The effect of platelet-rich plasma on patterns of gene expression in a dog model of anterior cruciate ligament reconstruction

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ARTICLE INFO

Article history:
Received 13 August 2012
Received in revised form
24 September 2012
Accepted 11 October 2012
Available online 8 November 2012

Keywords:
Platelet-rich plasma
Gene expression
Ligamentization
Extracellular matrix

ABSTRACT

Background: Autologous platelet-rich plasma (PRP) has been investigated as a potential promoter of tendon healing and has an enhancing effect on the anterior cruciate ligament (ACL) graft maturation process. However, the influence of PRP on the synthesis and degradation of the extracellular matrix during the ACL graft remodeling process has never been investigated.

Materials and methods: Healthy and mature beagle dogs were randomly assigned to one of four groups: in group I (PRP group), ACL grafts were treated with PRP; in group II (control group), ACL grafts were treated with saline; in group III (sham group), only the knee joints were exposed; in group IV (normal control group), no surgery was performed to the knees. Ligament tissue was dissected at 2, 6, and 12 wk after surgery, and real-time PCR was performed using primers for growth factor-

1. Introduction

Injuries to the anterior cruciate ligament (ACL) are very common. In the United States, it is estimated that 250,000 new ACL ruptures occur annually [1], making reconstruction of the ACL one of the most commonly performed procedures in sports medicine. The improvement of graft maturation after ACL reconstruction is very important for facilitating an early
and aggressive rehabilitation, which ensures a speedy recovery for the patient so they can return to normal daily activities. Many studies have shown that platelet-rich plasma (PRP) has an enhancing effect on the graft maturation process [2–4]; however, the reasons for this are still unclear.

PRP is a blood plasma that is enriched with platelets and can potentially enhance graft maturation by delivering various growth factors and cytokines from the a-granules contained within the platelets [2–5]. Previous studies have showed that PRP can enhance cell viability and promote collagen expression (type I and III) in ACL cells in vitro [6]. PRP can also promote the synthesis of the extracellular matrix (ECM) [7] and stimulate ACL healing at early time points [6,8,9]. Recently, PRP has been used to accelerate the ligamentization process of tendon graft in ACL reconstruction surgery [4,10,11]. However, to the best of our knowledge, no study has investigated the effects of PRP on the synthesis and degradation of the ECM in ACL grafts during the remodeling process.

In this study, we used dog models to examine the temporal gene expression of transforming growth factor-β1 (TGF-β1), collagen type I (COL1A1), collagen type III (COL3A1), decorin, biglycan, matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-13 (MMP-13), and tissue inhibitor of metalloproteinase-1 (TIMP-1) in ACL grafts via real-time quantitative PCR using SYBR-green. Our goal was to determine whether PRP would affect the patterns of gene expression in the autograft ligamentization process. We hypothesized that PRP would promote gene expression of graft collagen and proteoglycan, as well as affect gene expression of collagenase and its inhibitor during the remodeling process of the ACL grafts. In ACL reconstruction, identification of gene expression patterns that respond to PRP may permit more research into conditions that best support the expression of functional proteins critical for the ACL graft remodeling process. This research may also help to develop novel therapies to facilitate or inhibit the expression of these genes by local administration of exogenous growth factors.

2. Materials and methods

2.1. Animals and groups

Thirty-six healthy, skeletally mature, male beagle dogs weighing 12.50 ± 1.48 kg (mean ± SD) were used in this study. They were kept in cages measuring 120 cm × 100 cm × 75 cm where they were allowed full cage activity. This experimental study was approved by the Animal Experiment Ethics Committee of Shanghai Jiao Tong University, Shanghai, China.

Thirty-six dogs (72 knees) were randomly divided into four groups (18 knees per group). One knee of each dog was divided into group I or group II, and the other knee was divided into group III or group IV. In group I (18 knees), ACLs were reconstructed with flexor digitorum longus autografts treated with PRP. In group II (18 knees), ACLs were reconstructed with flexor digitorum longus autografts treated with saline. In group III (18 knees), the knees underwent a sham surgical operation. In group IV (18 knees), the knees did not undergo surgery. Four groups of animals (6 knees from each group) were sacrificed 2, 6, and 12 wk after the operation.

