Full Length Research

Preventive role of platelet rich plasma (PRP) in experimentally induced osteoarthritis in rabbit’s knee joint

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Received July, 2015; Accepted July, 2015

Osteoarthritis (OA) is the most common type of arthritis and the major cause of chronic musculoskeletal mobility disability in the elderly populations worldwide. In this study, scaffold free autogenous platelet rich plasma (PRP) was used in an experimental animal model of OA by direct intra articular injection. 15 white New Zealand adult rabbits of both sexes were used in this study. OA was induced by anterior cruciate ligament transection of the knee joints. Immediately following operation, a single dose of (0.5 ml) platelet rich plasma (PRP) was delivered to the injured knee by direct intra articular injection in the test groups. The control group received no PRP injection. The knees were examined after four and eight weeks following the operation histopathologically. Pathologic assessment confirmed development of OA changes after 8 weeks in the control group. Rabbits which received PRP showed lower degree of cartilage degeneration, osteophyte formation, and subchondral sclerosis than the control group at 8 weeks after surgery. In conclusion, PRP could be a valuable medium and a promising source for the prevention of the development of OA.

Key words: Prevention of osteoarthritis, platelet rich plasma, histopathologic assessment, stifle joint.

INTRODUCTION

Osteoarthritis (OA) is highly prevalent disease and the prevalence is expected to increase substantially as a greater proportion of the populations are facing with age. OA is a progressively debilitating disease that affects mostly cartilage, with associated changes in the bone. The insufficient therapeutic choices have led to focus on the potential use of platelet rich plasma (PRP) as a new strategy for cartilage repair. There are many studies indicating the use of PRP for the treatment of OA, but the studies on the preventive role of PRP in the developments of OA is scanty. In this study, scaffold free autogenous platelet rich plasma (PRP) was used immediately following the experimental induction of animal model of OA by direct intra articular injection to observe the preventive effects of PRP.
MATERIALS AND METHODS

Preparation of PRP

Four ml blood was obtained from the jugular vein of each rabbit collected into the Na citrate tube. The tubes were centrifuged at 1240 rpm for eight minutes. The tubes showed three different density compartment, the lower red blood cells, the middle Buffy coat of white blood cells, and the top plasma. The 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 platelet average plasma (PAP) and the lower platelet rich plasma (PRP). The first (PPP) and the second (PAP) layers were removed by pipette. The third (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 in the OA joints.

Surgical procedure

This study was approved by institutional ethical committee. Fifteen 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). Anterior cruciate ligament was transected (ACLT) under aseptic conditions through the skin incision in the medial para-patellar area of the left knee. To achieve optimal visualization of the anterior cruciate ligament, 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 silk suture (SUPA, Iran). Following surgery, rabbits were treated with the standard antibiotic (Penicillin, Zakaria laboratory Tabriz, Iran), and analgesic (Flunixin, Razak laboratories, Tehran-Iran) and allowed to resume normal cage activity for 8 weeks.

PRP injection

While the rabbits were not recovered yet 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 of the control group received no injection. All rabbits were allowed unrestricted cage activity without immobilization until sacrifice (Figure 1).

Pathologic evaluation

The samples (n: 15) were divided into three groups.

A- Group 1. PRP received preventive group (n=5) were euthanized on 4th week following operation.
B- Group 2. PRP received preventive group (n=5) were euthanized on 8th week following operation.
C – Group 3. No PRP control group (n=5) were euthanized on 8th week following operation.

Following euthanasia the operated leg was dissected and fixed in 10% formaldehyde, then they were decalcified by 7% nitric acid. The samples were cut in frontal plan, so that femur and tibia and joint space in between can be seen is a single plane, then were stained by toluidine blue solution. Evaluation of the quantitative measures on the samples include; cartilage thickness, chondrocyte count, presence and severity of cartilage tear(s), glycoprotein content, collagen staining quality and assessment of the proportion of live chondrocytes. Thickness of cartilage was measured by scaled graticule lens at 10× objective. This measurement was done on both femoral and tibial sides of the joint space in five randomly selected areas. To obtained chondrocytes quantity, the reticulated graticule lens was used to count the cells in five different, randomly selected areas. Presence and severity of cartilage tears was evaluated and ranked in the following grades:

Grade 0: no tears
Grade 1: tear in depleted glycoprotein layer.
Grade 2: tear up to calcified layer.
Grade 3: tear in calcified cartilage up to the underlying bone.
Grade 4: tear extending in to the bone.

