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The effect of autogenous platelet rich plasma on experimentally induced osteoarthritis in rabbit's stifles joint: a radiological assessment.

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Abstract

Osteoarthritis is the most common type of arthritis and the major cause of chronic disability in musculoskeletal mobility in the elderly populations worldwide. In this study, we used scaffold free authogenous platelet rich plasma (PRP) in an experimental animal model of osteoarthritis (OA) by direct intra articular injection. Thirty white New Zealand adult rabbits of both sexes were used in this study. Osteoarthritis was induced by anterior cruciate ligament transection of the left knee joints. The rabbits were divided into three groups randomly; Eight weeks after operation, a single dose (0.5 ml) of plate rich plasma (PRP) was delivered to the injured knee by direct intra articular injection (group 1, the treatment group). The preventive group received the same amount of PRP right after operation. The control group received no treatment. The knees were examined on 8, 12 and 16 weeks after the surgery. The repairing process was investigated radiologically. Radiological assessment confirmed development of OA changes after 8 weeks in rabbits of group 1 and 2. Rabbits received PRP (group 1, the treatment group) showed lower degree of cartilage degeneration, osteophyte formation, and subchondral sclerosis compared to the control group on16 weeks after surgery. In the preventive group, there was no OA development. PRP could be a valuable medium and the promising source for the treatment of osteoarthritis as well as for the prevention of the development of OA.

Key words: osteoarthritis, platelet rich plasma, radiology, stifle joint, prevention

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Introduction

Osteoarthritis (OA) is highly prevalent disease and the prevalence is expected to increase substantially as a greater proportion of the population is facing with age. OA-related complaints are the most common reasons for visits to health care providers (Hayes et al., 2005). It is a progressively debilitating disease that affects mostly cartilage, with associated changes in the bone. Vascular pathology might play a role in the initiation and progression of the major disease of joints such as osteoarthritis (Findlay, 2007). Although OA is characterized by progressive degenerative damage to articular cartilage, there are significant changes in the bone of affected joints. Bone changes in established OA include subchondral cysts, sclerosis and osteophyte formation. However, the detection of changes in the subchondral bone by magnetic resonance imaging (MRI), even in early OA have led to the suggestion that OA may arise as a bone disorder, affecting bone structure and remodeling (Hayes et al., 2005). However, lack of understanding of the underlying cause(s) for OA means that treatments remain largely palliative (Findlay, 2007). The insufficient therapeutic choices have led to focus on the potential of platelet-rich plasma (PRP) as new strategy for cartilage repair. It has been well established that articular cartilage has a limited capacity for self repair due to the low cellular mitotic activity of chondrocytes and its avascularity (Findlay, 2007). Large cartilage defects often fail to heal spontaneously and may result in progressive deterioration and eventually osteoarthritis. When the clinical application of growth factors was considered, PRP, as a rich source of autologous growth factors, can be regarded as an alternative approach (Gotterbarm, 2006). In this study, we used scaffold free authogenous PRP in an experimental animal model of OA by direct intra articular injection.

Material and Methods

Preparation of PRP

Two ml blood was obtained from the superficial ear vein of each rabbit collected into the sodium citrate tube. The tubes were centrifuged at 1240 rpm for 8 minutes. The top layer which was plasma had three distinct layers in ratio of 2:1:1 from the top. The first top layer was platelet-poor plasma (PPP), the middle layer was plasma-average platelet (PAP) and the lower layer was PRP. The PPP and PAP were removed by pipette. The PRP layer was carefully separated by pipette and centrifuged again for 5 minutes at the same rate. Then, the first layer (plasma) was discarded and the second layer (PRP) was collected for intra-articular injection into the induced-OA joints.