2.2. PRP

Immediately before surgery, 20 mL of whole blood was withdrawn from the jugular vein of each animal in group I and divide evenly (10 mL whole blood/tube) into two 15 mL centrifuge tubes each containing 1 mL of sodium citrate solution (2.5%). The blood was then centrifuged according to the technique described by Landesberg [12], which produces a high concentration of platelets and a low rate of activation. Landesberg’s method was described as a double spinning method because the samples are spun both steps spin at 200 g for 10 min at each step.

Two tubes were centrifuged at 200 g for 10 min at room temperature. Because of differential densities, the platelet-poor plasma (PPP) layer formed at the top, the PRP layer in the middle, and the red blood layer at the bottom (Fig. 1A). Using a pipette, the PPP and PRP layers were transferred to two new tubes without anticoagulant and centrifuged for 10 min at 200 g. The PPP was then removed from the upper layer of the supernatant leaving 1 mL of PRP at the bottom, and the tubes were stored on the ice until they were used (less than 1 h). Each tube of whole blood and PRP was sampled and sent to an independent diagnostic laboratory for platelet count. A mean value of whole blood platelets yielded 133 ± 67 × 10⁹ platelets/L (mean ± SD) and the platelet count of PRP was 669 ± 313 × 10⁹ platelets/L (mean ± SD). The platelet concentration procedure increased platelet numbers in PRP by an average of five times that of the baseline concentration found in whole blood.

2.3. Surgical procedures

2.3.1. Group I (PRP-treated graft as treated group)

The animals were anesthetized with intravenous pentobarbital sodium (30 mg/kg). The surgical procedure was performed according to the technique described by Huangfu and Zhao [13]. The tendon of the flexor digitorum longus (8 cm in length) was harvested (Fig. 1B), and both ends were sutured in a whip-stitch style with a no. 2 polyester suture (Ethibond; Ethicon, Somerville, NJ). The tendon was then folded in half to create a 4-cm-long double-stranded graft. To keep the cross-sectional area of each graft the same, the graft was trimmed to a diameter of 4 mm. Two no. 2 polyester sutures were placed through the graft loop for the fixation of the proximal end. The graft was sutured with a polyester suture 12 mm from the graft loop end, which was used as a marker to make sure that the graft length in the femoral tunnel was 12 mm and that the graft length in the tibial tunnel was approximately 18 mm. This procedure was done to ensure that all animals were subjected to the same conditions (Fig. 2B).

One milliliter of PRP was mixed with 0.05 mL of calcium chloride (10%) and then immediately injected into several areas along the graft length using a 2-mL syringe with a 19-gauge needle (Fig. 1C). Subsequently, the solution dripping from the injection sites was used to soak the graft until
implantation. This resulted in PRP gel formation (60 s after mixing) that covered the entire surface of the graft (Fig. 1D).

Using sterile techniques, the knee was exposed through an anteromedial incision followed by a medial parapatellar arthrotomy, and then the patella was dislocated laterally. The infrapatellar fat pad was partially removed, and the native ACL was excised from both its tibial and femoral footprint. Using a drill (4 mm in diameter), the tibial tunnel was created at a 45° angle to the tibial axis and at a 15° to 30° angle to the sagittal plane through the tibial insertion of the ACL. The entire femoral tunnel had a width of 4 mm and was drilled through to the tibial tunnel in 70° to 90° knee flexion. The graft was then inserted until the marker was pulled into the inlet of the femoral tunnel, and both ends were fixed by through-passing and in-braided sutures over post screws on the outer aspect of the bone. Another tube of PRP mixture was prepared by mixing 1 mL of PRP with 0.05 mL of calcium chloride (10%), and this PRP mixture was then injected into the outer parts of the tunnels through the openings. The tibial and femoral ends were injected with 0.3 mL each. The joint was irrigated, and the capsule and skin were closed with interrupted sutures.

