INFLUENCE OF VERTEBRAL DECORTICATION ON TISSUE NEOFORMATION AT BONE-GRAFT INTERFACE

FABIANO RICARDO TAVARES CANTO[1], SERGIO BRITO GARCIA[1], JOÃO PAULO MADERGAN ISSA[1], ANDERSON MARIN, ELAINE DEL-BEL[2], HELTON LUIZ APARECIDO DEFINO[1]

ABSTRACT

Objective: To determine the influence of vertebral posterior elements decortication in bone graft integration, considering a qualitative and quantitative evaluation of the (bone, cartilaginous, and fibrous) tissues in the interface between the receptor bed and the bone graft. Methods: Twenty-four Wistar rats were divided into two groups according to the decortication of the bone graft receptor bed. Autologous bone graft from the first and second lumbar vertebrae were used. The new tissue formation in the interface between the receptor bed and its bone graft was evaluated after three weeks by histomorphometric analyses. Results: In the animals group with the decorticated posterior bed the mean percentage of new bone formation was 40%±6.1, and 7.7%±3.5 in the not decorticated group (p=0.0001). The mean percentage of cartilaginous tissue formation in the decorticated group was 7.2%±3.5, and in the not decorticated 10.9%±5.6 (p=0.1123). The fibrous tissue formation in the decorticated group presented a mean of 8.6%±3.9 and, in the not decorticated group, of 24%±10.1, with which is a statistically significant difference (p=0.0002). Conclusion: Decortication accelerated the histologic process of bone graft integration. More production of new bone tissue and predominance of intramembranous type of ossification occurred in the decorticated group.

Keywords: Bone transplantation. Osteogenesis. Spine.

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INTRODUCTION

The concept of decortication of the bone graft receptor bed in vertebral arthrodeses was introduced by Hibbs.[1] Decortication involves the removal of the superficial part of the cortical bone covering the posterior vertebral elements (spinous process, lamina and articular facet), thus exposing the vertebral spongy bone. Consolidation of posterior arthrodesis of the spinal column depends on integration of the bone graft with its receptor bed, a process that is related to bone neoformation at this interface, where the bone, cartilaginous and fibrous tissues are present.[2,3] Decortication raises tissue metabolism at the interface through the increase of vascular flow to this region, accelerating the integration of the bone graft to the receptor bed, and triggering greater bone neoformation.[2,4] Although several studies demonstrate the effect of receptor bed decortication on bone graft integration,[5-7] the mechanisms involved in the osteogenesis of the receptor bed-graft interface are not totally clear.

The aim of this study was to determine the influence of posterior element decortication on bone graft integration, considering the quantitative and qualitative evaluation of the tissues (bone, cartilaginous and fibrous) present at the interface.

MATERIAL AND METHODS

Twenty-four male Wistar rats weighing between 250g and 350g were used in the study. The animals were divided into two experimental groups according to the decortication of the posterior vertebral elements. They were anesthetized by intraperitoneal injection of 0.1ml of Ketamine and 0.07ml of Xylazine for every 100 grams of weight of the animal. The first two lumbar vertebrae were bilaterally exposed through posterior longitudinal incision. The spinous processes of these vertebrae were sectioned at their base, divided in half in the sagittal plane and used as bone graft. (Figure 1)

In 12 animals the posterior vertebral elements (lamina, articular facet, transverse process) were decorticated, with an osteotome having been used for removal of the cortical bone, and in the other 12 animals the posterior vertebral elements were kept intact. The spinous process was placed on the posterior vertebral elements (decorticated or not decorticated) according to the experimental group and the surgical incision was closed by planes with absorbable sutures.

In the third postoperative week the animals were sacrificed with a lethal dose of anesthesia and the operated vertebral segment

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1 – Department of Biomechanics, Medicine and Rehabilitation of the Locomotor Apparatus - Faculdade de Medicina de Ribeirão Preto-USP
2 – Department of Morphology, Stomatology and Physiology of Faculdade de Odontologia de Ribeirão Preto -USP

Study conducted in the Department of Biomechanics, Medicine and Rehabilitation of the Locomotor Apparatus - Faculdade de Medicina de Ribeirão Preto-USP
Mailing Address: *Prof. Dr. Helton Luiz Aparecido Defino – Departamento de Biomecânica, Medicina e Reabilitação do Aparelho Locomotor - FMRP-USP. Av. Bandeirantes, 3900 – 11º andar – Hospital das Clinicas de Ribeirão Preto – Campus Universitário. Ribeirão Preto/SP, Brazil. CEP: 14048-900 – E-mail.hladefin@fmrp.usp.br*

