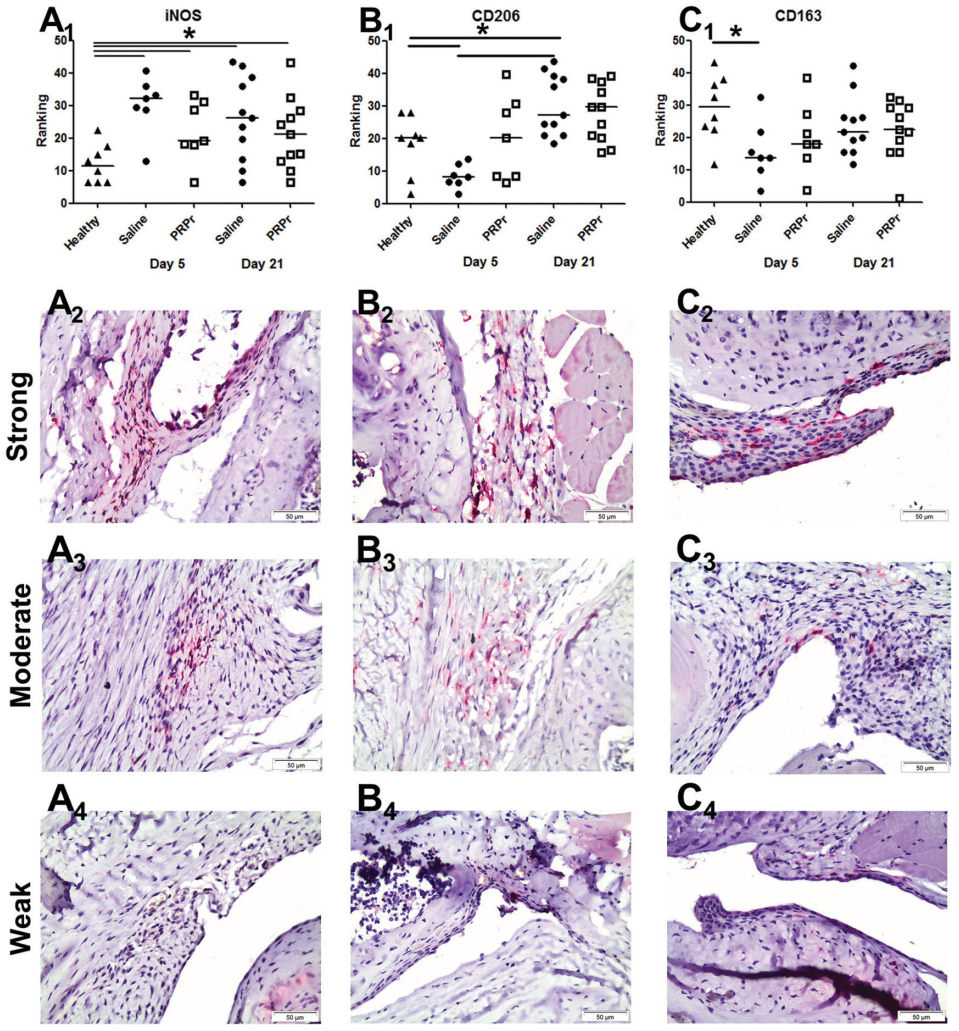

Figure 5. Cells positive for (A1-4) inducible nitric oxide (iNOS), (B1-4) cluster of differentiation 206 (CD206), and (C1-4) cluster of differentiation 163 (CD163) in the synovial membrane of healthy mice knees and osteoarthritic (OA) knees injected with platelet-rich plasma releasate (PRPr) or saline. Knees are ranked based on the presence of the marker of interest. Bars represent the median of each group. Day 5, n = 7 mice per group. Day 21, n = 11 mice per group. *$P < .05$.

inflammatory and anti-inflammatory macrophages in the synovial membrane, in particular by preventing the early decrease in anti-inflammatory macrophages seen after induction of CIOA. We also noted that although the association was not significant, PRPr-injected knees tended to have fewer proinflammatory iNOS positive macrophages than did saline-injected knees.

Collagenase injections induce joint inflammation, in particular in the first 2 weeks, making this model suitable for testing potential anti-inflammatory therapies. For example, others have shown that intra-articular injection of adipose-derived stem cells (ASCs) in this model reduces synovial inflammation at day 42 when ASCs are injected 1 week after induction of CIOA.[49] This demonstrates the possibility of restricting inflammation by using biological treatments in this model. Our results confirm that early intervention in this model can have beneficial effects.

PRPr injections reduced pain for 2 consecutive weeks. Mice with high pain levels at day 7 were very likely to have thicker synovial membranes 2 weeks later. The pathway by which PRPr reduces pain may involve inhibition of the production of prostaglandin E_2 (PGE_2). PGE_2 is a lipid mediator of inflammatory pain that causes pain hypersensitivity via nociceptor sensitization.[51] In an inflammatory environment, the main contributors to PGE_2 release are thought to be tissue-resident macrophages.[51] Our data suggest that PRPr-injected knees may have fewer proinflammatory and more anti-inflammatory macrophages, possibly resulting in lower PGE_2 production. It is known that PRP can promote the differentiation of monocytes toward repair and anti-inflammatory CD206 and CD163 positive macrophages.[40] This is supported by the finding in a rabbit knee OA model that intra-articular injections of leukocyte-poor PRP reduced PGE_2 concentrations.[57] Moreover, PRPr has been reported to