Postoperatively, each animal was allowed to move freely in its own cage without knee immobilization. Intramuscular injection with 3,200,000 U of penicillin and 1 g of streptomycin was administered for the first 3 d following surgery. The incision was cleansed with benzalkonium every 3 days.

2.3.2. Group II (saline-treated graft as control group)
In group II, animals underwent an identical surgical procedure except that the grafts were treated with saline instead of PRP.

2.3.3. Group III (sham group)
The same incision was made to expose the knee joint without operation, and the wound was closed in the same way.

2.3.4. Group IV (normal control without any surgery)
No surgery was performed in this group.

2.4. Sample collection
Animals were sacrificed by administering an overdose of sodium pentobarbital. The ACL autografts were resected, immediately frozen in liquid nitrogen, and then stored at −80°C.

2.5. Canine primers for PCR
The canine-specific primers used in this study are listed in Table 1.

2.6. RNA extraction and quality assessment
Total RNA was isolated from samples using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions.
Agarose gel electrophoretic (Mupid-EXu; Takara Shuzo, Shiga, Japan) analysis and spectrophotometry were used to assess the RNA integrity and concentration.

2.7. RNA reverse transcription and quantitative real-time polymerase chain reaction (PCR)

Total RNA (2 μg) was reverse transcribed into complementary DNA using an RNA PCR kit (Takara Shuzo) according to the manufacturer’s instructions. Quantitative reverse transcription-PCR was performed with a LightCycler (Roche Applied Science, Indianapolis, IN) using SYBR green (Toyobo, Osaka, Japan). PCR was initiated by denaturation at 94°C for 30 min. Following the initial denaturation step, the complementary DNA products were amplified for 50 PCR cycles consisting of a denaturation step for 10 s at 95°C, an annealing step for 30 s and an extension step at 72°C for 20 s. The endogenous glyceraldehyde 3-phosphate dehydrogenase housekeeping gene was used as an internal standard to normalize differences in total RNA levels within each sample. The threshold cycle (Ct) value of each marker was subtracted from the Ct value of glyceraldehyde 3-phosphate dehydrogenase to give the difference in Ct (ΔCt). The normalized expression of each marker was calculated as 2^(-ΔCt).

Fig. 2 – The flexor digitorum longus tendon was harvested (A) and then sutured (B). Normal ACL (C) before reconstruction (D) surgery. (Color version of figure is available online.)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
</tr>
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<td>COL1A1</td>
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<td></td>
<td>5’-AACTCTTCAGGGATTGGTG-3’ (reverse)</td>
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<tr>
<td>COL3A1</td>
<td>5’-GGAACCTGCCGGGACCTCAA-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-AACGCTCCGGGACTGCAATTCA-3’ (reverse)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>5’-TCCGCGAGCTCTACATTGACTTC-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-ACGCGCCCAGTGTGCTGTT-3’ (reverse)</td>
</tr>
<tr>
<td>Decorin</td>
<td>5’-GAGGAGGGCAAGAGAGACATC-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-GGGGGAGCAGACGGGACGCATT-3’ (reverse)</td>
</tr>
<tr>
<td>Biglycan</td>
<td>5’-GAGCCGGTGGGATTTCAGCCACCTAC-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-GGCGGAGCCTGCTGAGATCGT-3’ (reverse)</td>
</tr>
<tr>
<td>MMP-1</td>
<td>5’-ACTGATATGGGGCCTCTAGTGA-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-TCTCCCCCGATTTTGACTGATA-3’ (reverse)</td>
</tr>
<tr>
<td>MMP-13</td>
<td>5’-TCCGGCGACCTTATCTTCATCTC-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-AGCTCTCTCCCGCTGCTC-3’ (reverse)</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>5’-CCCTGGAAGCCTGCTGGGATAC-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-CAACCCCTACAGGCGACGATAG-3’ (reverse)</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-GCCGCAATGCCGAGGACACAG-3’ (forward)</td>
</tr>
<tr>
<td></td>
<td>5’-GGGGGATCACGAGAAGGACAG-3’ (reverse)</td>
</tr>
</tbody>
</table>

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
minimize variability due to human error, one operator was designated to perform all real-time reverse transcription-PCR analysis.

