GEN-PHOS® IMPLANT IN SURGICAL CAVITIES PERFORMED IN THE TIBIA OF RATS SUBMITTED TO EXPERIMENTAL CHRONIC ALCOHOLISM. A MICROSCOPIC STUDY

IMPLANTE DE GEN-PHOS® EM CAVIDADES CIRÚRGICAS REALIZADAS EM TÍBIA DE RATOS SUBMETIDOS AO ALCOOLISMO CRÔNICO EXPERIMENTAL. ESTUDO MICROSCÓPICO

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The reaction of bone tissue to the presence of Gen-phos® was studied in rats submitted to experimental chronic alcoholism. Sixty rats were divided into a control group receiving water as a liquid diet and an experimental group receiving 6% ethyl alcohol for 60 days. Bone repair was evaluated microscopically at 10, 20 and 40 days after the experimental surgery. The results obtained showed that the alcoholic diet led to a more discrete new bone formation in all specimens and at all time points, causing incomplete bone filling of the surgical cavity after 40 days of repair. New bone formation was more favorable in some specimens with the implantation of BioHapatitaâ after 20 days of repair.

UNITERMS: Alloplastic implant; Hydroxyapatite; Alcoholism.

INTRODUCTION

Alcoholism has been one of the major medical and social problems of all world societies during this century. The ingestion of alcohol leads to its distribution towards body tissues, where it causes damage to their normal function. In bone tissue, alcohol plays an important role as a cause of extensive bone losses, with osteoporosis being the predominant bone disease, by exerting a direct effect on bone remodeling[1].

Several alloplastic materials have been used to fill bone cavities in an attempt to induce bone repair. Starting in the 1970's, many investigators in this area began to use a group of synthetic materials consisting of calcium phosphate ceramics. In the 1980's, a very large number of papers was published all over the world for the evaluation of several aspects of these ceramics. Calcium phosphate ceramics are biocompatible by not inducing any type of adverse reaction and by consisting of calcium and phosphate, which are present in bone tissue[6].

Hydroxyapatite can be used as a bone graft in different areas of Medicine and Dentistry. In Dentistry, it has been exhaustively used to correction of bone deformities and for the filling of periodontal pockets [4,8] and in buccomaxillofacial surgery and traumatology for the regularization and maintenance

of bone margins [2].

Gen-phos® is an ultrafine high-purity and reabsorbable hydroxyapatite produced by Baumer S.A., with quality control performed at the Adolfo Lutz Institute of São Paulo[5]. Since this is a biomaterial widely used to treat bone defects, it is important to assess the reaction of bone tissue in contact with Gen-phos® implants associated or not with experimental alcoholism.

MATERIAL AND METHODS

Sixty male Wistar rats (Rattus norvegicus albinus) weighing 180-240 g were studied. The animals were divided into two groups: 1) Control group receiving tap water as the liquid diet, which was subdivided into group C1 with an empty surgical cavity and group C2 whose cavity was filled with Gen-phos®, and 2) Experimental group receiving ethyl alcohol diluted 6% in tap water, which was subdivided into group E1 with an empty surgical cavity and group E2 whose cavity was filled with Gen-phos®. The animals received solid ration before and during the experimental period (Nuvilab CR1 ration, Nuvital). The experimental group was gradually adapted to alcohol by receiving a liquid ethyl alcohol diet (absolute ethyl alcohol, Labsynth) at 2% dilution during the first week, 4% dilution during the second week, and 6% dilution during the third. After this time, the animals continued to receive a 6% alcohol diet for a period of 60 days. After the end of treatment, all animals (control and experimental groups) were submitted to experimental surgery.

The animals were submitted to general anesthesia with intramuscular injection of ketamine 50 (75mg/kg) + Rompun (1,5ml/kg) and the surgical area was shaved and disinfected with povidone-iodine 10% topic (Riodeine topic, Rioquimica). A 20-mm long longitudinal incision was made in the ventral region of the left pelvic leg of the animal with a surgical knife, divulsion was performed with a blunt instrument and the periosteum was incised and separated. A perforation in the tibia was made using a number 8 spherical burr at low rotational speed cooled with a 0.9% sodium chloride solution.In groups C1 and E1 the cavity was filled only with clot, and in groups C2 and E2 the cavity was filled with Gen-phos® (microgranular hydroxyapatite, Dentoflex) prepared during the surgical act by mixing with physiological saline until a dense paste was obtained. The tissues were repositioned and sutured with 4.0 Ethicon silk sutures.

Five animals from each group were sacrificed 10, 20 and 40 days after surgery. The left tibia was separated from the remaining anatomical structures, fixed in 10% formalin for 24 hours, and then decalcified in a solution of equal parts of sodium citrate and formic acid [12]. After decalcification, the pieces were routinely processed for paraffin embedding and 6-mm thick sections were stained with hematoxylin and eosin and with Masson trichrome for microscopic examination.

