Comparing PRP and bone marrow aspirate effects on cartilage defects associated with partial meniscectomy: a confocal microscopy study on animal model

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Abstract

Aim: The aim of our study was to assess the therapeutic effects of platelet-rich plasma (PRP) and bone marrow aspirate concentrate (BMAC) in an animal knee lesion complex associating a large osteochondral defect and meniscal defect resulted from partial meniscectomy, a clinical situation that occurs quite often in orthopedic practice. Materials and Methods: Twenty-one male rabbits were included in the study, and all underwent initial surgery on the right knee to create the osteochondral defect on the internal femoral condyle, and remove the anterior horn of the internal meniscus, simulating a clinical situation. Rabbits were separated in three study groups: control, PRP group, in which three PRP injections were administered, and BMAC group, in which one single BMAC injection was administered. At the end of the six months follow-up period, knees were harvested and further analyzed using confocal microscopy and three-dimensional (3D) reconstruction of the articular surface. Results: Therapeutic groups had better results concerning articular surface remodeling and joint degeneration indicators in comparison to trauma group. Conclusions: Our results suggest that using post-operative regenerative therapies does improve final results concerning surface contact remodeling that was investigated using confocal microscopy and should be considered a valid treatment adjuvant in managing patients with this type of lesion complex, as it improves global joint outcome.

Keywords: PRP, cartilage defect, meniscectomy, regenerative therapy, confocal microscopy.

Introduction

Osteochondral lesions occur quite often in active adults, usually discovered during arthroscopy for partial meniscectomy or meniscal repair. Because cartilage does not have the ability to heal full thickness lesions, the treatment options for defects in the knee joint remain a challenge for the practicing orthopedic surgeon. Present options are ranging from conservative measures, through simple arthroscopic interventions, marrow tapping techniques, osteochondral auto-allografting, cell-based techniques, growth factors and emerging gene therapy techniques [1]. Despite treatment option chosen, usually repair occurs by fibrocartilage which does not poses the biomechanical features of hyaline cartilage and ultimately leads to onset of knee osteoarthritis [1]. When a meniscal tear is associated and the need for partial meniscectomy is established, global knee prognosis is even worse.

There are two main types of chondral lesions taking into consideration the cause: focal lesions usually caused by sports injury, with well delineated defect, and degenerative defects, which are poorly delineated and usually a result of ligamentous instability, meniscal pathology, or osteoarthritis. Focal lesions can also be a result of osteochondritis dissecans or osteonecrosis [1]. Usually, knee trauma will cause an association of lesions within the joint. Curl et al. (1997) reported in a review of 31 516 knee arthroscopies, that 65% of the whole group associated chondral defects with either meniscal tears or ligament lesions, mainly anterior cruciate ligament lesions [2, 3]. Although many in vivo and in vitro studies in the literature are focused on platelet-rich plasma (PRP) and its potential to stimulate chondrogenesis [4–6], little data is found regarding complex lesion associations and relatively immediate post-traumatic or post-surgical administration of regenerative therapies.

Aim

The aim of our experimental study was to recreate a frequent scenario occurring in clinical practice, in which partial meniscectomy becomes the only option for a patient who associates an osteochondral defect, and to follow the evolution of this lesion complex without therapeutic
Interventions in comparison with intra-articular injections with PRP and bone marrow aspirate concentrate (BMAC).

Materials and Methods

Twenty-one male adult rabbits were selected for the experiment, all of them older than three years old to ensure adult biology associated with regenerative therapy results. Rabbits were separated in three experimental groups of seven individuals. The animals were individually identified by microchip, but group repartition was done randomly after the initial surgery, to ensure procedure uniformity in all individuals. The first surgical intervention consisted of creating the full thickness osteochondral defect on the weight bearing surface of the internal femoral condyle, and partial excision of the internal meniscus (Figures 1 and 2), under general anesthesia using Ketamine (35 mg/kg) and Xylazine (5 mg/kg), followed by anatomical closure and wound dressing with mean duration of surgery of 16 minutes and post-operative observation for six hours.

Three weeks after the initial surgery, when wound healing was completed, the animals were randomly split in four experimental groups, as follows:

- **Trauma group:** no further procedures were applied to the joint until final euthanasia and knee joint harvesting for further microscopy studies.
- **Trauma+PRP group:** three intra-articular injections were performed in the weeks 3, 6, and 9 after the initial surgery. PRP was prepared using sterile blood containers, 4% sodium citrate solution for preventing blood clotting and a standard centrifuge for separation. Blood was drawn from the saphenous vein in the amount of 8 mL. Approximately 0.8 mL of PRP was finally obtained through means of centrifugation according to protocol data found in the literature and injected intra-articular, the entire procedure being performed under anesthesia.
- **Trauma+BMAC group:** a single intra-articular injection was performed in week 4 after initial surgery. Bone marrow was harvested from the posterior iliac crest in the amount of 2 mL and filtered through 200 μm mesh filter. Final product was obtained through means of differential centrifugation according to protocol data found in literature [7]. At the end of the follow-up period, seven healthy left knee specimens were also harvested in a random manner from the experimental groups, to create the Control group with measurement values corresponding to perfectly healthy cartilage.