2.8. Statistical analysis

Data are expressed as the mean ± SD. The control value is set at 1 for messenger RNA (mRNA). Statistical analysis (GraphPad software Inc., La Jolla, CA) was performed using the one-way ANOVA or Student’s t-test. Differences between groups were considered significant at P values < 0.05.

3. Results

During the remodeling process, significant differences in the mRNA levels of many target genes were observed in the PRP-treated group (group I) compared to the control group (group II).

3.1. Effect of PRP on COL1A1 and COL3A1 mRNA levels

COL1A1 and COL3A1 mRNA levels were significantly higher in group I compared with group II at 2 and 6 wk after surgery (P < 0.05). COL1A1 and COL3A1 mRNA expression in groups I and II both peaked at 6 wk and then declined dramatically 12 wk after surgery. No significant changes were observed between group III (sham group) and group IV (normal control group) at any time point (Fig. 3A and B).

3.2. Effect of PRP on TGF-β1 mRNA levels

Expression of TGF-β1 was significantly higher in group I than in group II, 6 and 12 wk after the operation (P < 0.05). Group I also showed a sustained increase at all time points compared with group IV (P < 0.05). Two wk after surgery, expression of TGF-β1 was significantly higher in group III than in group IV. Then the levels of TGF-β1 returned to normal 6 wk after the operation (Fig. 3C).

3.3. Effect of PRP on decorin mRNA levels

In groups I and II, decorin mRNA expression significantly decreased (P < 0.05) 2 and 6 wk after surgery and then returned to normal by 12 wk (P > 0.05). The levels of decorin mRNA expression were significantly elevated in group I compared with group II 6 wk after surgery (P = 0.03). No significant differences were observed between groups III and IV at any time points (Fig. 3D).

3.4. Effect of PRP on biglycan mRNA levels

The levels of biglycan mRNA expression were significantly elevated in group I compared with group II at all time points (P < 0.05). The biglycan mRNA levels in group I were higher than in group IV 2 wk after surgery (P > 0.05), peaking at 6 wk (P < 0.05), and then decreased 12 wk after surgery (P < 0.05). In group II, biglycan mRNA expression was lower than that in group IV 2 wk after surgery (P = 0.03), peaking at 6 wk (P < 0.05), and then returning to preoperative levels 12 wk after surgery (P > 0.05) (Fig. 3E).

3.5. Effect of PRP on MMP-1 and MMP-13 mRNA levels

In group I, MMP-1 mRNA levels were significantly higher compared with that in other groups (P < 0.05) at all time points (Fig. 3F). The levels of MMP-13 mRNA significantly decreased in group I compared with that in group II at 2 and 6 wk after surgery (P < 0.05). In group III, expression of MMP-13 was higher than that in group IV 2 wk after the operation (P = 0.046). (Fig. 3G).

3.6. Effect of PRP on TIMP-1 mRNA levels

In group I, a significant increase in TIMP-1 expression was observed 2 and 6 wk after surgery compared with all other groups (P < 0.05). In group II, expression of TIMP-1 mRNA was significantly higher 2 and 6 wk after surgery compared with group III than group IV (P < 0.05), and high levels were sustained until 12 wk after surgery (P = 0.33). No significant difference was seen in group III and group IV (Fig. 3H).

3.7. Effect of Sham Surgery on Gene Expression

The effects of sham surgery on mRNA levels of target genes in the ACL are shown in Table 2. Sham surgery induced sustained increased in MMP-13 and TGF-1 mRNA in the ACL.