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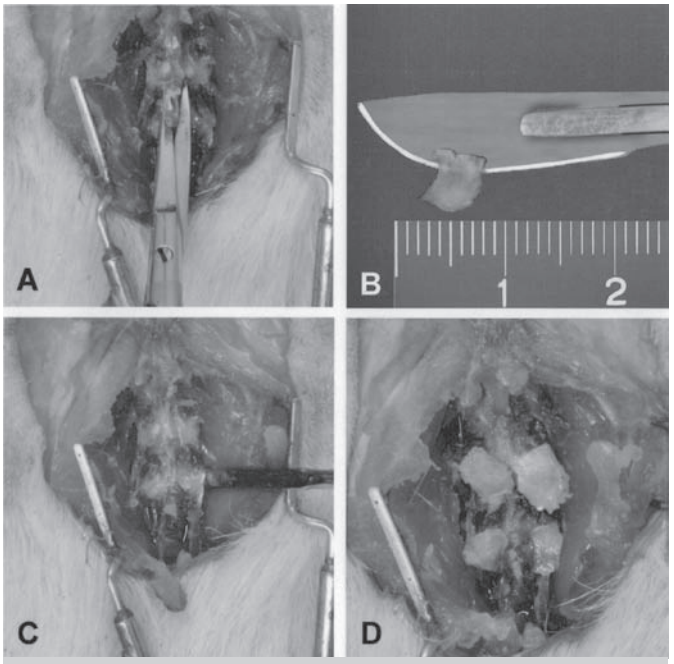

Figure 1 – *Photograph illustrating the main stages of the surgical procedure. Surgical exposure of the first two lumbar vertebrae. A) removal of the graft from the spinous process. B) subdivision of the graft into smaller fragments. C) decortication of the posterior elements. D) apposition of the bone graft on its receptor bed.*

was removed and prepared for histological study by means of fixation in formaldehyde at 10% and decalcification in a solution at 30% of trichloroacetic acid. The parts were embedded in paraffin blocks and submitted to 5 μm histological cuts that were stained with Masson's trichome, for the study of the interface between the posterior elements of the spinal column and the bone graft. This interface was the study target, with the neoformed bone tissue, the cartilaginous tissue and the fibrous tissue present in the histological section having been quantified. The histomorphometric study was performed with a zoom of 100X.

The tissues analyzed presented different colorations. The old bone tissue exhibited a dark red color, the cartilaginous tissue a light blue color, the fibrous tissue a dark blue color and the neoformed bone tissue a blue color midway between the light blue of the cartilaginous tissue and the dark blue of the fibrous tissue.

The total area of the histological section was considered as having a rectangular shape due to the dimensions presented, with the calculation of the area of this geometric figure having been used for the measurement of the total area of the histological section in the quantitative evaluation by means of histomorphometry.

With a ruler in decimal scale coupled to the microscope objective the participants calculated the total area of the histological section and the area of each one of the different neoformed histological tissues present in the section, with 25 measurements by histological section. The area of the three neoformed tissues was expressed in percentage in relation to the total area of the histological section. The intersection in parallel lines is a histomorphometric method described in literature and that makes it possible to estimate the percentage occupied by each type of tissue on parallel lines marked in scales.[8-11]

The statistical analysis of the comparison of quantitative results of the neoformed tissues was conducted by means of the Kurtosis test for evaluation of sample normality. The normality of the sample was not verified by means of this test, with the Mann-Whitney test having been used to compare the variables studied. A significance level of 5% was adopted.

RESULTS

The quantitative evaluation of the neoformed bone, cartilaginous tissue and fibrous tissue present at the receptor bed and bone graft interface exhibited a difference among the animals considering the performance of decortication.

