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Platelet-rich plasma enhances autograft revascularization and reinnervation in a dog model of anterior cruciate ligament reconstruction

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ARTICLE INFO

Article history:
Received 30 October 2012
Received in revised form
10 December 2012
Accepted 10 January 2013
Available online 31 January 2013

Keywords:
Platelet-rich plasma
Gene expression
Ligamentization
Revascularization
Reinnervation

ABSTRACT

Background: Autologous platelet-rich plasma (PRP) has been investigated as a potential promoter of tendon healing that affects the anterior cruciate ligament (ACL) graft maturation process. However, the influence of PRP on revascularization and reinnervation during the ACL graft remodeling has never been investigated.

Materials and methods: We randomly assigned healthy and mature beagles to one of four groups. In group 1 (PRP group), we treated the ACL grafts with PRP. In group 2 (control group), we treated the ACL grafts with saline. In group 3 (sham group), we exposed only the knee joints. In group 4 (normal control group), no surgery was performed on the knees. We dissected the ligament tissue at 2, 6, and 12 wk after surgery and performed real-time polymerase chain reaction using primers for cluster of differentiation molecule 31, vascular endothelial growth factor, thrombospondin-1 (TSP-1), neurotrophin-3, growth-associated protein-43 (GAP-43), and nerve growth factor.

Results: We observed the increased expression of vascular endothelial growth factor, TSP-1, neurotrophin-3, GAP-43, and nerve growth factor mRNA in group 1 at 2, 6, and 12 wk after surgery, compared with that in group 2 ($P < 0.05$). We also detected increased levels of cluster of differentiation molecule 31 expression in group 1 ($P < 0.05$) at 2 and 6 wk after surgery. The levels of TSP-1 and GAP-43 mRNA were significantly increased in group 3 compared with those in group 4 at 2 wk after surgery ($P < 0.05$).

Conclusions: During graft remodeling, we observed a time-dependent change in gene expression after ACL reconstruction surgery. In addition, these results demonstrate that PRP alters the expression of some target genes at certain times, particularly during the early stages of graft remodeling. Platelet-rich plasma could promote revascularization and reinnervation, which might explain the enhancing effect of PRP on ACL graft maturation.

1. Introduction

Injuries to the anterior cruciate ligament (ACL) are common. In the United States, it is estimated that 250,000 new ACL ruptures occur annually [1], which makes ACL reconstruction one of the most commonly performed procedures in sports medicine. Slow graft maturation might result in graft failure or elongation during postoperative rehabilitation; however,

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2013.01.020

the cause of this phenomenon remains unknown [2]. Recently, several studies have suggested that the addition of platelet concentrates to ACL reconstruction might have a beneficial effect on graft maturation [3–5]. The reasons for these positive effects, however, remain unclear.

Platelet-rich plasma (PRP) is a concentrate of platelets and associated growth factors that potentially enhances graft maturation through the delivery of various growth factors and cytokines from the alpha-granules contained in platelets [4,6–8]. Previous studies have shown that PRP significantly enhances neovascularization during tendon and bone healing [9–11], which might also accelerate tissue healing. The local administration of PRP measurably affects facial nerve regeneration after transection in a rat model [12] and is considered to be a promising method for the treatment of peripheral nerve regeneration after nerve injury [13]. However, to our knowledge, the use of PRP to accelerate revascularization and reinnervation of the ACL graft during graft maturation has not been reported in animals.

In this study, we examined the temporal gene expression of cluster of differentiation molecule 31 (CD31), vascular endothelial growth factor (VEGF), thrombospondin-1 (TSP-1), neurotrophin-3 (NT-3), growth-associated protein-43 (GAP-43), and nerve growth factor (NGF) in ACL grafts using quantitative real-time polymerase chain reaction (PCR) with SYBR Green in a dog ACL reconstruction model. The purpose of this study was to determine whether PRP affects gene expression during autograft ligamentization. We hypothesized that PRP would affect the gene expression associated with revascularization and reinnervation during ACL graft remodeling. Consequently, characterizing the expression patterns of the genes that respond to PRP during ACL reconstruction might encourage future research into the conditions that support the expression of functional proteins critical for ACL reconstruction, and facilitate the development of novel therapies to induce or inhibit the expression of these genes through the local administration of exogenous growth factors.