Surgical procedure

This study was approved by institutional ethical committee of the Shiraz University. Thirty adult New Zealand white rabbits weighing 2.22 ± 0.12 kg were used in this study. The rabbits were

anesthetized with intramuscular administration of 50 mg/kg of ketamine (Alfasan, Woerden-Holand) and 10 mg/kg of Xylazine (Rompun, Bayer AG, Leverkusen). The anterior cruciate ligament (ACL) was transected under aseptic conditions through the skin incision in the medial parapatellar area of the left knee. To achieve optimal visualization of the anterior cruciate ligament (ACL), the patella was displaced laterally and the knee was placed in full flexion, then the ACL was transected. The joint capsule and subcutaneous tissue were closed using 3-0 Polydioxanone suture (ETHICON, INC). The skin was closed using braided silk suture (SUPA, Iran). Following the operation, the rabbits were treated by Penicillin (Zakaria, laboratory, Tabriz, Iran), and non-steroidal anti-inflammatory drug (Flunixin, Razak laboratories, Tehran, Iran) and allowed to resume normal cage activity. The rabbits were divided into three groups randomly as:

1- Treatment group which received autogenous PRP by intra articular injection on 8th week (56 days) post operation.
2- Preventive group which received autogenous PRP right after operation by intra articular injection.
3- Control group which did not receive any treatment at all.

PRP injection

The rabbits in group 1 (the treatment group) were anesthetized at the end of 8 weeks following the operation. The knee joint was prepared for aseptic injection. 0.5 ml PRP was injected into the medial compartment of the operated joint. The rabbits in the control group did not receive any PRP injection and the rabbits in the preventive group received PRP injection right after operation (day 0). All rabbits were allowed unrestricted cage activity without immobilization.

Radiological evaluation

Radiographs of the left knee joint were obtained in craniocaudal and lateral views, prior to operation, 8th, 12th and 16th weeks following operation using 42 kV, 20 mAs and 75 cm film-focus distance. Radiography was performed by the same operator with the same equipment (Toshiba Japan, IME-124M). For craniocaudal view, rabbits were placed in dorsal recumbency with legs extended caudally. Lateral radiographs were done on semi flexed knee joints. Osteoarthritis was evaluated radiologically by the grading system proposed by Kellgren et al. (1957). In this system, based on radiological features including: narrowing of joint space, presence of osteophytes, sclerosis of subchondral bone and deformity of bone ends, osteoarthritis was divided into five grades: 0 (None), 1 (Doubtful), 2 (Minimal), 3 (Moderate), and 4 (Severe). To improve qualitative data recordings, two independent readers who were blinded to all data of the rabbits scored the radiographs.

Statistical Analysis

The statistical analysis was performed using the Kruskal-Wallis test to test for significant difference among groups and pair-wise comparison was performed using the Mann-whitney test.

Results

The normal radiological appearance of the knee joint in rabbits is shown in the Fig. 1 which was taken prior to the knee operation. Eight weeks after surgery, all knees subjected to ACL transection showed radiological signs of OA including marginal osteophytes, narrowing of the joint space and subchondral bone sclerosis (Fig. 2 compared to Fig. 1). At 12 weeks, radiological score of OA decreased slightly or stayed unchanged in PRP-treated group in comparison with 8^{th} weeks ($P < 0.05$) (Fig. 3). In the control group, the radiological score of OA increased at 12 weeks (Table 1 and Fig. 2) compared to the 8^{th} weeks post operation. At 16 weeks in the PRP treated joints, the degree of osteophyte formation, and subchondral bone sclerosis were all reduced compared with that of 8^{th} week ($P < 0.05$) (Fig. 4), and the control group ($P < 0.05$) (Fig. 5). We observed a decrease in OA score in PRP treated group and a significant increase in OA score in the control group. The rabbits in the preventive group did not show any sign of OA at the end of 8^{th} week (Table 1 and Fig. 6).

Discussion

Our study describes the process of development of osteoarthritis (OA) in rabbits by radiological investigation and also the effects of platelet rich plasma on repair process and conversion of osteoarthritis changes to normal articular structure (table 1). Injection of PRP in the joints affected by OA changes at eight weeks post operation of 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 in this study. Osteoarthritis have been induced in rabbits to study the effect of infra patellar fat pad derived stem cells in and subcutaneous derived Mesenchymal stem cells in repair of osteoarthritis

Table 1. Radiological score of osteoarthritis based on grading systems reported by Kellgren at weeks 1 to 16 following transection of the ACL.The score of Osteoarthritis was 0 (none), 1(doubtful), 2 (minimal), 3 (moderate), and 4 (severe). Similar letters reflects significant difference between each two groups ($P < 0.05$) in rabbits.

