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Radiological study of the effect of Platelet Rich Plasma (PRP) on experimentally induced Osteoarthritis in Guinea Pig's stifle joint

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Abstract

The purpose of this radiologic, longitudinal study was to estimate the effects of the platelet rich plasma (PRP) on improvement of the defective articular cartilage in an osteoarthritis (OA) model. The PRP is an autologous product that concentrates a high number of platelets in a small volume of plasma. It mimics the last step of the coagulation cascade, leading to formation of a fibrin clot, which consolidates and adheres to the application site in a short period of time. Twenty adult guinea pigs (treatment group, n = 10; control group, n = 10) were anesthetized and the anterior cruciate ligament of the left knee was transected through a para-patellar approach. The animals were allowed to resume normal cage activity for 12 weeks. For the preparation of PRP, 2 ml blood was collected into a Na-citrate tube by direct heart puncture. Twelve weeks post-operation PRP was injected into the OA joint of the treatment group only. The animals were observed for further 8 weeks. At 20 weeks radiological score of OA increased in control group. Reduction in the degree of osteophyte formation, and subchondral bone sclerosis were detected in the PRP-treated joints. In conclusion, this study shows beneficial effects of the PRP on improvement of the defective articular cartilage in the OA joint.

Keywords: Platelet rich plasma, Osteoarthritis, Stifle joint, Guinea pigs, Radiological study

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Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease characterized by reduction of extracellular matrices in joint cartilage, bone and eventually, loss of joint function (Ringe and Stinger, 2009). Although articular cartilage is a metabolically active tissue, the chondrocytes in the matrix have a relatively slow state of turnover. The tissue itself lacks a blood supply to support repair and remodeling (Chang et al., 2005). Because of the limited capacity for spontaneous repair, today's treatment strategies are inefficacious and restricted to relieving the symptoms. Most treatment options are restricted to different surgical procedures including debridement, drilling, osteochondral transplantation, autologous perichondral and periosteal grafts, and autologous chondrocyte implantation (Hunziker, 2002). Platelet-rich plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above the baseline (Foster et al., 2009). The PRP releases a high concentration of multiple growth factors that can modulate healing processes (Foster et al., 2009). In this study, PRP from autologous blood was used by direct intra-articular injection in an experimental model of OA (Gimeno et al., 2006). The purpose of this radiologic, longitudinal study was to estimate effects of the PRP on improvement of the defective articular cartilage in an OA model**.**

Material and Method

Twenty adult guinea pigs (700.0 ± 57.0 g, Mean ± SD) were used in this study. Animals were randomly assigned into two groups: the PRP treated group (n = 10), and control group (n = 10). This study was approved by the institutional ethical committee of the Shiraz University.

Preparation of PRP: Two mL blood was collected directly from the heart into a Na-citrate tube. The tubes were centrifuged at 2000 rpm for five minutes. Three different density compartments were detectable after the preliminary centrifuging; the lower consisted of red blood cells, the middle buffy coat of white blood cells, and the top plasma. The plasma on its own had three distinct layers in ratio of 2:1:1 from the top. The top layer was the platelet-poor plasma (PPP), the middle plasma-average platelet (PAP) and the lowest, the PRP. The first (PPP) and the second (PAP) layers were removed by a pipette. The third (PRP) layer was carefully separated by pipette and centrifuged again at 5000 rpm for 2 minutes. Following the second centrifuging the top layer in the tube (plasma) was discarded and the second layer (PRP) was collected for intra-articular injection (0.4 mL) in the OA joint.

Surgical procedure: The guinea pigs were anesthetized by intramuscular administration of 50 mg/kg of ketamin (Alfasan, Woerden-Holand) and 10 mg/kg xylazine (Rompun, Bayer AG, Leverkusen). The anterior cruciate ligament (ACL) of the left stifle was transected under aseptic conditions through a para-patellar skin approach. To achieve optimal visualization of the anterior cruciate ligament for transection, the patella was displaced laterally and the knee was placed in a full flexion. The joint capsule and subcutaneous tissue were closed using 4-0 polydioxanone suture (PDS™ II, Eticon, Inc.). The skin was closed using 3-0 silk surgical suture (SUPA, Iran). Following surgery, animals were treated by penicillin (Zakaria Co., Tabriz, Iran), and Flunixin Meglumine (Razak Co., Tehran, Iran), and allowed to resume the normal cage activity for 12 weeks.

