STUDY ABOUT FUSION USING CERAMIC WITH PLATELET-RICH PLASMA IN THE SPINE OF RATS

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ABSTRACT

Objective: To assess the osteoinductive action of platelet-rich plasma when associated to ceramics in the spine of rats. Material and method: Laminectomy was performed in 16 isogenic Lewis rats for posterior ceramic grafting. PRP was prepared intraoperatively using blood collected from two other rats. Study and control groups were set by randomization, with the study group receiving ceramics associated to PRP, and the control group receiving only ceramics. The animals were sacrificed for histopathological analysis after 10 weeks. Results: Strong osteoblastic and osteoclastic activity and full re-absorption of ceramics were found on study group. In control group, small bone islands across fibrous tissue and non-reabsorbed were seen. Discussion: growth factors released by platelets bind to osteoblasts and fibroblasts surfaces, stimulating collagen synthesis to form bone matrix. Activated macrophages keep releasing growth factors and stimulating osteogenesis. Conclusion: The use of PRP associated to ceramics showed stronger osteoblastic and osteoclastic activity and full ceramics re-absorption compared to standalone grafting on the spine of rats.

Keywords: *Platelet-rich plasma. Ceramic. Ossification.*

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INTRODUCTION

Surgical arthrodesis interventions are becoming increasingly popular in the practice of surgeons involved with the treatment of spine conditions, notably in the last twenty years. The understanding of spine biomechanics and the development of surgical techniques and rigid instrumentation materials led to the rationalization of these procedures, making them easier to perform, less morbid e more effective, with resultant reduction of hospitalization time and better postoperative recovery of patients.

Bone fusion process is critical for postoperative stabilization of spinal arthrodesis. For that, the use of grafting material favoring bone neoformation between osteotomized vertebral segments is required during surgical procedure. Bone bridges leading to fusion and, subsequently, to spine stabilization involve a number of physiopathological events in which osteoprogenitor mesenchymal cells are activated by inducing growth factors differentiating into osteoblasts, responsible for osteogenesis and subsequent remodeling along a structurally conductive surface.

Autologous bone graft serves as an optimal material for grafting for presenting three major properties: presence of osteogenic cells, osteoconductive structure and osteoinductive matrix. No other material comprises all advantages mentioned above[1]. Autologous grafting implies in higher operative morbidity to obtain a small amount of graft, hence the need of seeking biomaterial alternatives favoring bone fusion in arthrodeses. The most frequently employed ones today are ceramics, homologous bone grafts and demineralized bone matrix. These are usually employed in combination with autologous graft or added to other materials providing them osteoinductive properties: the growth factors.[2]

Many factors interfere on bone fusion process and extend beyond grafting material properties. Local biomechanical changes resulting in instability impair bone neoformation process. The mineralization status of the host bed, the presence of local pathologic changes such as ischemia, infections and neoplasms, as well as hormonal unbalances, use of drugs and toxic substances (notably tobacco) are worsening factors that negatively interfere on the bone fusion process.[3-6] Therefore, it is understandable that fusion failure rates on intertransversal lumbar arthrodeses, just to mention one of the most common approaches for the treatment of lumbar spine injuries, range from 5 to 35%.[5]

Several research lines and clinical studies currently address this issue, pursuing the optimal graft: a material containing osteoinductive, osteogenic and osteoconductive properties, that is easy to achieve and unlimitedly available, biocompatible and reabsorbable, immunologically inert, affordable, and that favors bone tissue neoformation despite of the presence of local tissue changes and systemic diseases. There is clearly a long road to go.

The present study is aimed to assess the osteoinductive effect of growth factors present on platelet-rich plasma (PRP) when associated to ceramics on bone neoformation process of rats' spine.

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MATERIALS AND METHODS

The experimental model constituted of male adult isogenic Lewis rats weighting 300 g in average. The use of isogenic animals produces a homogenous sample and allows for reducing it, which makes study development easier. Thus, two groups (study and control) were built with seven animals each. Two rats were separately used for obtaining PRP.

Surgical procedures were performed at the same day by the researcher, The animals were anesthetized with intramuscular injection of 2% xylazine and 5% ketamine at a proportion of 1:0,2 ml/ 100 g. A skin incision at the level of iliac crests was made on lumbar region with subsequent dissection of fascias, muscular aponeurosis exposure, subperiostal dissection of the paravertebral musculature and laminectomy of two continuous levels of the spine. The wounds were temporarily covered and PRP was prepared separately by centrifuging the blood collected from two other rats, obtained by direct catheterism of the aorta exposed by thoracolaparotomy.

