Effects of ethanol on the osteogenesis around porous hydroxyapatite implants

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(With 1 figure)

Abstract

Alcohol consumption compromises bone tissue, and thus may either impair or stop the fixation and maintenance of osseointegrated implants. To evaluate the effects of 5% and 15% ethanol on bone neoformation around porous hydroxiapatite implants. Fifteen rats were separated into 3 groups of 5 animals each: control (CT); 5% alcohol (A); and 15% alcohol (AA). After four weeks of ethanol consumption, the rats received porous hydroxiapatite implants into surgically made cavities in the femur. After surgery, the animals continued to consume ethanol until day 90 of the experiment, when they were euthanised and their femurs removed for histological processing. Bone tissue was found around the ceramic specimens of all the animals. The largest volume of neoformed bone around ceramic specimens occurred in the CT group, and the smallest in the AA group, followed by the A group. It was concluded that ethanol consumption produced a negative effect on osteogenesis around hydroxyapatite implants. Even small doses, such as the 5% ethanol dilution can interfere with bone repair.

Keywords: hydroxyapatite, alcoholism, ethanol, bone.

Efeitos do etanol sobre a osteogênese ao redor de implantes de hidroxiapatita porosa

Resumo

O consumo de álcool é prejudicial à integridade do tecido ósseo; consequentemente, pode dificultar ou até mesmo impedir a fixação e manutenção dos implantes osseointegráveis. Avaliar os efeitos do etanol (5% e 15%) sobre a neoformação óssea ao redor de implantes de hidroxiapatita porosa. Foram utilizados 15 ratos divididos em três grupos de 5: controle (CT); álcool 5% (A); e álcool 15% (AA). Após quatro semanas de consumo de etanol, a hidroxiapatita porosa foi implantada em cavidades produzidas cirurgicamente nos fêmures dos animais. Após as cirurgias, os animais continuaram a consumir etanol até completar 90 dias de experimento, quando foram sacrificados e os fêmures isolados para o processamento histológico. Em todos os animais foi encontrado tecido ósseo junto aos corpos cerâmicos. O volume de osso formado ao redor dos corpos cerâmicos foi maior no grupo CT em relação aos demais grupos. Os animais do grupo AA foram aqueles que apresentaram menor volume de osso neoformado, seguidos dos animais do grupo A. Conclui-se que o consumo de etanol produziu efeito negativo sobre a osteogênese ao redor dos implantes de HAP, e que mesmo doses pequenas, como a diluição de etanol 5%, podem interferir na reparação óssea.
Palavras-chave: hidroxiapatita, alcoolismo, etanol, osso.

1. Introduction

Bones are rigid structures for supporting the body, but they are subject to losses and defects due to fractures, malformations or tumor resection (Sakakura et al., 2001). Although capable of spontaneous repair, large bone losses require intervention such as bone grafts and biomaterial implants (Camilli et al., 2004; Caria et al., 2007).

The search for biocompatible, osteoconductive materials, which do not cause immune reactions, brought forth hydroxiapatite (HA), a human-bone resembling bioceramic, with strong chemical stability, used in dentistry and orthopedics (Legeros, 2002; Andrade et al., 2002). In dentistry, it has been exhaustively used for the correction

of bone deformities and filling of periodontal pockets and in bucomaxillofacial surgery and traumatology for the regularisation and maintenance of bone margins (Buchaim et al., 2002). It can be produced in the form of powder or either dense (DHA) or porous (PHA) blocks, with different granules, and associated with collagen, demineralised bone matrix and osteoconductive proteins (Vidigal and Goisman, 2003).