TABLE 1
Selection of Correlations Coefficients Between the Parameters Tested[a]

Parameters Tested by Spearman Rho		Correlation Coefficient	Strength	*P* Value	Interpretation
Pain reduction day 7	Synovial thickness day 21	–0.622	Strong	.002	Less pain at day 7 associated with less synovial inflammation at day 21[b]
Pain reduction day 21	Synovial thickness day 21	–0.098	Very weak	.670	No association between pain at day 21 and synovial inflammation at day 21
CD206 macrophage marker	Synovial thickness	–0.440	Moderate	.007	More CD206+ is associated with thinner synovial membrane
CD206 macrophage marker	CD163 macrophage marker	0.560	Moderate	<.000	Presence of CD163+ and CD206+ subtypes associated with each other
iNOS macrophage marker	Lateral OA score	0.492	Moderate	.020	Presence of iNOS+ is associated with more cartilage damage lateral compartment

[a]The correlations involving macrophage data and synovial thickness are based on pooled data from day 5 and 21 histologic testing. The correlation involving OA score is based on day 21 histological data. CD, cluster of differentiation; iNOS, inducible nitric oxide; OA, osteoarthritis.

[b]Weight distribution on the affected limb was measured as an indicator of pain; the higher the percentage of weight on the affected limb, the less pain, thus giving a negative association with synovial thickness.

contain high levels of interleukin 1 receptor antagonist (IL-1Ra)[35] that can inhibit acute inflammation caused by IL-1 and promote macrophage polarization toward an M2 phenotype.[28] Our finding that animals in neither the treated nor the untreated group appeared to experience any pain at 3 weeks may be partly due to the fact that acute inflammation weakens in time after collagenase injection, thereby reducing nociceptive input to the central nervous system. Less nociceptive input can be preceded by desensitization of the mouse nervous system for pain. In the latter case, the threshold for the activation of the joint nociceptors is reduced, and thus a bigger stimulus is needed to register pain.[31]

Besides synovial inflammation, cartilage damage is an important hallmark of OA. In this study, 28 days after OA induction, cartilage damage in the lateral joint compartment after multiple PRPr injections was not different from that in the healthy knees. The severity of the cartilage damage, however, was significantly increased in the lateral compartment of the CIOA joints treated with saline. The mild cartilage damage we observed in our study made it difficult to detect differences between PRPr and saline groups. The absence of a difference between PRPr-treated CIOA mice and healthy mice could be an indication of a chondroprotective effect of PRPr injections. No correlation between pain and cartilage damage at any time point was found, confirming previous findings in the field.[32]

We used a commercially available human-derived PRP, which was pooled from 5 healthy human donors. Pooling PRP donors can reduce the interdonor variability described previously.[30,56] This PRP is poor in leukocytes and in this study was activated before injection in mice. In contrast, other investigators injected nonactivated PRP and relied on activation in vivo.[4,8,12,41] We chose to activate the PRP product before injection because, first, it is difficult to control the activation of PRP in vivo and thus difficult to draw conclusions about the working mechanism of PRP without knowing the level of activation.[9] Some of the disappointing results from other studies might be attributed to less than optimal activation. Second, activating PRP with $CaCl_2$ leads to higher levels of PDGF-AA and -BB than other activation methods such as freeze-thaw.[46] Third, the activation of PRP results in the formation of a so-called cloth, which catches any remaining erythrocytes and leukocytes, making the end product low in cells and high in growth factors. Although possible positive effects of a PRP product rich in leukocytes are still being debated,[2] a product depleted from allogenic or in this case xenogenic cells will cause less immunoreaction. Fourth, by using a PRP product low in leukocytes and short storage before activation, we can reduce the catabolic factors in PRP or PRPr.[50] Since leukocytes are the main contributors to tumor necrosis factor α (TNF-α) levels in PRP—but also to levels of interleukins 6 and 8—these levels might increase further in the period during which leukocyte-rich PRP is stored.[10,23] Although the working mechanism of PRP is not fully understood, the current knowledge about its active components is improving. This will likely help optimize the PRPr product by filtering out components such as TNF-α and vascular endothelial growth factor or increasing the concentrations of other components such as PDGF. Other investigators have reported that freeze-drying of PRP into a powder increases its efficacy. Freeze-drying could also prolong the lifetime of the PRP product, resulting in an off-the-shelf product with a longer lifetime.[21,36] We could therefore reduce the levels of catabolic factors in PRPr by selecting a PRP product low in leukocytes and minimizing the duration of storage before activation.[50] Nevertheless, clinical studies typically use nonactivated PRP, making our results not fully comparable or immediately clinically translatable.

Here we have demonstrated in an OA model that multiple PRPr injections reduce pain and synovial membrane thickness and that PRPr appears to modulate the phenotype of synovial macrophages. We believe that PRPr injections are more potent when used as therapy for early stage intervention after trauma and early OA rather than as

a treatment for end-stage OA. The latter is confirmed in a few clinical trials, where PRP injections did not affect patients with end-stage OA.[18] This knowledge can be used in future experiments to determine the best time point for intra-articular PRP injections after trauma and to further evaluate and confirm the chondroprotective effects of PRP in the long term. Together with improvements of the PRP or PRPr product itself, these findings could help to make PRP a suitable treatment shortly after joint trauma or for patients with low-grade OA, both to relieve pain and to inhibit the progression of OA.

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