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Use of Platelet-rich Plasma in an Experimental Rheumatoid Arthritis Model

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Abstract: *Rheumatoid arthritis (RA) is characterized by an autoimmune activity against the synovial membrane, leading to a clinical picture of synovitis. Platelet-rich plasma (PRP) can be an alternative treatment for RA. This study evaluated PRP as a treatment for RA by controlling osteogenesis and inflammatory process in rats. Animals were randomly separated into: Control Group 7 (CG7) and 21 (CG21) days; Experimental Group 7 (EG7) and 21 (EG21) days. RA was induced through intra-articular knee with 100 µL Freud's complete adjuvant. On day 10, in GE7 and 21 were injected 100 µL PRP while those in GC7 and CG21 were injected 100 µL phosphate-buffered saline (PBS). On day 8 animals in EG7 and CG7 groups were euthanized. A second injection of 100 µL PRP and 100 µL PBS were performed on day 8 in EG21 and CG21, respectively. As result, we observed that granulation and necrosis tissue were intensely formed in CG7 while in EG7 was lightly formed with moderate osteogenesis and neovascularization. CG21 still presented moderate necrosis tissue, polymorphonuclear cells, and moderate emergence of capillaries, while EG21 showed decreased intensity of capillaries with low granulation tissue. EG21 showed bone tissue cells at a moderate level and subchondral bone formation. In conclusion, PRP intra-articular can be used as a co-adjuvant RA treatment because it was effective in controlling osteogenesis and stimulating the deposit of collagen fibers in cartilage.*

Key words: *Platelet-Rich Plasma; Rheumatoid Arthritis; Growth Factors; Rats.*

1. Introduction

Rheumatoid arthritis (RA) is a chronic and systemic disease with long and progressive evolution. It is known that some phenotypic variations may contribute to disease emergence such as smoking, infections, and hormonal alterations [1]. However, despite the fact that the human leukocyte antigen class II DRB1 is considered the main genetic factor related to this disease, its etiology remains unclear. The pathology is characterized by an autoimmune activity against the synovial membrane, leading to a clinical frame of synovitis with a significant increase in synovial fluid and membrane tissue proliferation called "pannus" [2]. The formation of new blood vessels allows the influx of inflammatory cells into the joint's interior, resulting in tissue growth on the articular cartilage and degradation of collagen type II [3].

B lymphocytes and macrophages migrate into the tissue, intensely producing pro-inflammatory cytokines, mainly TNF-alpha, which further exacerbates the inflammatory process. The cellular infiltration generates a disorganized process of destruction and bone remodeling [4]. This process expands into the peripheral tissues, causing erosion in the articular cartilage and subchondral bone, further leading to joint deformities and muscle and bone atrophy. There is also an imbalance between bone resorption and bone formation because the resorption metabolism, mediated by osteoclasts and multinucleated cells, and present in the "pannus," is increased [5,6].

Different classes of medications are used for RA treatment, including painkillers, nonsteroidal anti-inflammatory drugs, corticosteroids, and drugs aimed at preventing and reducing joint damage. However, it is known that long-term use of these drugs may produce different side effects such as insufficient heart function and kidney damage [7]. Platelet-rich plasma (PRP) can be an alternative treatment for several diseases including RA. It is advantageous because as a low-cost human byproduct it decreases the chances of adverse effects and rejection. PRP is composed of plasma, leukocytes, and platelets, and is a rich source of essential growth factors (GF) and osteoconductive proteins [8]. Platelets originate in the bone marrow and are anucleated cells with a complex cytoplasm; they are rich in mucopolysaccharides, glycoproteins, and

phospholipids, which are responsible for platelet adhesion and aggregability [9].

Growth factors consist of a group of polypeptides related to tissue repair because they regulate and stimulate the cellular processes of mitogenesis, chemotaxis, differentiation, and metabolism.[10] Platelets can release GFs that stimulate angiogenesis, promoting vascular growth and proliferation of fibroblasts, which in turn leads to an increase in collagen synthesis. The number of platelets in the human body ranges from 150,000 to 400,000 per µL; the concentration of platelets in PRP for therapeutic purposes must be significantly greater than that of plasma in order to provide a proper release of GFs in the lesion site [8,11].