The follow-up period was six months after initial surgery, at the end of which knees were harvested for further study consisting of macroscopic examination, confocal microscopy study and three-dimensional (3D) reconstruction of articular surface and standard histological examination using Harris Hematoxylin for control.

As complications, there were three superficial wound complications that resolved with treatment and one case in the control group that had complete joint degeneration from possible septic arthritis. The diagnosis was established at the end of the follow-up period when joints were harvested and macroscopically examined.

Freshly resected osteochondral fragments were immediately scanned on an Olympus Fluoview FV1000 confocal laser scanning microscope, with a UPLSAPO 10× objective [numerical aperture (NA) 0.40], using 405 nm laser excitation and detection of the emitted light at 461 nm. Z-stacks of 1024×1024 pixels images were taken at 1.5 μm intervals. The resulting datasets were 3D reconstructed on a Bitplane Imaris v7.4 platform [8]. On the 3D reconstructions, the most significant surface variations found on each image were measured and the mean values of four measurements were used to compare surface defects and depth.

For morphological examination, specimens were prepared using standard histological technique. Fixation was achieved with 10% neutral buffered formalin for 48 hours. Decalcification was done using a rapid decalcifier (TUNIC) for 24 hours. Paraffin inclusion was done using an automated Thermo Shandon device. Further processing of the specimens was standard and final mount was done using permanent Leica CV mount.

Statistics

Obtained data was categorized in four groups and further analyzed in a comparative manner using one-way analysis of variance (ANOVA) test. Three distinct joint locations have been studied to assess defect area remodeling, but also entire joint degenerative process, as follows: internal femoral condyle (defect area), internal tibia condyle directly opposed to the defect (“kissing lesion effect”) [9], and external femoral condyle (secondary joint degenerative process).
Ethics statement

The present research was conducted according to the Declaration of Helsinki and Good Practice in Biomedical Research, with the Approval (No. 16b/26.06.2018) of the Bioethics Committee of the Victor Babes University of Medicine and Pharmacy, Timișoara, and Approval (No. 87/07.05.2018) of the Bioethics Committee of The King Michael I of Romania Banat University of Agricultural Sciences and Veterinary Medicine, Timișoara, Romania.

Results

Macroscopic evaluation of the cartilage defect was in accordance with the ulterior confocal microscopy results. Regarding the meniscal lesion, two surprising findings were noticed in the Trauma+PRP group, where the anterior horn of the internal meniscus appeared to be regenerated as shown in Figure 3. Macroscopically, the boundary between the natural meniscus and the tissue resulted from the healing process can be seen. Also, the newly formed anterior horn appeared to be attached anteriorly in the intercondylar fossa. In the great majority of cases, at the site of meniscectomy we observed osteophyte formation and degeneration of the residual meniscus which became thin and brittle (Figure 3).

Reconstructions of the articular surface resulted in extremely detailed images of the articular contact surface ranging from completely degenerated to healthy cartilage (Figures 4–7).

Confocal microscopy imaging was used to assess the quality of contact surface resulting from the healing process at the end of the six months follow-up period with or without regenerative therapy treatments.

Measurements of the surface defects revealed differences between experimental groups, as follows:

- The internal femoral condyle (Figure 8) was the election area to place the cartilage defect because of biomechanical stress and resulting images and measurements are most representative for local healing potential of a cartilage lesion. In healthy cartilage, depth variation was a median of 27.25 μm, a median of 74 μm in Trauma, 64 μm in Trauma+PRP group, and a median of 88.35 μm in Trauma+BMAC group, with a p-value of 0.02 (<0.05). Results suggest that at the site of the focal defect, PRP slightly improved final healing pattern resulting in a better joint contact surface of the cartilage but with no statistical difference.

- The external femoral condyle (Figure 9) was elected as an indicator for joint surface degeneration in the presence of a cartilage defect and partial meniscectomy. In the healthy joint or the Control group, external femoral condyle surface presents depth variations with a median of 20.57 μm, in the Trauma group a median of 45.39 μm, Trauma+PRP group 34.07 μm, and Trauma+BMAC group 34.32 μm, with a p-value of 0.0002. Data suggests that both therapies improve global joint healing and/or limit joint degeneration PRP being slightly more effective.

Figure 3 – Subject 8352 from the Trauma+PRP group, macroscopic aspect: (A) Internal femoral condyle defect; (B) Residual meniscus after partial meniscectomy; (C) Resection line; (D) Newly formed tissue attached to the residual meniscus and to the anterior intercondylar area after the tibia. PRP: Platelet-rich plasma.

Figure 4 – Digital image obtained after 3D reconstruction from the data resulted from confocal microscopy study of Trauma+BMAC knee specimen. Defect was created on the internal femoral condyle. Image is showing surface defects of articular surface. 3D: Three-dimensional; BMAC: Bone marrow aspirate concentrate.