Glycoprotein content was measured by using tide mark level of cartilage (boundary between glycoprotein layer and calcified cartilage) as a guideline (Griffin et al., 2009). The collagen staining quality was assessed by toluidine blue staining intensity (Xiaofeng et al., 2011). Cartilage cell distribution was evaluated by cell distribution pattern as: columnar, columnar and cluster, cluster (Xiaofeng et al., 2011). Live chondrocyte proportion was measured by counting cartilage lacunae that contained visible chondrocytes with intact nucleus and cell membrane in five different randomly selected areas (Xiaofeng et al., 2011).

**Statistical analysis**

Statistical analysis was done using version 18 SPSS and P< 0.05 considered significant.

**RESULTS**

The results of measured data in preventive groups on 4th, 8th weeks and control group on 8th week are summarized in Tables 1 to 3, Figures 2 to 4 and Histograms 1 and 2.

**DISCUSSION**

Osteoarthritis is one of the most debilitating joint diseases and has major influences on the life style, and characterized by cartilage deterioration, loss of joint space, osteophyte and loss of joint function. Several studies has assessed the role of PRP in healing of many diseases, Griffin (2009). PRP contains various growth factors that play important roles in cell proliferation, chemotaxis, cell differentiation, and angiogenesis concentrations. The basic cytokines identified include platelet-derived growth factor, transforming growth factor β, vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor, epidermal growth factor, and endothelial cell growth factor. The clinical benefit of these growth factors contained in PRP for the treatment of pathologies of the foot or ankle is derived from these biological properties (Xiaofeng, 2011).

Gruber et al. (2002) found that platelets and thrombin activated platelet products induced mitogenic activity of cultured human trabecular bone derived cells and that platelet concentrates also enhance the proliferation of human osteoblast like cells (Weibrich, 2002). Interestingly, Han et al. (2009) found that PRP augmented the quantity of marrow stromal cells in a dose dependent manner at 48 hours but that thrombin-activated PRP did not do so. When using a rat model, PRP appeared to amplify in vivo demineralized bone matrix osteoinductivity. Chondrogenesis was seen in 2 weeks and osteogenesis in 4 weeks in the non activated PRP cohort. PRP also contains Platelet Activation Factor (PAF) that produces several biochemical responses associated with inflammation and wound healing, inducing rapid activation of PLA2 and release of AA (Hurst and Bazan, 1995). It also activates mitogen-activated protein kinases (Bazan and Varier, 1997) and stimulates both calcium influx into cells and the expression of the cyclooxygenase (COX-2) enzyme, an inducible isofrom responsible for synthesis of various prostaglandins associated with inflammation (Bazan et al., 1997). In addition, PAF activates the gene expression of selective metalloproteinases (MMPs) involved in tissue remodeling (Bazan et al., 1993; Tao
et al., 1995; Tao et al., 1996), and their inhibitors, including tissue inhibitors TIMP-1 and -2 and plasminogen activator inhibitor PAI-1(Ottino et al., 2002). PRP also has been studied in the context of discogenic regeneration in hopes of finding a novel therapeutic option for back pain (Chen et al., 2006) and found that administering PRP to human nucleus pulposus (NP) cells resulted in NP proliferation and differentiation, accelerated proteoglycan matrix accumulation, and decreased apoptotic cell numbers. Furthermore, PRP contributed to tissue engineering of NP by using type I and II collagen scaffolds, indicating evidence of chondrogenesis. PRP has typically been derived from blood and its method of use is by intra articular injection.

Our study describes the process of development of Osteoarthritis (OA) in rabbits of the control group by pathological investigation and also the effects of platelet rich plasma on repair process or prevention of the development of osteoarthritis changes to restore normal articular structure. In this study the live cell population and cell distribution were much better than the control group (p<0.05), also the staining properties of the articular cartilage matrix in the preventive group was superior to the control group (P<0.05). There was a lot of cartilage tears in the control group (P<0.05), all indicating the effective influence of PRP in the prevention of the development of OA syndromes due to transection of ACL in the stifle joint.

Vascular pathology might play a role in the initiation and progression of the major disease of joints such as osteoarthritis (OA). Although OA is characterized by progressive degenerative damage to articular cartilage, there are, as the
Table 1. Measured data and their grading system.