The software employed in this study was the Smart Prep (Harvest Technologies Inc., Plymouth, MA). Study and control groups were randomly determined. PRP was activated by adding calcium gluconate at a proportion of 1:10 and the resulting clog was immediately combined with the ceramics (Osteosynt, Einco Biomaterial Ltda., Belo Horizonte,MG). The animals in the study group received ceramic graft with PRP and control group animals, only ceramics. The wounds were closed at two planes and the animals were postoperatively kept in the animal lab for 10 weeks, at uniform lighting and temperature conditions and with water and food *ad libitum.* After that period, the animals were sacrificed with a lethal injection and their spines removed in block on the operated segments for anatomical-pathological study. Stains used for the analysis were Hematoxylin-eosin and Masson trichrome. The anatomical-pathological analysis of the material comprehended two phases:

1) Qualitative study, where histopathological aspects of the bone neoformation process were assessed: presence of loose connective tissue, cartilaginous and bone tissue, and respective proportions; osteogenic and osteoclastic activity; presence and type of associated inflammatory process and ceramic's degree of reabsorption.

2) Quantitative study. This was conducted with the aid of an analysis digital system consisting of an Olympus BX40 microscope with plan-achromatic objective lenses attached to an Oly video camera and to an Intel Celeron, 768 Mhz / 1,00 GB RAM personal computer with built-in image digitalizer plate running with an UTHSCA (The University of Texas Health Science Center in San Antonio) Image Tool software release 3.0. Images were captured with 200 x magnification, to view the whole operative bed. Once captured, digitalized images were processed on Adobe Photoshop 7.0, with the aid of the "eraser" tool, erasing all non-bone structures of the image. From that processed image, bone area was measured with the Image Tool software, with the value expressed as squared micrometers and exporting it to an Excel sheet.

RESULTS

The histopathological analysis evidenced differences between groups. In the study group, topic and heterotopic bone tissue neoformation was found, sometimes as a continuation with cartilaginous tissue (endochondral ossification), strong osteoblastic and osteoclastic activity with signs of bone remodeling with several bone cement lines *restitutio ad integrum* of the bone marrow and full reabsorption of the implanted ceramics (Figure 1A,1B). On control group, small islands of mature bone were observed around loose connective tissue, with a subtler osteoblastic activity, but no osteoclastic activity and with a large portion of non-reabsorbed ceramics (Figure 2A,2B). Specific and non-specific inflammatory process was found on both groups, randomly. The mean area values measured by histometry were higher in the study group as compared to the control group.

The histometric values measured for both groups are presented on Table 1.

Table 1 – *Histometry (in squared micrometers)*

	Study Group	Control Group
Animal 1	4587291	1974169
Animal 2	11161126	6293622
Animal 3	4586735	5242105
Animal 4	10101187	5251582
Animal 5	4291024	1767348
Animal 6	2756362	3704697
Animal 7	26564948	3114449

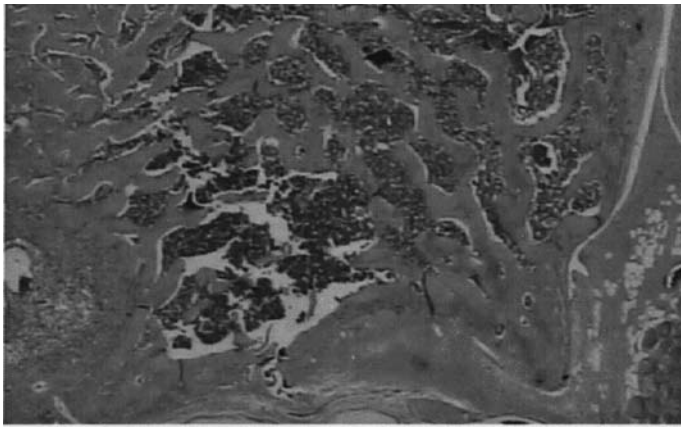

Figure 1 (a) – *Histologic slide stained with Hematoxylin-eosin (magnification: 200 x), showing a bone neoformation area adjacent to vertebral canal in an animal of the study group.*