4. Discussion

In the present study, we demonstrated that there were time-dependent changes of target genes expression, including collagens, proteoglycans, collagenase, and its inhibitor, in group I (PRP-treated group) and group II (control group), during the graft remodeling process after ACL reconstruction. We also showed that there were significant differences in target genes expression between group I and group II at some time points during the graft remodeling process. Our findings suggest that PRP has a role in promoting synthesis of ECM after ACL reconstruction surgery. To our knowledge, this is the first study to clarify the effect of PRP on the synthesis and degradation of the ECM in the graft following ACL reconstruction surgery.

The levels of COL1A1 and COL3A1 were monitored because these genes encode for collagen type I and III. Collagen type I is a major component of the ligament and is crucial in providing the high tensile strength of the tendon. Collagen type III, a fibrillar collagen, is present in the immature ligament and ligament scar tissue and is over expressed during the process of remodeling and incorporation of ACL grafts [14]. In a rabbit model, Amiel et al. [15] evaluated collagen types in the patellar tendon graft after ACL reconstruction surgery. They detected collagen type III in the patellar tendon graft as early as 2 wk after the operation. Collagen type III reached maximum levels 6 wk after surgery. In a rat model, the expression of COL3A1 in the freeze-thawed tendon significantly increased compared to the sham group at 6 and 12 wk. Additionally, positive collagen type III staining around cells at 3, 6, and 12 wk was noted [16]. In a rabbit model, the gene expression of COL1A1 and COL3A1 was significantly elevated at 6 and 12 wk after ACL reconstruction surgery [17]. Various
in vitro studies have shown that PRP can enhance cell viability and promote collagen type I and III expression in ACL or tendon cells [6,7,18,19]. Similarly, our study demonstrated a significant elevation in COL1A1 and COL3A1 levels in group I compared with group II at 2 and 6 wk after surgery. Our results support the idea that PRP promotes the synthesis of collagen and may promote the remodeling process of the ACL graft after reconstruction surgery.

TGF-β1 is a multifunctional peptide growth factor known to affect the production of extracellular matrix, therefore playing a critical role in the regulation of the healing and remodeling process of ligaments. TGF-β1 also plays an important role in the development of ligaments, fibrosis, and collagen synthesis [20]. In a dog model, increased levels of TGF-β1 were observed 1 to 6 wk after surgery, which then decreased rather rapidly, returning to original levels by 12 wk [21]. In a rabbit remnant preservation ACL reconstruction model, a notable increase in TGF-β1 mRNA levels was observed when compared with the control group at 2 and 6 wk after surgery [17]. Marieke et al. [22] demonstrated that PRP could increase the expression of

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Fig. 3 — Relative mRNA levels for (A) COL1A1, (B) COL3A1, (C) TGF-β1, (D) decorin, (E) biglycan, (F) MMP-1, (G) MMP-13, and (H) TIMP-1 in each group at the three given time points after surgery. The results are expressed as a ratio of the mRNA levels of the reference gene GAPDH. The results are shown as the mean ± standard deviation (*P < 0.05 versus group IV (normal control group); **P < 0.05 versus group III (sham group); ***P < 0.05 versus group I (PRP group), n = 6 in each group).
TGF-β1 at early time points in human tenocyte culture. In a rabbit Achilles tendon model [23], PRP up-regulated the expression of TGF-β1 in the first 2 wk. Similarly, our study demonstrated that there is a sustained increase in the levels of TGF-β1 mRNA in group I. Our results suggest that PRP may play a key role in promoting the synthesis of TGF-β1, which is crucial for regulating the healing and remodeling process of the ACL graft. This may be explained that PRP is known to promote viability and proliferation of cells [6,7,18,19], including connective tissue cells such as fibroblasts and ligament cells [24], which could produce TGF-β1. However, further studies are still needed to prove this.