Blood was also collected from the animals on the day of sacrifice by intracardiac puncture and analyzed by gas chromatography with flame ionization detection [16].

RESULTS

Blood ethanol determination showed total absence of alcohol in all control animals and a mean level of 1.05 ± 0.74 g/l in the experimental animals.

Histomorphological analysis of the different groups is described as a function of postoperative time.

Ten-day group

In group C1, the surgical cavity was occupied by immature bone tissue leaving an ample intertrabecular space filled with well-vascularized and fibroblast-rich connective tissue (Figure 1). In group C2, trabecular bone was thinner and isolated compared to group C1 (Figure 2). In group E1, new bone formation was less pronounced in all specimens compared to the control group (Figure 3). In group E2, the surgical cavity was occupied by thin immature trabecular bone comparable to that of group C2 (Figure 4). Implanted material was absent in all specimens of groups C2 and E2.

Twenty-day group

Compared to the previous stage, group C1 showed a surgical cavity with more developed bone tissue but still with thin trabecular bone and with an ample intertrabecular space occupied by well-vascularized and fibroblast-rich connective tissue (Figure 5). In some specimens of group C2, thicker trabecular bone partially occupying the surgical cavity was observed (Figure 6). In group E1 the surgical cavity showed discrete ossification, with extensive areas occupied by connective tissue without bone

differentiation (Figure 7). In some specimens of group E2, new bone formation was more intense and was observed close to the margins of the surgical cavity (Figure 8). Implanted material was absent in all specimens of groups C2 and E2.

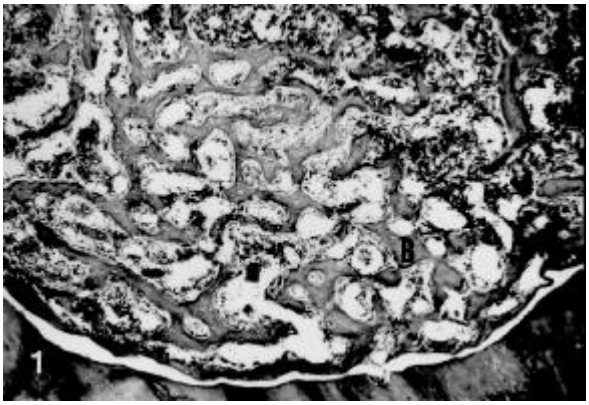
Forty-day group

In groups C1 and C2 the surgical cavity was completely filled with well-developed newly formed bone tissue with defined medullary canals (Figure 9). In groups E1 and E2 the surgical cavity showed the presence of thick and well-defined trabecular bone, although areas without bone differentiation could be observed (Figure 10). Implanted material was absent in all specimens of groups C2 and E2.

DISCUSSION

The prolonged and excessive use of alcohol causes several clinical, biochemical and electrophysiological abnormalities associated with various diseases, especially of the liver, neuromuscular system, heart and brain, with a consequent increase in arterial pressure and the occurrence of cardiomyopathies[18], testicular reduction [20], decreased blood cell production[19], delayed bone growth[15], loss of bone mineral density, and osteoporosis [11]. The interference of alcohol in the process of bone repair occurs through the inhibition of bone remodeling, contributing to progressive bone demineralization, usually by inhibition of osteoblast proliferation[9,10].

Concern about the negative action of alcohol on bone tissue, the increasing rates of osteoporosis, and



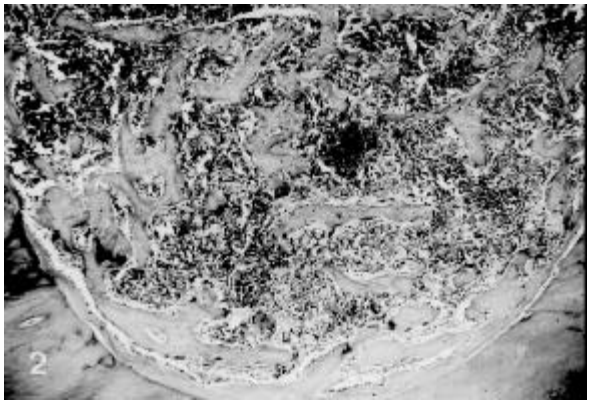
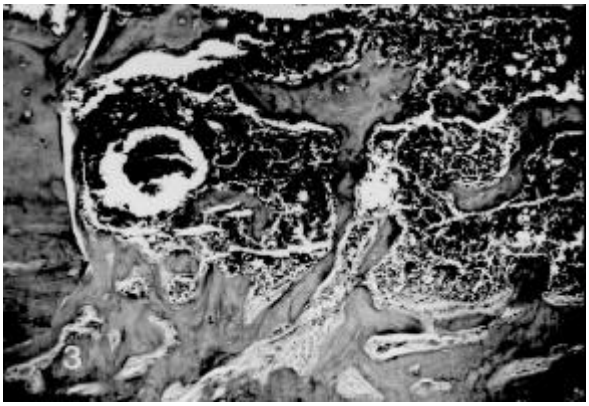


FIGURE 1- Group C1. Ten days. Surgical cavity showing immature bone tissue (B) with an ample intertrabecular space filled with connective tissue. HE. 63X.