Figure 5 – Digital image obtained after 3D reconstruction from the data resulted from confocal microscopy study of Trauma+PRP knee specimen. Defect was created on the internal femoral condyle. Image is showing surface defects of articular surface and remodeling result after PRP therapy was applied, demonstrating a smooth remodeling pattern in the Trauma+PRP group. 3D: Three-dimensional; PRP: Platelet-rich plasma.
Figure 6 – Digital image obtained after 3D reconstruction from the data resulted from confocal microscopy study of Trauma knee specimen. Defect was created on the internal femoral condyle. Image is showing surface defects of articular surface and remodeling result with no therapeutic interventions, demonstrating advanced degeneration of contact surface.

Figure 7 – Digital image obtained after 3D reconstruction from the data resulted from confocal microscopy study of Control knee specimen (healthy joint). Osteochondral fragment was harvested from the internal femoral condyle. Image is demonstrating perfectly healthy articular surface, which is smooth and uniform.

Figure 8 – Values resulting from measurements on the defect area on the internal femoral condyle. BMAC: Bone marrow aspirate concentrate; PRP: Platelet-rich plasma.

Figure 9 – Values resulting from measurements on the external femoral condyle. BMAC: Bone marrow aspirate concentrate; PRP: Platelet-rich plasma.

Figure 10 – Values resulting from measurements on the internal femoral condyle, the area opposed to the cartilage defect created. BMAC: Bone marrow aspirate concentrate; PRP: Platelet-rich plasma.

Discussions

Regenerative therapies are becoming more popular in many surgical fields [10, 11], and Evans et al. [12] introduced the new concept of facilitated endogenous repair in which molecular signals are the starting point of...
the healing process using regenerative potential of local tissues. PRP and BMAC fall in this category of therapies that rely on local biological potential that unfortunately cannot be quantified or objectified by regular investigation methods. Taking this into consideration, results using this type of therapies can be fairly unpredictable for the individual. However, results in the literature, the same as the present experimental results suggest improvements using PRP as an adjuvant therapy in treating osteochondral defects and degenerative lesions of the cartilage because it results in better contact surface remodeling. Considering cost and the extent to which a procedure is more invasive than the other PRP also offers advantages in comparison to BMAC.

Present study results using BMAC are not as predicted, because of preparation difficulties and administration protocol, thus making final data and conclusions focused on PRP therapy rather than BMAC.

Using PRP therapy in meniscal lesions is also appealing but still lacks a satisfactory amount of data with only few publications dealing with meniscal injections of PRP [13–15]. Blanke et al. studied effect of PRP therapy administered percutaneous for intra-substance lesions of the meniscus [13] and demonstrated the ability to improve pain and stop lesion progression over six months follow-up. However, only four out of 10 patients had morphological lesion improvement [13], raising questions about the unpredictability of PRP results but also accuracy of injectate placement. In the present study, two individuals out of seven presented an apparently regenerated meniscus with no degeneration of the residual meniscus after partial meniscectomy, while the rest of the animals had completely degenerated residual meniscus similar to all individuals in the control group. These results are motivating for further research in understanding the unpredictability factor of PRP treatment in meniscal and cartilage lesions, and methods to enhance these results.

Creating a full thickness cartilage defect in the experimental model, one factor that needs to be considered is the importance of subchondral bone healing as much as the neocartilaginous tissue. Many publications focus on PRP ability to stimulate bone formation [16–18] but it is impossible to state the influence of subchondral bone healing in the presence of PRP on final results reflected in the quality of the articular surface at the end of the follow-up period.

Conclusions

Experimental results suggest improvements in healing and overall joint outcome after trauma that results in an association of cartilage lesion and partial meniscectomy using PRP relatively soon after the surgical intervention with a protocol of three injections followed by joint loading to the pain threshold (animals were not limited in any way after surgery and PRP injection procedures). In the animal experimental model, weight bearing limitations cannot be applied but might have effects on end stage outcome in clinical practice and should be further investigated in real clinical setting. Regenerative therapy using PRP improved the joint healing process thus validating this therapeutic procedure as an option to enhance surgical outcome in the particular clinical context of an osteochondral lesion associated to post-partial meniscectomy status after knee surgery in young patients. BMAC cannot be prepared in rabbit model due to small quantity of bone marrow that can be harvested, and thus leading to inconstant final produce quality and non-reproducible scientific results. Studies looking at effects of BMAC must be conducted on different animal models, and further research is mandatory to understand its importance in the therapeutic management of cartilage lesions and orthopedic pathology. Results do encourage the orthopedic surgeon to take into consideration PRP therapy in post-traumatic and/or post-operative context. However, this should be part of a broad traumatic management plan and not as single therapeutic procedure in the treatment of such complex joint pathology.

Conflict of interests

The authors declare that they have no conflict of interests.

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