<table>
<thead>
<tr>
<th>Title</th>
<th>Explanation</th>
<th>Grade</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartilage tear</td>
<td>Smooth and normal</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to condensed glycoprotein layer(thickness of depleted glycoprotein layer)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Up to calcified cartilage(thickness of glycoprotein layer)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deep to calcified layer</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole cartilage thickness</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Columnar</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Columnar-cluster</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cluster</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irregular/single cells</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mostly live cells</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cellular distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live cell population</td>
<td>Few live cells</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less than 10% live cells</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal to near normal</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Staining of cartilage matrix</td>
<td>Good staining</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium staining</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weak staining</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cartilage thickness</td>
<td>Whole thickness will be increased due to increase in depleted glycoprotein layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcified cartilage</td>
<td>Part of cartilage below tide mark</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condensed Glycoprotein layer thickness</td>
<td>In normal joint thickness and staining are higher than in osteoarthritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depleted glycoprotein layer thickness</td>
<td>In osteoarthritis thickness of this layer will be increased. Normal cartilage lacks this layer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Measured data in three different groups. Significant statistical difference (P<0.05) between control and preventive group on 8th week, except articular cartilage matrix staining (P > 0.05)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Live cell population</th>
<th>Cellular distribution</th>
<th>Articular cartilage matrix staining</th>
<th>Cartilage tears</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th prevention</td>
<td>2.5±0.22</td>
<td>2.3±0.21</td>
<td>2.0±0.0</td>
<td>0.5±0.18</td>
</tr>
<tr>
<td>8th prevention</td>
<td>2.6±0.16</td>
<td>2.1±0.1</td>
<td>2.1±0.23</td>
<td>0.5±0.16</td>
</tr>
<tr>
<td>8th control</td>
<td>1.62±0.18</td>
<td>1.25±0.25</td>
<td>1.5±0.18</td>
<td>3.0±0.42</td>
</tr>
</tbody>
</table>

Table 3. Measured data in three different groups. Only thickness of depleted glycoprotein layer had significant statistical difference (P<0.05) between preventive and control group on 8th week.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Thickness of condensed glycoprotein layer</th>
<th>Thickness of depleted glycoprotein layer</th>
<th>Thickness of Calcified cartilage</th>
<th>Total cartilage thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th prevention</td>
<td>437.23±88.37</td>
<td>76.22±41.47</td>
<td>175.32±63.68</td>
<td>688.67±170.99</td>
</tr>
<tr>
<td>8th prevention</td>
<td>332.57±25.96</td>
<td>9.58±2.73</td>
<td>138.95±13.13</td>
<td>498.54±25.27</td>
</tr>
<tr>
<td>8th control</td>
<td>264.89±60.36</td>
<td>155.18±41.81</td>
<td>101.54±13.19</td>
<td>530.88±84.95</td>
</tr>
</tbody>
</table>

name suggests, significant changes in the bone of affected joints (Findlay, 2007; Sanchez et al., 2012). Bone changes in established OA include subchondral cysts, and osteophyte formation. However, the detection of changes in the subchondral bone by MRI, even in early OA has led to the suggestion that OA may arise as a bone disorder, affecting bone structure and remodeling and lack of understanding of the underlying cause(s) for OA means that treatments remain largely palliative (Findlay, 2007). 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 deterioration, soft tissue or vascular changes in the joint and resulted in an almost sound joint. Therefore it can be suggested that in conditions such as traumatic insults to the joint surface or joint ligaments especially in athletes, the preventive use of PRP can be
Histogram 1. Measured data in 4th and 8th week. All data between prevention and control groups in 8th week have significant statistical difference (p<0.05), except articular cartilage matrix staining (p>0.05).

Histogram 2. Measured data in three different groups. Only thickness of depleted glycoprotein layer has significant statistical difference (P<0.05) between preventive and control group on 8th week.

valuable and may reduce further abnormal developments of joint structures. Platelet rich plasma (PRP) 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 (Ahmed et al., 2012; Gotherbarn et al., 2006). Because it can be used autogenously, it poses no risk of transmissible diseases. Furthermore, PRP can easily be obtained on the day of surgery by two centrifugation steps from autogenous whole blood. PRP has been extensively investigated for bone regeneration and soft tissue healing and many reports claim a positive effect of PRP (Wiltfang et al., 2004; Sun et al., 2010). Complicated diabetic patients show impaired delayed wound healing caused by multiple factors. A study on wound healing showed that platelet-rich plasma (PRP) was effective in normal tissue regeneration. Nonetheless, there is no evidence that when platelet rich plasma is applied to diabetic wounds, it normalizes the diabetic wound healing process (Shin et al., 2012). In a study it was indicated that the extracellular matrix-regulating effect has been observed by PRP. Therefore the acceleration of wound healing events by PRP under hyperglycemic conditions might be a useful clue for future clinical treatment for diabetic wounds. The normal healing of a cutaneous wound is achieved via a complex biological and molecular process (Shin et al., 2012; Madlener et al., 1998). The literature search suggests that the preventive role of PRP has not been studied earlier. This study proved that PRP can prevent the development of OA syndromes in the experimentally traumatized joints.

**Conflict of interest**

Authors have none to declare

**REFERENCES**