Radiological study: Cranio-caudal and lateral radiographs of the stifle joint were obtained at the 12th and 20th week post-surgery (Toshiba X-ray, Toshiba, Japan). The exposure factors were 42 kV, 20 mAs, and 75 cm film-focus distance. For the cranio-caudal view, the guinea pigs were placed in a dorsal recumbency with legs extended caudally. For the lateral view, animals were positioned on the lateral side with half-flexed knee joints. Radiological OA was assessed by means of the grading system proposed by Kellgren and Lawrence (1957). In this system, based on radiological features including narrowing of the joint space, presence of osteophytes, sclerosis of subchondral bone and deformity of bone ends, OA was categorized into five grades 0 (None), 1 (Doubtful), 2 (Minimal), 3 (Moderate), and 4 (Severe). Two independent evaluators blinded to treatment allocation scored the radiographs.

Statistical evaluation: The data were analyzed statistically by SPSS version 16.0 using Mann-Whitney test and Npar tests to compare the groups together. The *P*-values less than 0.05 were considered as significant.

Results

All stifle joints subjected to anterior cruciate ligament transection showed radiological signs of OA at twelve weeks post-surgery. Signs of OA included marginal osteophytes, narrowing of joint space and subchondral bone sclerosis. Radiological score 1 was found in 2 joints, score 2 in 8 joints, score 3 in 8 joints, and score 4 in 2 joints (Table 1). At 20 weeks radiological score of OA increased in control group in comparison with 12 weeks (average increase of 2 scores). In the PRP-treated joints, the degree of osteophyte formation, and subchondral bone sclerosis were all reduced compared with that at 12 week (average decrease of 2 scores; Table 1).

Table 1. The degree of Osteoarthritis following surgical resection of ACL ligament at 12 and 20 weeks post operation in the control and treatment groups (5).

Osteoarthritis degree	12 weeks post op. total animals	12 weeks post op. control group	12 weeks post op. Treated group	20 weeks post op. control group	20 weeks post op. Treated group
0	-	-	-	-	-
1	2	1	1	-	3
2	8	6	4	4	6
3	8	2	4	3	1
4	2	1	1	3	-
Total	20	10	10	10	10

Figure 1. The stifle joint (lateral position) at 12th week post operation (no treatment). Reduced joint space, sclerosis of subchondral bone, joint surface irregularities, and osteophytes were observed.

Figure 2. The stifle joint (lateral position) in the treatment group (intra articular PRP injection 12 weeks post operation) at 20th week post operation. "Note the reduction of the OA lesions in the joint"

Figure 3. The stifle joint (lateral position) in the control group at 20th week post operation (no intra articular PRP injection). Severely reduced joint space, sclerosis of subchondral bone, joint surface irregularities, and osteophytes formation at 20th weeks post operation.

Discussion

Osteoarthritis is associated with cartilage destruction, subchondral bone remodeling and inflammation of the synovial membrane, although the etiology and pathogenesis underlying this debilitating disease are poorly understood (Kapoor et al., 2011). Since the biochemical and pathological changes are identical to those seen in human OA, the ACL transected model in guinea pigs has been proved as a useful model in investigation of OA development (Jean et al., 2006). In this study, all radiographic signs of OA, including periarticular osteophytosis, sclerosis of subchondral bone and deformity of bone ends were observed at 12 weeks after surgical induction of the joint instability in guinea pigs by the ACL transection. Treatment by PRP was associated with a significant improvement in the joint cartilage by week 20 of this study. The severe cases of OA changes were improved to milder forms of OA so that by the end of 20 weeks there were more cases of score 1 and 2 of OA rather than Score 3 and 4 compared to the control group.

Recent investigations have focused on employing PRP for repairing cartilage lesions (Kalbkhani et al., 2013). The PRP is an autologous product that concentrates a high number of platelets in a small volume of plasma (Foster et al., 2009). This product mimics the last step of the coagulation cascade, leading to the formation of a fibrin clot, which consolidates and adheres to the application site in a short period of time (Gimeno et al., 2006).

The PRP has been utilized in surgery for 2 decades. A recent interest in the use of PRP for the treatment of sports-related injuries has been evolving. The PRP contains growth factors and bioactive proteins that influence the healing of tendon, ligament, muscle, and bone tissue (Foster et al., 2009). The therapeutic use of PRP is an autologous biotechnology that relies on the local delivery of a wide range of growth factors and cytokines with the aim of enhancing the tissue healing (Andia, 2010; Mankin et al., 1971). The PRP has been used in humans in different kinds of transplant procedures such as dentistry, orthopedics, maxillofacial surgery, plastic surgery and ophthalmology. In addition, PRP may be considered a carrier for biological active agents and a healing substance causing less post-surgical pain (Gimeno et al., 2006). Injection of PRP in the joints affected by OA changes at 12 weeks post ACL transections stopped further deterioration of the joints as well as repaired the process of healing by reducing the OA changes toward a normal joint ($P < 0.05$). The results show the beneficial effects of the PRP on improvement of the defective articular cartilage in the OA joint.

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