Decorin is the most prevalent small leucine-rich proteoglycan and has been shown to play a diverse role in connective tissues. It is known to bind collagen fibrils to regulate fibril formation and to influence the mechanical properties of tissue. It also modulates TGF-β1 activity by binding and inactivating TGF-β1 [25] and potentially assists in fibril orientation [26]. Previous studies suggested that platelet-released growth factors significantly increase the mRNA level of decorin compared with the control [7,27]. Similarly, we observed a significant increase of decorin mRNA expression in group I compared with group II 6 wk after surgery. This result may suggest that PRP promotes the synthesis of decorin at early time points during the graft remodeling process. However, decorin mRNA expression decreased in the ACL grafts compared with that in the normal ACLs.

Biglycan, similar to decorin, is another small leucine-rich proteoglycan that plays an important role in regulating the formation rate, distribution, and orientation of collagen fibrils by combining with fibrils or by directly incorporating itself into the fibrils [28,29]. Previous studies have shown that abundant amounts of biglycan accumulate in the repairing ligament 3 to 6 wk after MCL injury in a rabbit model and that biglycan may interfere with the collagen network remodeling and matrix turnover process [30]. In a rabbit model, mRNA expression of biglycan increased 3 wk after MCL injury and declined by 6 and 14 wk post-injury. However, levels were still elevated compared to the control, which suggests that biglycan may directly affect matrix assembly and biomechanical strength in healing [31]. Similarly, our results demonstrated a significant increase of biglycan mRNA expression in group I compared with group II at all time points. Our findings suggest that PRP may enhance the synthesis of biglycan, which is related to tendon repair, matrix organization and binding of growth factors.

Matrix metalloproteinases (MMPs) play an important role in tendon matrix remodeling processes by degrading collagen and proteoglycans in healthy and sick conditions through their broad proteolytic capabilities. Tissue inhibitor of metalloproteinases is thought to play an essential role in development, morphogenesis, reproduction, tissue remodeling, and disease processes such as rheumatoid arthritis and osteoarthritis [32]. To our knowledge, this is the first study to show that MMP-1, MMP-13, and TIMP-1 mRNA are expressed during the remodeling of ACL grafts in a canine model.

MMP-1 has the capability of cleaving interstitial collagens I, II and III at specific sites relative to the N terminus [33]. Roseti et al. [34] suggested that MMP-1 was expressed at higher levels in human ACL grafts years after ACL reconstruction surgery. Our data demonstrated a significant increase of MMP-1 mRNA expression in both groups I and II compared with that in group IV after ACL reconstruction surgery.

MMP-13 is a member of the MMPs family, and it collectively degrades essentially all components of the extracellular matrix. Therefore, it plays an important role in tendon healing, matrix remodeling and degeneration processes [35]. In a rabbit ACL injury model, significant increases in the mRNA levels for the matrix remodeling protein MMP-13 were observed in an ACL-injured group from 3 days to 12 wk after surgery [36]. Lo et al. [35] demonstrated a sustained increase in MMP-13 mRNA levels until 12 wk after injury in a partial ACL-injured sheep model. Similarly, our study demonstrated that MMP-13 mRNA expression was markedly higher in group I and group II compared with that in group III and group IV at all time points after surgery. Increased expression of MMP-13 could result in a net degradative or catabolic response in the ACL graft after surgery, which suggests matrix remodeling and possibly degradation in the ACL graft after reconstruction surgery.

In a rabbit ACL injury model, the mRNA levels for TIMP-1 were significantly higher in the ACL injury group compared with the control group and persisted until 6 wk after surgery [36]. In a rabbit MCL injury model, expression of TIMP-1 mRNA was significantly elevated at 3 wk post-injury, but levels

