Is a 5 mm rat calvarium defect really critical?[1]

Um defeito de 5 mm em calota craniana de rato é realmente critico?

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ABSTRACT

PURPOSE: To evaluate bone regeneration in critical defects in the rats´ calvarium.

METHODS: Eighteen rats Wistar were divided into three groups of six animals each according to the time of evaluation (15, 30 and 60 days). One calvarium defect of 5mm was made in the parietal bone of each animal under general anesthesia. After the time of evaluation, the animals were killed, when the bone was histological studied and classified according to the type of tissue found: fibrosis or bone.

RESULTS: The results showed that in the group of 15 days, in five animals there was only fibrosis. In the group of 30 days, the process of regeneration was growing and in four animals was found bone, in three with partial filling and in the other one with complete filling of the defect. In the group of 60 days, out of the three animals with bone, two had a complete filling of the defect.

CONCLUSIONS: There was no bone regeneration in critical defects in 15 days. There was regeneration in the most part of the animals in 30 and 60 days.

Key words: Bone Regeneration. Skull. Neurosurgery. Rats.

RESUMO

OBJETIVO: Avaliar a regeneração óssea de defeitos críticos em calota craniana de ratos.

MÉTODOS: Foram utilizados 18 ratos Wistar que foram distribuídos em três grupos de acordo com o tempo de avaliação (15, 30 e 60 dias). Na calota craniana desses animais foi realizado defeito de 5mm, após anestesia geral prévia. Após o tempo de avaliação, os animais foram submetidos à eutanásia e a calota foi enviada para estudo histológico, quando foi classificada de acordo o tecido encontrado: fibroso ou ósseo.

RESULTADOS: Diante dos achados deste estudo, pode-se observar que para o grupo de 15 dias na maioria dos animais, n=5, foi encontrado apenas fibrose. Com o passar do tempo de avaliação, no grupo de 30 dias, o processo de reparo foi evoluindo e em quatro animais já foi encontrado osso, sendo em três com preenchimento parcial e em um completo. No grupo de 60 dias, o processo praticamente permaneceu o mesmo, onde em três animais foi encontrado osso, e, desses, em dois o preenchimento foi completo.

CONCLUSÕES: Não houve regeneração óssea do defeito crítico para o período de avaliação de 15 dias. Houve regeneração óssea em grande parte dos animais para o período de avaliação de 30 e 60 dias.

Descritores: Regeneração Óssea. Crânio. Neurocirurgia. Ratos.

Introduction

The restoration of bone defects in the maxillofacial skeleton remains one of the most challenging problems faced by oral and maxillofacial surgeons[1]. Trauma, osteotomy sites, oncologic resections, infections, or developmental anomalies are clinical situations in which defects need to be reconstructed. In adults, only minor bone defects are capable of healing spontaneously[2]. The reason for this impaired defect healing seems to be related to the fast ingrowth of soft tissue, thus blocking bone formation at the edges of the defect and thereby preventing bone healing[3].

Currently, autogenous bone grafts are the preferred material for craniofacial reconstruction. Despite being highly effective, there are several problems associated with their use. Clinically, autogenous bone grafts may increase operative time and morbidity, they are often mechanically unstable during surgery, may be insufficient for reconstruction of large defects, and undergo significant resorption, thus requiring further bone grafting procedures[4]. Recently, to minimize these problems, interest has been shown in the use of allogenic and alloplastic bone substitutes. The ideal graft should be osteoinductive to stimulate osteogenesis and osteoconductive to provide a scaffold for establishing optimal conditions for ingrowth of blood vessels and cells with osteogenic potential[5]. Therefore more studies need to be developed in order to devise innovative treatments aimed at the ideal graft.

For this it is necessary to create a critical bone defect so it will not regenerate spontaneously unless a graft is used. Some studies have considered as critical diameter a bone defect of 5mm[6]. However clinical observations by the authors lead to this experimental study in the aim of re-evaluate the 5mm bone defect as critical.

Methods

The trial protocol was approved by the University´s Ethics Committee (009686/2007-09).

The sample comprised of 18 male Wistar rats. They were divided into groups according to the type of the time of euthanasia (15, 30 and 60 days).

The animals were subjected to surgery under general anesthesia, which was induced by muscular injection of ketamine and xylaxine diluted 1:1 in a dose of 0.1ml per 100g of weight. For local anesthesia 0.2ml of lidocaine (1:200.000) was infiltrated. A sagittal incision of approximately 10mm was made from the fronto-nasal region to the occipital protuberance. Blunt dissection was created and the calvarium was exposed. A defect of 5mm on the right side of the median suture was performed using a trephine, always taking care to avoid injuring the dura mater. The procedure was concluded with suture in layers using nylon.

The euthanasia was performed after the evaluation period with intra-cardiac injection of 1ml solution of potassium chloride in conjunction with prior general anesthesia.

After the euthanasia, the calvarias were submitted to histological study in order to classify the bone repair using scores, in which the main characteristics of fibrous invasion, bone partial filling and bone complete filling were used (Chart 1). The area considered for this classification was the overall area of the defect. The purpose was to detect the presence or absence of the tissues rather than quantify them.