PRP has the property of pro-healing, adhesion, increased cell division capacity and collagen synthesis, and acceleration of the cellular differentiation process. Studies have shown that it is a product capable of enhancing grafts integration, healing of wounds and pressure sores, and bone and connective tissue formation [12]. In RA, PRP has proven to mitigate chondral and synovial alterations in pig experimental models using one single PRP application [13]. Thus, the present study evaluated if the use of PRP can be an adjuvant treatment for RA, controlling osteogenesis and the inflammatory process in a rat model.

2. Material and Methods

Animals

This study was approved by the Ethics Committee for the Use of Animals (CEUA) of Midwestern State University (UNICENTRO) under protocol 0852013. Twenty male *Rattus norvegicus* Wistar strain rats with an average weight of 200 g were used. The animals were provided by the Vivarium of the Pontifical Catholic University of Paraná and kept at 23 ± 1 °C in an acclimated room with 12:12 light:dark cycle in the Animal Experimentation Vivarium of the Laboratory of Neuroanatomy and Neurophysiology of UNICENTRO. Five rats were placed in each cage, receiving rodent standard ration and water *ad libitum* during the experiment.

Procedures

The animals were randomly separated into four groups, each with n = 5: Control Group 7 Days (GC7); Experimental Group 7 Days (GE7); Control Group 21 Days (GC21); and Experimental Group 21 Days (GC21). RA was induced through an intra-articular right knee injection of 100 µL Freud's complete adjuvant on day 0 [14]. On day 10, animals in the GE7 and GE21 groups were injected with 100 µL PRP in the same body site while those in GC7 and GC21 groups were injected with 100 µL phosphate buffered saline (PBS). Animals in groups GE7 and GC7 were euthanized on day 17. On day 8, animals in the GE21 group were injected with a second dose of 100 µL PRP and those in the GC21 group with 100 µL PBS; these animals were euthanized on day 21.

A mixture of ketamine hydrochloride (80 mg/Kg) and xylazine (20 mg/Kg) was used for euthanasia; 0.1 mL per 100 g of weight was used intraperitoneally; animals were subsequently placed in a CO_2 chamber. Biological material was disposed of through a specialized company.

Obtaining platelet-rich plasma (PRP)

Four milliliters of blood were collected by cardiac puncture in previously sedated animals and stored in tubes with sodium citrate anticoagulant [15]. The procedure was conducted in sterile conditions and lysing or damaging platelets was avoided to prevent the loss of their ability to secrete growth factors. Tubes with collected blood samples were centrifuged at 900 rpm for 10 min to separate red, white, and platelet cells [16]. The upper portion of the supernatant, up to the edge of the fog zone that corresponds to plasma and platelets, was collected into new tubes [17]. These tubes were centrifuged at 1800 rpm for 10 min [16]; about 50% of the plasma portion was removed and stored in another tube (portion considered as plasma poor in platelets, PPP). The remaining material containing the platelet pellet was resuspended, originating the PRP portion [17]. PPP presented 733,000 platelets per µL; PRP presented 2,300,000 platelets per µL and was considered suitable for the study's purpose [1].

Basic histology

The right knee joint was histologically examined in each animal. Five micrometers thick tissue samples were stained with hematoxylin and eosin, and Trichrome Gomori. Lesions were observed in an optical microscope (MO) at 40 x magnification [19].

All aspects relevant for the anatomopathological analysis were evaluated in the slides with emphasis on cartilage, trabecular bone, periosteum, and adjacent structures.

3. Results

Granulation tissue was intensely formed (+++) in GC7 animals and lightly formed (+) in GE7 animals at the same time point during the experiment. Angiogenesis was lightly (+) observed in GC7 and moderately (++) in GE7. Tissue necrosis was moderate (++) in GC7 and light (+) in GE7. A moderate number (+ +) of osteoclasts, low number (+) of osteoblasts, and high number (+++) of osteocytes were observed in GC7. A low number (+)

of osteoblasts and moderate number (++) of osteocytes were observed in GE7; osteoclasts were absent. Osteogenesis was observed as a light (+) and moderate (++) bone neoformation in GC7 and GE7 animals, respectively (Figure 1).