2. Materials and methods

2.1. Animals and groups

We used 36 healthy, skeletally mature male beagles weighing 12.50 ± 1.48 kg (mean $\pm$ standard deviation [SD]) in this study. We maintained the dogs in cages measuring $120 \times 100 \times 75$ cm with full cage activity. The Animal Experiment Ethics Committee of Shanghai Jiao Tong University, Shanghai, China, approved this experimental study.

We randomly divided the 36 dogs (72 knees) into four groups (18 knees/group). One knee from each dog was assigned to group 1 or 2; the other was assigned to group 3 or 4. In group 1 (18 knees), we reconstructed the ACLs using flexor digitorum longus autografts treated with PRP. In group 2 (18 knees), we reconstructed the ACLs using flexor digitorum longus autografts treated with saline. In group 3 (18 knees), we subjected the knees to a sham surgical operation. In group 4 (18 knees), the knees were not subjected to surgery. We killed the four groups of animals (six knees from each group) at 2, 6, and 12 wk after surgery.

2.2. Preparation of PRP

Immediately before surgery, we withdrew 20 mL of whole blood from the jugular vein of each animal in group 1 and divided it evenly (10 mL whole blood/tube) into two 15-mL centrifuge tubes, each containing 1 mL sodium citrate solution (2.5%). The blood was subsequently centrifuged according to the method of Landesberg *et al.* [14], which produces a high concentration of platelets and a low rate of activation. This method involves a double-spinning technique in which both samples undergo two rounds of centrifugation.

We centrifuged the two tubes at 200 *g* for 10 min at room temperature. Because of the differential densities, a platelet-poor plasma (PPP) layer formed at the top, a PRP layer formed in the middle, and a red blood layer formed at the bottom of the tubes (Fig. 1A). Using a pipette, we transferred the PPP and PRP layers to two new tubes without anticoagulant and centrifuged them for 10 min at 200 *g*. The PPP was subsequently removed from the upper layer of the supernatant, leaving 1 mL PRP at the bottom of the tubes, which we subsequently stored on ice (Fig. 1B) until further use (within 1 h). Each tube of whole blood and PRP was sampled and transported to an independent diagnostic laboratory to obtain the platelet count. A mean value of whole blood platelets yielded $133 \pm 33 \times 10^9$ platelets/L (mean $\pm$ SD) and the platelet count of PRP was $669 \pm 51 \times 10^9$ platelets/L (mean $\pm$ SD). The platelets were subsequently concentrated to increase the platelet numbers in the PRP to an average of five times that of the baseline concentration in whole blood.

2.3. Surgical procedures

2.3.1. Group 1 (PRP-treated graft)

We anesthetized the animals intravenously with sodium pentobarbital (30 mg/kg). The surgical procedure was performed according to the technique of Huangfu and Zhao [15]. We harvested the tendon of the flexor digitorum longus (8 cm in length) (Fig. 2A) and sutured both ends using a whipstitch with a number 2 polyester suture (Ethibond; Ethicon, Somerville, NJ). The tendon was subsequently folded in half to create a 4-cm-long, double-stranded graft (Fig. 2B). To maintain a consistent cross-sectional area between each graft, we trimmed the graft to a diameter of 4 mm. We placed two number 2 polyester sutures through the graft loop to fix the proximal end. We sutured the graft with a polyester suture at 12 mm from the graft loop end, which we used as a marker to ensure that the graft length in the femoral tunnel was 12 mm and the graft length in the tibial tunnel was approximately 18 mm. We performed these procedures to ensure that all animals were subjected to the same conditions.

The PRP (1 mL) was mixed with 10% calcium chloride (0.05 mL) and injected into the graft through a 2-mL syringe with a 19-gauge needle, with several punctures performed along the graft length (within 40–50 s after mixing) (Fig. 2C). We subsequently used the solution dripping from the injection sites to soak the graft until implantation (Fig. 2D), which resulted in the formation of a PRP gel (60 s after mixing) that covered the entire surface of the graft.

Using sterile technique, we exposed the knee through an anteromedial incision, followed by medial parapatellar