CHART 1 - Histopathological scores.

1st Category: type of tissue found in the bone defect
1 – Presence of fibrous connective tissue
2 – Partial filling with bone tissue
3 – Complete filling with bone tissue

Histological study

The specimens were fixed in formaldehyde for 48 hours and then were decalcified in 5% nitric acid for approximately 10 days. Semi-serial sections 5µm-thick were cut in the sagittal plane and stained with haematoxylin and eosin.

Results

The mean duration of surgery for the bone-graft group was 11 ± 2.43 minutes. In relation to the histological scores, in the 15 day group almost all animals had fibrosis (n=5) (Figure 1). In the 30 day group, there was new bone formation in four animals; out of this one had a complete filling with bone tissue (Figure 1). In the 60 day group, in three animals there was bone formation; out of this two had a complete filling with bone tissue (Figure 2).

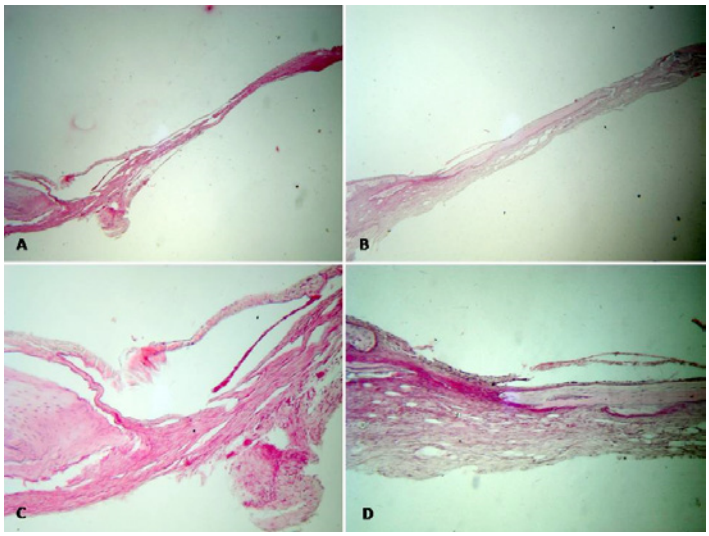

FIGURE 1 – A. Presence of fibrosis (HE 40x); **B.** Close up of the bone defect margin and the fibrous tissue (HE 100x); **C.** Partial bone filling with fibrosis (HE 40x); **D.** Close up of the partial bone filling with fibrosis (HE 100x).

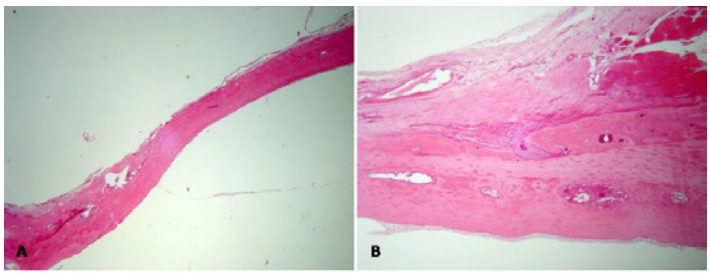

FIGURE 2 – A. Complete bone filling of the bone defect (HE 40x); **B.** Close up of the bone defect margin and the new bone formation (HE 100x).

It is observed in Table 1 the number of animals according to the histopathological scores and groups.

TABLE 1 - Number of animals per type of tissue found in the bone defect using categories according to the period of evaluation.

Group	Category			Total
	1	2	3	
15 days	5	1	0	6
30 days	2	3	1	6
60 days	3	1	2	6
Total	10	5	3	18
Percentage	55.55%	27.78%	16.67%	100%

Discussion

The regeneration capacity of bone presents limitations and in many cases the reconstruction of the defects is necessary, as in traumas, oncologic resections, infections, or prior to the placement of dental implants[7,8]. The reconstructions may be done with autogenous bone and synthetic or natural material[6,9].

The ideal graft should be osteoinductive to stimulate osteogenesis and osteoconductive to provide a scaffold for establishing optimal conditions for ingrowth of blood vessels and cells with osteogenic potential[5]. Therefore more studies need to be developed in order to devise innovative treatments aimed at the ideal graft.

For this it is necessary to create a critical bone defect so it will not regenerate spontaneously unless a graft is used. Rats are animals that are easy to handle and less expensive to maintain in a bioterium, so it is convenient to use them in experimental studies in the quest for finding the ideal graft. Some studies have been done in such animal finding as critical diameter a bone defect of 5mm[6]. However clinical observations made by the authors lead to this experimental study questioning if this size is really critical.

This study found that when using a 5mm sized defect, there was some kind of bone formation in a great amount of the animals (44%), considering periods of evaluation superior to 30 days. With these findings, it is suggested that this size may not be used as a critical defect for periods of evaluation of more than 30 days in this specie, since they intend to repair without using any kind of graft. The defect size of 5mm shall be used in studies of a 15 day maximum evaluation period.

Conclusions

There was no bone regeneration in critical defects in 15 days. There was regeneration in the most part of the animals in 30 and 60 days.

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