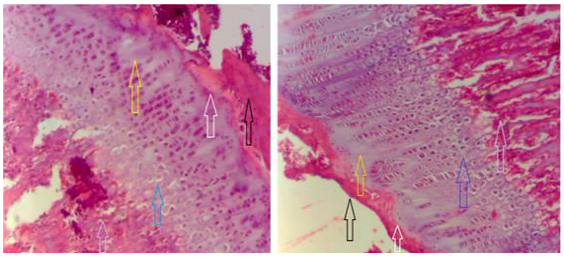

Figure 1: Photomicrograph at 40 x magnification with HE staining showing bone regions in GC7 and GE7 animals. The yellow arrow indicates the serial layer; the blue arrow indicates the hypertrophic layer; the light blue arrow indicates the ossification zone; the white arrow indicates the cartilage zone at rest; and the black arrow indicates the beginning of the epiphyseal region.

Analyses on day 21 showed that animals in the GC21 group presented moderate (+ +) polymorphonuclear cells and those in GE21 did not present these cells. GC21 animals showed a moderated (+ +) emergence of blood vessels; GE21 animals showed decreased intensity of capillaries with low granulation tissue (+). GC21 animals showed moderate intensity (++) in tissue necrosis, similar to those in GC7; this necrosis went from light (+) to moderate (++) in GE21 animals. GC21 animals showed a light (+) presence of osteoblasts and osteocytes and the absence of osteoclasts. GE21 animals showed bone tissue cells (osteoblasts, osteoclasts, and osteocytes) at a moderate level (+ +). The increase of subchondral bone was observed as moderate (+ +) in GC21 animals and intense (+++) in GE21 animals (Figure 2).

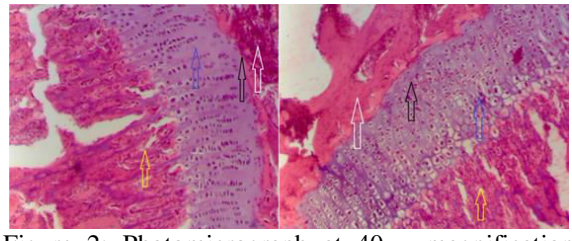

Figure 2: Photomicrograph at 40 x magnification with HE staining demonstrating bone regions in GC21 and GE21 animals. The yellow arrow indicates the serial layer; the blue arrow indicates the hypertrophic layer; the white arrow indicates the cartilage zone at rest; and the black arrow indicates the beginning of the epiphyseal region.

Figure 3 shows the cartilage regions in the epiphyseal region. GC7 animals showed chondrocytes cell death, which acquired eosinophilic staining and are normally basophils, in addition to cartilage degeneration. GE7 animals showed more aligned cells and higher tissue integrity than those in GC7.

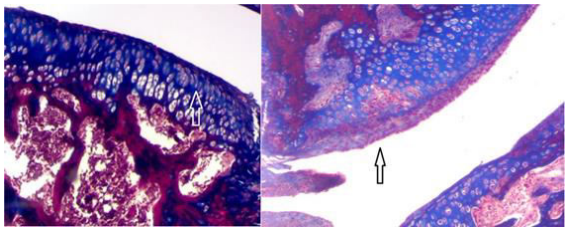

Figure 3: Photomicrograph at 40 x magnification with Trichrome Gomori staining demonstrating the cartilage region in GC7 and GE7 animals. The black arrow indicates the epiphyseal region.

Increased chondrocytes death and degradation of type II collagen fibers were observed in GC21 animals. However, those in GE21 showed chondrocytes conservation with many of them in "nests"; a deposition of collagen type II fibers was also observed in this group, demonstrating minor joint cartilage aggression (Figure 4).

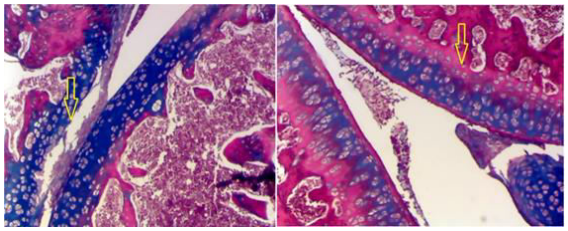

Figure 4: Photomicrograph at 40 x magnification with Trichrome Gomori staining demonstrating the cartilage region in GC21 and GE21 animals. The yellow arrow indicates collagen deposition.