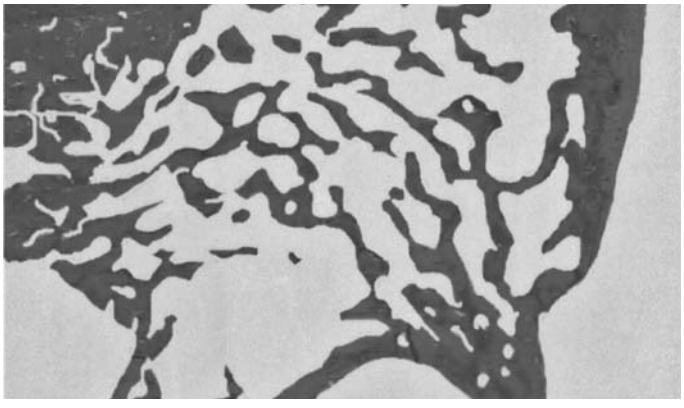

Figure 1 (b) – *Image processed on UTHSCSA's (The University of Texas Health Science Center in San Antonio) Image Tool software, from the previous (a), where histological elements different from bone were excluded for subsequent histometric measurement of the area.*

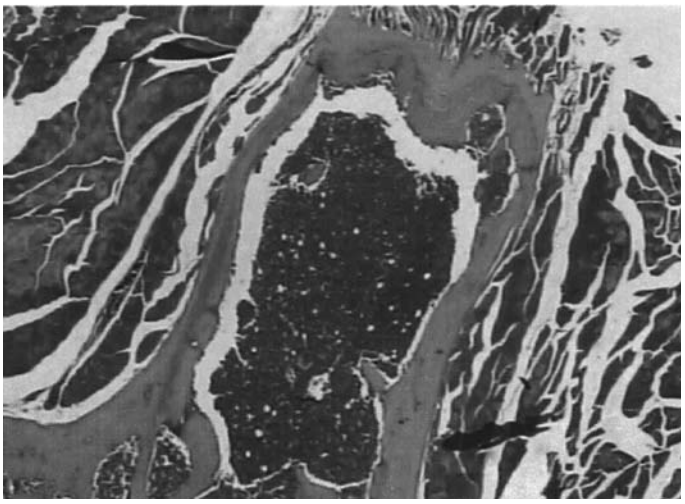

Figure 2 (a) – *Histologic slide stained with Hematoxylin-eosin (magnification: 200 x), showing a bone neoformation area adjacent to vertebral canal in an animal of the control group.*

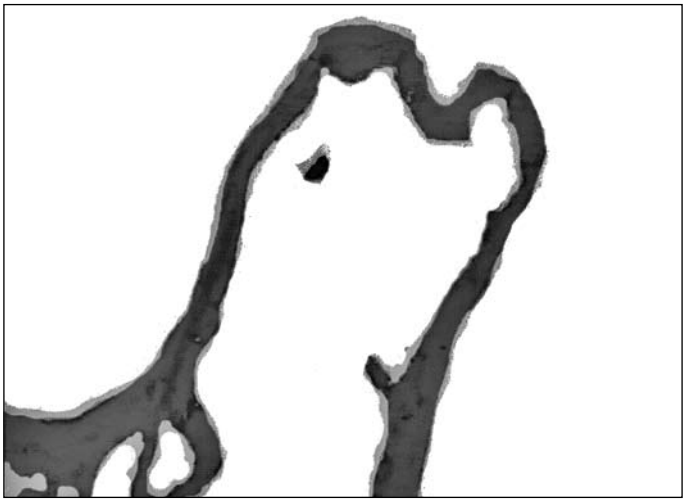

Figure 2 (b) – *Image processed on UTHSCSA's (The University of Texas Health Science Center in San Antonio) Image Tool software, from the previous (a), where histological elements different from bone were excluded for subsequent histometric measurement of the area.*

DISCUSSION

Blood platelets contain essential proteins that are released to start the healing process of any wound in human body: the platelet-derived growth factor (PDGF, released as three isomeric forms: PDGFαα, PDGFββ and PDGFαβ), the transforming growth factors (TGF, TGFβ1 and TGFβ2 variants), the vascular endothelium growth factor (VEGF) and the epithelial growth factor (EGF).

PDGF is the first growth factor released upon any tissue lesion. Its action triggers connective tissue induction and differentiation process, ultimately leading to lesion repair. PDGF's specific activities include enhancement of mitogenesis and angiogenesis and macrophages activation, which are responsible for early debridement of the wound through cell degradation residues phagocytosis. Subsequently, these cells will also release PDGF, perpetuating the osteoinductive stimulation and local differentiation of mesenchymal cells. Blood platelets are PDGF-rich; about 1200 molecules of this growth factor are estimated to be presynthesized on the granules of a single platelet.