FIGURE 2- Group C2. Ten days. Thin and isolated trabecular bone partially occupying the cavity and separated by abundant connective tissue. HE. 63X.



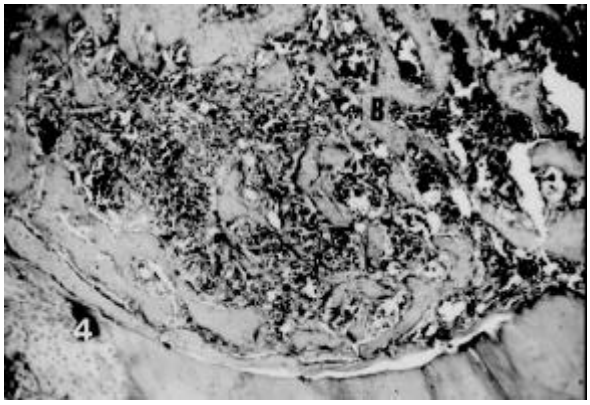
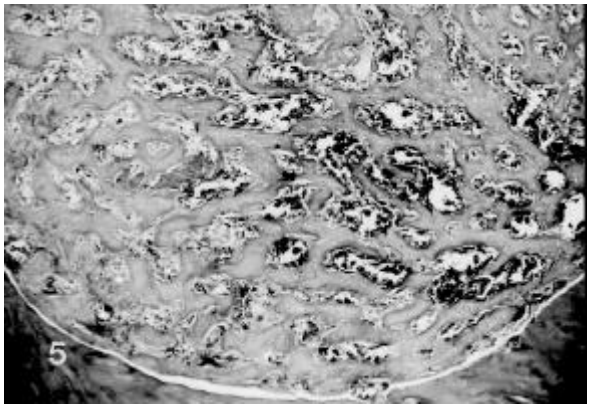


FIGURE 3- Group E1. Ten days. Surgical cavity partially occupied by thin and isolated trabecular bone and by connective tissue without bone differentiation. HE. 63X.

FIGURE 4- Group E2. Ten days. Surgical cavity showing immature thin trabecular bone (B) and connective tissue without bone differentiation. HE. 63X.



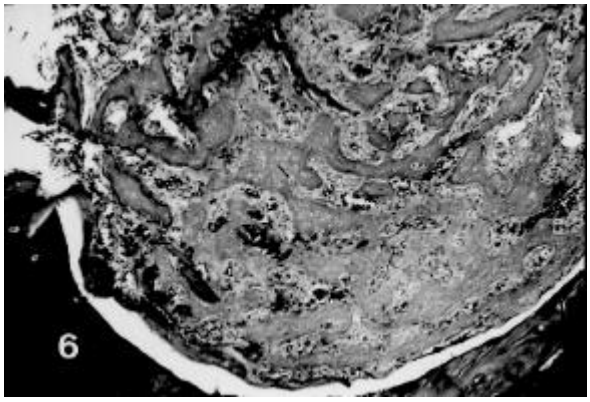


FIGURE 5- Group C1. Twenty days. Thin trabecular bone partially occupying the surgical cavity, with an ample intertrabecular space occupied by connective tissue. HE. 63X.

FIGURE 6- Group C2. Twenty days. Thicker trabecular bone compared to the preceding period. HE. 63X.

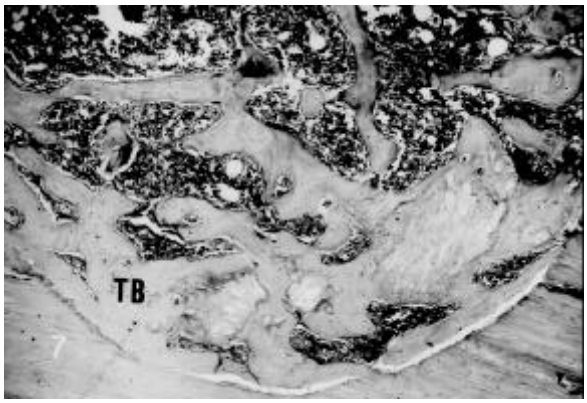


FIGURE 7- Group E1. Twenty days. Presence of thicker trabecular bone close to the margins of the surgical cavity (TB). HE. 63X.