4. Discussion

A It is known that an imbalance in rates of bone formation and resorption occurs in RA, and osteoclasts are considered the main cell involved in these alterations of focal loss in the marginal and subchondral bone [6,20]. The comparison between animals in the control and experimental groups showed that those in control groups had higher numbers of cells belonging to bone tissue (which showed decreased numbers during the chronic phase) than those in experimental groups. Conversely, animals in GE21 showed an increase in osteoprogenitor cells, corroborating the findings of Miller et al [6] and Kon et al [20].

PRP can be considered as a co-adjunct RA treatment when injected into the joint. It will release growth factors such as the Epidermal Growth Factor (EGF) that act on bone proliferation, promoting the formation of periosteal bone and increasing endosteal

resorption. The absence of receptors for this GF can cause a delay in the primary ossification of cartilage and recruitment of osteoclasts and osteoblasts [21]. The Platelet-Derived Growth Factor (PDGF) is another notorious growth factor with activity in bone metabolism, influencing the mitogenic activity of osteoclasts and osteocytes; its controlled release at the lesion site has been shown effective for bone regeneration [22,23]. Therefore, our results are in agreement with those reported in other studies, [21-23] because despite the fact that PRP stimulates bone proliferation, it generates a response of primary ossification cell recruitment that is more effective than that found in controls.

The GF present in PRP contributes to the regeneration of cartilaginous lesions. The Transforming Growth Factor (TGF) acts to increase the phenotypic expression of chondrocytes in chondrogenic differentiation of mesenchymal stem cells, and to stimulate extracellular matrix deposition. In addition, it decreases the suppressive effects of the inflammatory mediator Interleukin-1 in the synthesis of proteoglycan in cartilage. PDGF acts on chondrocytes proliferation and synthesis of proteoglycans; the insulin-like growth factor (IGF) stimulates the synthesis of proteoglycans and slows their catabolism [24]. PRP was effective in controlling synovial membrane hyperplasia through inhibiting the expression of nuclear factors and blocking the action of pro-inflammatory metabolites that play key roles in the initiation and perpetuation of cellular chronic inflammation in RA [22]. In this study, PRP stimulated the deposition of collagen type II fibers and regeneration of articular cartilage. These findings corroborate previously reported results [8,18,20,23,25].

One of the RA pathological mechanisms is related to the emergence of new blood vessels in the injured area, causing an increased flow of inflammatory cells. However, the GF present in platelets also stimulates angiogenesis, particularly EGF and Vascular Endothelial Growth Factor (VEGF), acting on the hypervascularization of inflamed tissue, mitogenesis, and vascular permeability [20]. In this study, PRP contained this process in the chronic phase. Although the pathways in which PRP acts are still unknown, it is estimated that the use of PRP in rheumatoid arthritis processes influences growth factors and controls anti-inflammatory processes through the production of arachidonic acid.

The inflammatory process can be destructive, but it can also promote tissue regeneration in the acute lesion phase because it will more intensely stimulate a cascade of events that culminate with tissue reconstruction, inducing quick proliferation and tissue maturation [26]. This mechanism was observed in the animals injected with PRP in the experimental groups.

The findings of this study corroborate those reported by Lippross et al [13] in 6-month- old pigs after inducing RA through intra-articular injections of bovine serum albumin (BSA) in the pigs' knees and injecting PRP two weeks later. These authors observed that an attenuation of arthritic alterations in the synovial membrane occurred, decreasing the levels of interleukin-6, PDGF, and TGF, which are essential GFs for inflammation control and tissue regeneration [13, 25].

5. Conclusion

Therefore, according to our results, intra-articular PRP injections can be used as RA treatment in the experimental conditions evaluated in this study. The use of PRP was effective in controlling osteogenesis and stimulating the deposition of collagen fibers in cartilage. However, an established protocol for obtaining PRP and information about the rate and frequency of its application are still lacking in the literature.

6. Conflict of Interest

There is no conflict of interest by authors.

7. References

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