In the group of animals in which the decortication was performed a greater quantity of neoformed bone and lower quantity of cartilaginous tissue and fibrous tissue was observed in comparison to the non-decorticated group. (Tables 1 and 2)

Table 1 – *Percentages of the neoformed tissues of the decorticated group with the respective mean values and standard deviations.*

	new bone	cartilage	fibrosis
1	35.20%	10.00%	16.40%
2	41.20%	9.20%	14.40%
3	43.20%	8.00%	8.40%
4	44.40%	7.20%	11.60%
5	34.00%	4.40%	9.60%
6	47.20%	6.40%	5.60%
7	42.40%	8.00%	7.60%
8	49.20%	7.60%	7.60%
9	41.60%	1.60%	6.00%
10	28.40%	8.40%	6.00%
11	39.60%	8.00%	6.80%
12	33.60%	7.20%	2.80%
mean	40%	7.20%	8.60%
standard deviation	6.1	3.5	3.9

Table 2 – *Percentages of the neoformed tissues of the non-decorticated group with the respective mean values and standard deviations.*

	new bone	cartilage	fibrosis
1	5.20%	5.60%	35.20%
2	6.00%	11.60%	25.20%
3	3.60%	3.60%	27.20%
4	5.60%	18.80%	13.60%
5	10.00%	16.40%	25.60%
6	6.40%	19.60%	30.80%
7	8.80%	9.60%	16.00%
8	8.40%	15.60%	16.00%
9	4.40%	10.00%	9.20%
10	8.80%	5.20%	19.60%
11	16.80%	5.20%	45.60%
12	8.40%	9.20%	24.80%
mean	7.70%	10.90%	24.00%
standard deviation	3.5	5.6	10.1

In the group of animals in which the decortication was performed the mean neoformed bone was 40% ± 6.1, and in the non-decorticated group 7.7%±3.5, with statistical difference having been observed between the two groups (p=0.0001). As regards cartilaginous tissue formation the decorticated group presented mean value of 7.2% ± 3.5 and the non-corticated group of 10.9% ± 5.6, whereas this difference was not statistically significant (p=0.1123).

The decorticated group presented mean value of 8.6% ± 3.9 of neoformed fibrous tissue and the non-decorticated group of 24% ± 10.1, with statistical difference having been observed between the two groups (p=0.0002). (Figure 2)

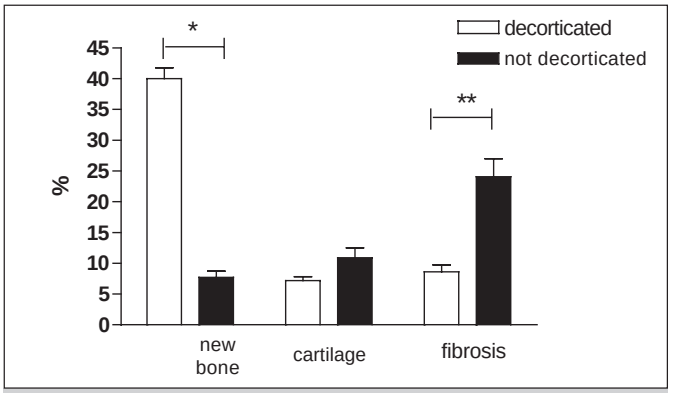

Figure 2 – *Tissue neoformation in the two groups of animals analyzed. * p=0.0001 ** p=0.0002.*

The histomorphometric evaluation of the interface between receptor bed and bone graft showed different percentages of neoformed tissues in the two experimental groups.

The observational histological analysis showed that the osteogenesis mechanism at the receptor bed and bone graft interface was not similar in the two groups studied. In the decorticated animals there was osteogenesis by the endochondral and intramembranous ossification mechanism, with predominance of intramembranous ossification, while in the non-decorticated animals osteogenesis occurred due to endochondral ossification. (Figure 3)

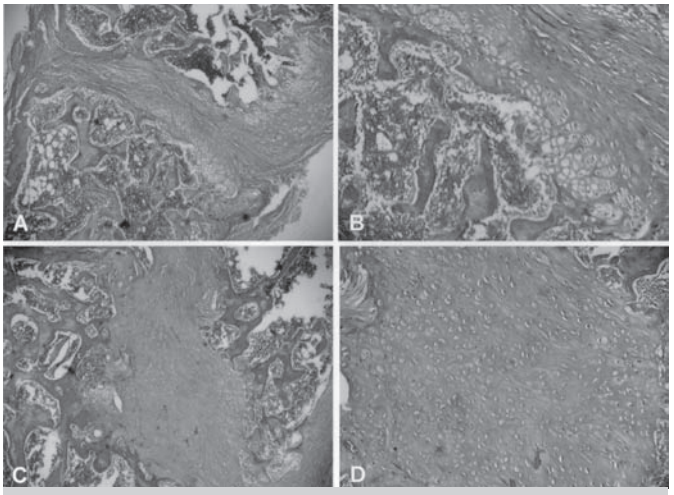

Figure 3 – *A and B Photomicrography of the group of non-decorticated animals, demonstrating pattern of endochondral ossification, A 28X and B 103X. C and D Photomicrography of the group of decorticated animals, demonstrating a predominantly intramembranous pattern of ossification.*