TGFβ1 and TGFβ2 belong to the same family as bone morphogenetic proteins (BMP). They are similarly synthesized by macrophages and other cells, and, when released, have a paracrine action over fibroblasts, mesenchymal cells of bone marrow and pre-osteoblasts. Their specific actions include mitogenesis stimulation, chemotaxis of osteoprogenitor cells, osteoblasts stimulation to release collagen matrix for osteoid formation and osteoclastic activity inhibition.

Considering the properties of the growth factors found on platelets, and having in mind that at least two of them (PDGF and TGF) stimulate osteoblastic activity[7] and, whereas TGF comprehends in its family all BMP variables[8], it is reasonable to assume that these growth factors have a similar osteoinductive ability to that attributed to BMP. Thus, these factors could have an enhanced osteoinductive ability by obtaining a blood platelet concentrate when deposited into a bone continuity solution.

PRP consists of an autologous platelet concentrate into a small plasma volume, obtained from centrifugation, which separates platelets from other blood elements without damaging them. PDGF and TGF levels were identified and quantified by radioimmune assay (ELISA) by Landsberg.[9] Haynesworth et al.[10] found a correlation between osteoinductive ability and local concentration of platelets, establishing osteoinductive platelet concentration as 1.000.000/ ml PRP.

Growth factors are released by platelet degranulation and act by binding to osteoblasts, fibroblasts, endothelial and epithelial cell surfaces through membrane receptors, of which activation results in the induction of proteins into cell cytoplasm, which, in turn, stimulates the expression of a characteristic genetic sequence of the cell. The resulting protein synthesis implies in cell proliferation and collagen synthesis for bone matrix or osteoid formation. Thus, growth factors do not penetrate the cell or are mutagenic: they simply at as stimulating factors to physiologic growth processes. Platelets keep osteoinductive stimulus for seven days, then experience degradation. Osteoinduction is taken from there by previously activated macrophages.

Obtaining PRP and recognizing its osteoinductive properties is a recent matter of study. There are few experimental studies, and its clinical application is now starting to be assessed in some situations. Marx et al.[8] and Kassolis et.[11] report a clinical use for PRP in preparing jaw grafts in various situations and in the ossification of dental implants, just like Garg[12] and Anitua[13,] with good results. Man et al.[14] and Abuzeni et al.[15] suggest using it in Plastic Surgery, reporting improved and earlier healing, with superior clinical evolution and cosmetic outcome in incisions where PRP is used as a sealing agent. Fennis et al.[16] reported significant outcomes in an experimental study conducted on goats, where animals' jaw osteotomy kept with rigid fixation showed superior bone repair when grafts were added by PRP. Aghaloo et al.[17] found better bone neoformation in New Zealand rabbits' calvaria using PRP versus autologous repair.

To date, we haven't found any publication in literature involving the use of PRP associated to ceramics in the repair of spinal osteotomies on any animal experimental model. The present study is a novelty in this sense.

Histopathological findings reported in our study for the study group are similar to those described by literature where ceram-

ics is associated to some kind of BMP, usually rhBMP-2. From the eighth evolution week, the animals are going through reabsorption and remodeling phase of the bone repair process, with mature bone formation distributed as trabeculae and apposed lamellas and bone marrow restructuring *ad integrum*. There are osteoclasts and osteoblasts present, as well as bone remodeling signs with several bone cement lines and full ceramic reabsorption. On control group, loose or fibrous tissue was found permeating small islands of bone tissue of various sizes and a large amount of granules of non-reabsorbed ceramics. Histometry compared by the mean bone areas measurement was three times higher in the study group than in control group. Therefore, PRP may potentially have accelerated bone neoformation process on the study group, justifying ceramic graft's full reabsorption and the better organized tissue appearance, with more evident remodeling. An earlier mature bone formation might mean an accelerated and more effective fusion process, which encourages us to conduct further studies on more evolved animals aiming to extrapolate the synergistic association of ceramics and PRP to future clinical studies.

CONCLUSION

The use of PRP associated to ceramics as synergistic grafting in spine osteotomies of isogenic rats has evidenced marked histopathological differences when compared to ceramic grafting alone. Stronger osteoblastic and osteoclastic activity, better tissue structuring of newly-formed bone and full ceramics reabsorption were found with the use of PRP.

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