<p>| Table 2 — mRNA expression in ACL from the normal control group compared with ACL from the other groups.* |
|--------------------------------------|-------------------------------------|-------------------------------------|</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>2 wk</th>
<th>6 wk</th>
<th>12 wk</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>COL1A1</td>
<td>† (9.3)</td>
<td>† (2.6)</td>
<td>† (1.7)</td>
</tr>
<tr>
<td>COL5A1</td>
<td>† (1.2)</td>
<td>† (2.2)</td>
<td>† (2.3)</td>
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<tr>
<td>TGF-β1</td>
<td>(0.83)</td>
<td>(0.87)</td>
<td>(0.9)</td>
</tr>
<tr>
<td>Decorin</td>
<td>(1.4)</td>
<td>(1.2)</td>
<td>(1.2)</td>
</tr>
<tr>
<td>Biglycan</td>
<td>(3.2)</td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>MMP-1</td>
<td>(2)</td>
<td>(1.7)</td>
<td>(1.7)</td>
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<tr>
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<td>TIMP-1</td>
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<td>(1.5)</td>
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<td>(1.3)</td>
<td>(1.3)</td>
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* PRP treated group (I), saline treated group (II), and sham group (III) increased (†), decreased (†), and no statistically significant (—) gene expression compared with normal control group (P ≤ 0.05). Magnitude of change (fold change compared with normal groups) is indicated in parentheses.
declined to near non-injured levels by 6 wk [37]. Similarly, our study demonstrated a significant increase of TIMP-1 mRNA expression in groups I and II compared with group IV at 2 and 6 wk after surgery. TIMP-1 mRNA levels then declined by 12 wk post-operation; however, levels were still elevated compared to group IV.

Sham surgery increased the levels of MMP-13 and TGF-β1 mRNA in ACL grafts 2 wk after surgery (P < 0.05). Xie et al. [17] demonstrated persistent increases of TGF-β1 and GAP-43 mRNA levels in ACL tissue in the sham group compared with the non-surgery group. Beye et al. [36] revealed persistent increased levels of MMP-3 and TGF-β1 mRNA in rabbit ACLs in the sham surgery group compared with the normal control group. It is known that TGF-β1 plays an important role in anti-inflammation and is up-regulated in response to inflammation [17]. Increased mRNA levels of MMP-13 might be from capsulotomy-induced inflammation; however, further studies are needed.

TGF-β1, PDGF and IGF-I in the PRP may promote synthesis of the ECM [38] during the ACL graft remodeling process. Although several growth factors abundant in PRP, have been extensively studied in tissue regeneration, the key factors are unknown. No simple rule can be generalized to describe how PRP works during the ACL graft remodeling process. For future studies, a major challenge is to disentangle the relative effects of the various components of PRP and to understand how they influence the ACL graft maturation process [39].

As with any study, some potential limitations and assumptions of this study should be considered. First, even though each knee of the animals was randomly assigned to one group, each animal underwent an ACL reconstruction on at least one side, while the contralateral side underwent a sham surgery/control; therefore, because these samples are not truly independent, there is no true sham or control group that did not have ACL reconstruction surgery. To date, there is no compelling evidence of the systemic effects of local PRP injection [39]. However, local administration of PRP could affect the results not only for the ipsilateral knee but also for the contralateral side. Second, the reported results may be species-specific. Therefore, these results may not extend to clinical ACL reconstruction surgery. In the current study, only of the mRNA expression of genes was monitored and no investigation was performed at the protein level; therefore, and it is unclear how much of the mRNA that was translated into functional protein. Nonetheless, previous studies of ligament tissue have demonstrated a significant correlation between some mRNA and protein expression [36].

In summary, our study demonstrated that PRP affect the synthesis and degradation of the ECM in ACL grafts. The levels of the genes are elevated and are time-dependent during the graft remodeling process in both group I and group II, although the changes are more significant in group I for some target genes at certain time points. The present study has provided novel insights into the effect of PRP on ACL graft remodeling, as well as optimal treatment protocols to accelerate ACL graft remodeling. In addition, this study has provided evidence for the local administration of PRP during ACL reconstruction surgery in the clinic. However further studies are needed to elucidate whether the changes in animal models are related to differences in clinical outcomes.

Acknowledgments

The authors thank Feng Ni, MD and Lin Sha, MD for their technical assistance.

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