FIGURE 8- Group E2. Twenty days. Presence of more intense new bone formation close to the margins of the surgical cavity (B) and a moderate area occupied by connective tissue without bone differentiation (CT). HE. 63X.

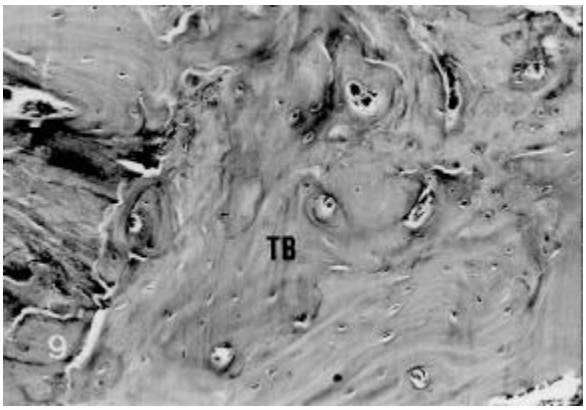


FIGURE 9- Groups C1 and C2. Forty days. Surgical cavity showing well-developed trabecular bone (TB) with defined medullary canals . HE. 160X.

FIGURE 10- Groups E1 and E2. Forty days. Presence of thick trabecular bone (TB) and areas without bone differentiation (arrow). HE. 160X.

the search for alloplastic that might aid the recovery of this tissue have led to the study of hydroxyapatite, a biocompatible calcium phosphate ceramic.

Gen-phos®, desveloped at the Dental School of Bauru/USP, is indicated by the manufacturer (Dentoflex) for the reconstruction of bone tissue in all areas of dentistry, where it accelerates new bone and tissue formation by releasing calcium and phosphate ions, which stimulate the action of fibroblasts, macrophages and osteoblasts. Among synthetic materials, hydroxyapatites are considered by researchers to be the most biocompatible materials since they do not induce any type of adverse reaction and chemically consist of calcium and phosphate ions, elements occurring in bone tissue [3,14].

Gen-phos® has shown no undesirable effect on subcutaneous tissue such as cytotoxicity or exacerbated immunological involvement [6]. In the present experiment, in the 10-day group the animals that received Gen-phos® presented lower new bone formation because hydroxyapatite induces acute inflammation, especially during the first 24 hours, and exuberant chronic granulomatous inflammation starting on the seventh day [13]. In order to induce bone tissue formation, a biomaterial must overcome a phase of biological receptivity. In the 20-day group, the animals that received the biomaterial implant presented better new bone formation than the remaining ones, perhaps because by that time calcium and phosphate incorporation into the tissues phagocytized by the osteoblasts had already occurred[17]. Gen-phos® is a microgranular and reabsorbable hydroxyapatite with particles of 10-15 mm in diameter. Because of this small diameter and because of its porosity, microgranular hydroxyapatite can be phagocytized by osteoblasts, where gene transcription and protein biosynthesis are increased, characterizing a greater metabolic activity[7].

In the 40-day group, only experimental animals did not present newly formed bone tissue and continued to present areas without bone differentiation. This fact is due to the negative effects of chronic alcoholism on bone tissue, even when Gen-phos® is used, which, if it did not eliminate the negative effects of alcohol, at least minimized them.

CONCLUSIONS

On the basis of the conditions employed in the present study, we may conclude that:

1- The alcoholic diet interfered in a negative manner with new bone formation in all specimens, with delayed filling of the surgical cavity, which was still incomplete after 40 days of repair.

2- With the use of Gen-phos®, new bone formation was more intense, especially close to the margins of the surgical cavity.

3- In the control group, the results obtained with Gen-phos® implantation were similar to those obtained without implantation of the material, with the surgical cavity being totally filled with newly formed bone tissue after 40 days of repair.

4- Gen-phos® was reabsorbed in all implanted specimens.

RESUMO

No presente trabalho foi avaliada em ratos, submetidos ao alcoolismo crônico experimental, a reação do tecido ósseo na presença da Gen-phos®. Para tanto foram utilizados 60 ratos divididos em grupo controle, que receberam água como dieta líquida, e o grupo experimental, que receberam álcool etílico a 6% durante 60 dias. O reparo ósseo foi avaliado microscopicamente aos 10, 20 e 40 dias após a cirurgia experimental. Os resultados obtidos mostram que a dieta alcoólica provocou uma neoformação óssea mais discreta em todos os espécimes e em todos os períodos, ocasionando um preenchimento ósseo incompleto da loja cirúrgica até os 40 dias de reparação. A neoformação óssea foi mais favorável em alguns espécimes com a implantação da Gen-phos®, aos 20 dias de reparação.

UNITERMOS: Implante aloplástico; Hidroxiapatita; Alcoolismo.

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