	Radiological Score	Week 1	Week 8	Week 12	Week16
		(no. in each score)	(no. in each score)	(no. in each score)	(no. in each score)
Treatment group[1]	0	10	0	4	6
A	1	0	1	2	3
	2	0	5	2	1
	3	0	4	2	0
	4	0	0	0	0
Control group[2]	0	10	0	0	0
B	1	0	2	0	0
	2	0	4	3	1
	3	0	3	3	3
	4	0	1	4	6
Preventive group[3]	0	8	9	10	10
B	1	2	1	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0

[1]Received PRP on 8th week post op; [2]Did not receive any PRP on 8th weeks post op; [3]Received PRP on the same day right after operation

Fig. 1. Lateral radiograph of a normal rabbit joint.

Fig. 2 .The lateral radiograph of the rabbit at 8th weeks following ACL transection showing score 4 of the osteoarthritis. (knee joint with osteoarthritis. Note subchondral bone sclerosis and marginal osteophytes).

Fig 3. The lateral radiograph of a rabbit at 12th weeks following treatment by PRP, showing reduction of radiological score of osteoarthritis.

Fig 4. The lateral radiograph of a rabbit at 16 weeks (8 weeks following injection of PRP into the joint space) showing improvement of score 4 to moderate osteoarthritis radiological score.

Fig 5. The lateral radiograph of the rabbit stifle (knee) joint in the control group without any treatment on 16th weeks following anterio cruciate ligament transection, showing radiological score 4 of osteoarthritis. Note subchondral bone sclerosis and marginal osteophytes.

Fig. 6. The lateral radiograph of the knee joint of a rabbit in the preventive group receiving platelet rich plasma right after anterior cruciate ligament transection, showing no sign of oteoarthritis on 8 weeks after anterior cruciate ligament transection.

(Toghraei et al., 2011; Toghraei et al., 2012). Early phases of osteoarthritis have been studied by radiological study (Nikahval et al., 2011). They concluded that osteoarthritic changes of the joints can be detectable 1 month post injury by MRI. Meniscal degeneration and subchondral bone irregularity were detectable diagnostic signs.The disease which affects 60–70 % of people over the age of 70 years, causing considerable pain and loss of mobility and thus reducing the quality of life for millions of elderly people. Neither cause nor cure is known at present (Helmtrud, 2008).

As OA progresses, there is evidence of vascular invasion and advancement of this zone of calcified cartilage into the articular cartilage that further contributes to a decrease in articular cartilage thickness (Goldring and Goldring, 2006). In our study the preventive group which received the PRP right after the ACL transection, the PRP has prevented development of bone, soft tissue or vascular changes in the joint and resulted in an almost sound joint after 8 weeks following surgical resection of ACL (Table 1).

Craniocaudal radiographs of the knee were obtained in a weight-bearing extended position by using a standard radiographic technique. One experienced musculoskeletal radiologist and one experienced rheumatologist independently assigned scores to all radiographs by using the Kellgren and Lawrence scoring system (Kellgren and Lawrence, 1957) and found that the craniocaudal views characterize OA of the knee in the medial and lateral femorotibial compartments, with exclusion of the patellofemoral compartment (Hayes et al., 2005).

The digital x-ray technique without fluoroscopy is a simple and reliable technique for measuring the joint space width (JSW) in the normal knee. The greatest source of variation was due to the subject (Oksendahla et al., 2009). Nikahwal et al. (2011) also concluded that the early radiographic changes detectable in one month post OA were mild joint narrowing.