DISCUSSION

Integration of the bone graft in vertebral arthrodesis is related to local and systemic factors and the bone graft receptor bed acts as an important local factor for graft integration.[12]

Decortication of the receptor bone bed of the graft accelerates bone integration.[4] The biological factors involved in this phenomenon are not yet fully known,[13] having been the motivation for the preparation of the experimental model, in which a small animal like the rat was used to quantify the phenomenon of osteogenesis between the decorticated or non-decorticated receptor bed and the bone graft.

The bone graft integration time is variable in the different species, between 2 and 3 weeks in rats, 3 and 4 weeks in rabbits and a few months in humans and primates.[2,14] Based on this information the sacrifice time of the animals was selected in the third week.

The histomorphometric method that uses a ruler coupled to the microscope objective and the total area of a flat geometric figure makes it possible to satisfactorily quantify the different tissues formed in the osteogenesis process, and is a method of easy use and low cost.[8,15]

The capacity for induction and acceleration of bone formation in the decorticated areas has been experimentally observed.[4,5]

Histological studies with vascular neoformation markers indicate that the initial vascular supply to the arthrodesis mass originates from the decorticated transverse processes and not from the adjacent soft tissues.[16] This is allegedly the explanation for greater bone neoformation in the group of decorticated animals. The profusion of nutrients provided by the more abundant blood irrigation represents an important osteogenesis stimulus factor.[17] Moreover, decortication of the posterior elements places the bone graft in direct contact with cells from the reticulo-endothelial system and with osteoinductor and osteogenic factors present in the bloodstream.[16] The bone graft undergoes a process of necrosis and resorption.[18] The cells of the reticuloendothelial system are attracted and when in contact with this environment have the ability to transform into progenitor cells of the osteoblast lineage.[18] The direct contact of the bone graft with the open area is said to allow greater abundance of the inducer and osteogenic factors, such as the bone morphogenic proteins at the posterior bed and graft interface.[19] The more detailed study of the interface between the bone graft and the receptor bed allowed the observation and the quantification of the different neoformed tissues. The larger quantity of neoformed bone tissue, with a lower quantity of cartilaginous and fibrous tissue at the interface in the decorticated group, is apparently more advantageous for bone graft integration, because the bone bridge forms more quickly, guaranteeing greater resistance for bone graft integration.[5]

The quantity of neoformed bone tissue and of fibrous tissue presented statistical difference in the experimental groups, showing interference of decortication in bone graft integration. Not only the quantity of neoformed tissues, but also the osteogenesis process of bone graft integration was modified by decortication.

Osteogenesis at the interface was not similar between the decorticated and non-decorticated animals. In the group of decorticated animals there was osteogenesis by the intramembranous and endochondral ossification mechanism, with predominance of intramembranous ossification, while in the non-decorticated group endochondral ossification was predominant.

The greater abundance of oxygen at the decorticated interface could induce ossification of intramembranous origin. Under conditions of low oxygen concentration there is a tendency for the endochondral ossification model.[10] The increase of the transfer of morphogenic proteins from the bone to the interface would be another possible stimulus for the occurrence of intramembranous ossification.[16]

The larger quantity of fibrous tissue at the interface of the animals from the non-decorticated group could be related to the greater slowness of the endochondral ossification model in forming a stable bone bridge between the posterior bed and the bone graft and also to the reduced stability at the interface, conferred by the smaller area of contact between the posterior bed and the bone graft. It has been observed that in regions of lower stability there is formation of fibrous tissue.[20]

Decortication of the bone graft receptor bed influenced the integration process of the bone graft with its receptor bed. A difference was observed in the quantity of neoformed bone tissue, of fibrous tissue at the interface and also of the osteogenesis process. Decortication interferes in the bone graft integration process, allowing its faster integration, due to increased formation of neoformed bone tissue and predominance of intramembranous ossification.

CONCLUSION

Decortication of the bone graft receptor bed interferes in its histological integration process. It accelerates the integration of the graft with its bed, with greater production of neoformed bone tissue and predominance of intramembranous ossification at the interface between the bone graft and its receptor bed.

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