Platelet-derived growth factors (GFs) are biologically active peptides that enhance tissue repair mechanisms such as angiogenesis, extracellular matrix remodeling, and cellular effects as stem cells recruitment, chemotaxis, cell proliferation, and differentiation. Platelet-rich plasma is used in a variety of clinical applications, based on the premise that higher GF content should promote better healing. Platelet derivatives represent a promising therapeutic modality, offering opportunities for treatment of wounds, ulcers, soft-tissue injuries, and various other applications in cell therapy (Sanchez- Gonzalez et al., 2012).

Platelet rich plasma is an autologous blood-derived product that has an increased concentration of platelets that are rich in growth factors, and has the potential to enhance the healing of tissue at the cellular level via the recruitment, proliferation, and differentiation of cells involved in tissue regeneration (Ahmad et al., 2012). Because, it can be used autogenously, it poses no risk of transmissible diseases (Shin et al., 2012). Furthermore, PRP can easily be obtained on the day of surgery by two centrifugation

steps from autogenous whole blood. Platelet-rich plasma has been extensively investigated for bone regeneration and soft tissue healing and reports claim a positive effect of PRP (Wiltfang et al., 2004). Thus, if PRP growth factors were also effective for cartilage tissue, PRP could be an attractive clinical source for osteochondral tissue regeneration (Sun et al., 2010). Complicated diabetic patients show impairment and delayed wound healing caused by multiple factors. A study on wound healing showed that PRP was effective in normal tissue regeneration. For examination of new biomarkers and their relevance for radiographic severity in knee osteoarthritis new imaging approaches to assess structural deterioration and correlation with biomarker levels are warranted to advance in OA research (Anitua et al., 2009). Autologous mesenchymal stem cell injection, in conjunction with hyaluronic acid, platelet rich plasma and calcium chloride, is a promising minimally invasive therapy for osteonecrosis of femoral head and, with low-dose dexamethasone, for osteoarthritis of human knees (Madlener et al., 1998). Platelet-rich plasma was used as a source of growth factor and as a differentiating agent for the mesenchymal stem cells (Jaewoo, 2011). Moreover, strong evidence from well-designed clinical trials to support the PRP therapy for osteoarthritis of the knee joint is yet scanty. As a matter of fact, PRP protocols are currently approved only for use in clinical trials and research, and are not allowed for treatment purpose by any institutions in Korea (Yong- Geun et al., 2012). Platelet-derived Growth Factors (GFs) are biologically active peptides that enhance tissue repair mechanisms such as angiogenesis, extracellular matrix remodeling, and cellular effects as stem cells recruitment, chemotaxis, cell proliferation, and differentiation. Platelet-rich plasma is used in a variety of clinical applications, based on the premise that higher GF content should promote better healing. Platelet derivatives represent a promising therapeutic modality, offering opportunities for treatment of wounds, ulcers, soft-tissue injuries, and various other applications in cell therapy (Gotterbarm, 2006). PRP can be combined with cell-based therapies such as adipose-derived stem cells, regenerative cell therapy, and transfer factors therapy (Sanchez- Gonzalez et al., 2012). In Europe and the United States, there is an increasing prevalence of the use of autologous blood products to facilitate healing in a variety of applications (Ahmad et al., 2012). With that knowledge, there is abundant enthusiasm in the application of concentrated platelets, which release a supra-maximal quantity of these growth factors to stimulate recovery in non-healing injuries. However, as clinical use increases, more controlled studies are needed to further understand this treatment (Sampson et al., 2012). Platelet gel is really valuable in the management of alveolar bone loss because of its already reported antiseptic, adhesive and osteoregenerative properties. It is well tolerated and does not cause any harmful side effects. Platelet gel is rich source of growth factors and represents a versatile home-made product (Dominijanni et al., 2012).

In our study, intra- articular injection of PRP right after surgical transection of ACL prevented the development of the early phases of the OA as compared to the control group. The OA changes in the rabbits of the control group at eight weeks post operation has been compared to the no OA changes or almost normal joints of the rabbits in the preventive group at eight weeks post operation. Also, injection of PRP in the joints affected by OA changes at eight 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.

In conclusion, from the radiological point of view the PRP could be a valuable medium and the promising source for the treatment of osteoarthritis as well as for the prevention of development of OA.

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