Alcoholism is a chronic disease that affects 13% of the world population, causing psychic, organic and socioeconomic disorders (Kopelman et al., 2009). Ethanol, the main component of alcoholic beverages, is toxic to vital organs and even to resistant tissues such as bones (Soares et al., 2010). Friday and Howard (1991) demonstrated that ethanol reduced cell proliferation, protein synthesis and the alkaline phosphatase activity in "in vitro" human bone cells. In addition, alcoholic patients were shown to be susceptible to fractures, besides presenting decreased number of bone cells, and alterations in bone repair (Klein, 1996). Histomorphometrical studies in young rats submitted to chronic ingestion of ethanol showed delayed endosteal and periosteal bone formation, and compromised mechanical properties of bone (Nyquist et al., 2002).

Different methods have been used to evaluate the effects of alcohol on bone tissue. Diez et al. (1997) submitted rats to a liquid diet with 30% ethanol for 6 weeks, and histochemically verified that ethanol reduced bone mineral metabolism. Sampson et al. (1998) used that same concentration during 4 weeks, and concluded that ethanol altered the homeostasis of hormones acting bones. Nyquist et al. (1999) gave rats a liquid diet with 15% ethanol for 5 weeks and observed that such alcoholism either inhibited or delayed the process of bone repair. Camilli et al. (2004) used 20% ethanol during 4 seeks, and then PHA implants were subperiosteally placed in the tibia and skull of rats, and the treatment was maintained until day 140 of the experiment. They concluded that the rate of osteogenesis and the volume of neoformed bone in alcohol-treated rats were inferior to those of the non-treated animals. Soares et al. (2010) gave rats a liquid diet with 10% ethanol associated with daily subcutaneous doses of nicotine (0.125 mg/100 g of the animal) during 90 days, and observed that the blood calcium level, the osseous mechanical resistance and the bone neoformation around DHA and PHA implants were reduced when compared to the control group.

According to Albrektsson et al. (1981), ethanol interferes negatively with the process of biomaterial osseointegration, and thus hinders the prognosis of bone repair.

When using biomaterials, the practitioner must be careful of the patient's habits, such as alcohol, tobacco, and drug abuse, which can compromise the success of osseointegrated implants. Chronic and heavy alcohol consumption is known to contribute to low bone mass, decreased bone formation, increased incidence of fractures, and delayed fracture healing. Given the high number of alcoholics and scarcity of studies about the effects of low concentrations of ethanol on osteogenesis and osseointegration, our study aims at evaluating the effects of 5% and 15% ethanol on bone neoformation around porous hydroxyapatite implants.

2. Material and Methods

2.1. Animal protocol

Fifteen 45-day-old male Wistar rats, weighing 180±2.5 g, were randomly separated into three groups (n=5): control (CT); 5%-alcoholist (A); and 15%-alcoholist (AA).

All the animals were fed a solid diet (Nuvilab®, Colombo, PR, Brasil). Regarding the liquid diet, the CT group received water ad libitum; the treated groups received alcohol diluted with water ad libitum: group A, 5% ethanol; group AA, 15% ethanol.

During 90 days, water was changed and new dilutions of ethanol were made every 48 hours. Every week, the animals were weighed and the solid and liquid consumptions were measured to calculate the mean caloric ingestion.

2.2. Surgery

After four weeks of treatment, all the animals were submitted to surgery for the hydroxyapatite implantation in the right femur. The animals were anesthetised with an IM injection of 1:1 solution of ketamin (Francotar®) and xylazin (Virbaxyl® 2%) at 0.10 mL/100 g. The skin from the femur bone shaved and longitudinally incised, the periosteum of the medial face of the distal epiphysis of the femur was detached and the bone cortex exposed.

With a dental bur (2 mm) coupled to the handpiece of a low speed mini-motor a cavity was prepared and filled with a 2 mm × 3 mm porous hydroxyapatite (PHA) implant, furnished by Unicamp Institute of Chemistry. After implantation, the periosteum was repositioned with silk sutures no. 8.0, and the skin was closed with cotton sutures no. 4.0. Soon after surgery, the animals received a single IM dose of dipirone at 160 mg/Kg. Then the animals returned to their respective diets until the 90th day of the experiment. Twenty-four hours after surgery, the animals walked without significant restrictions. Finally, the animals were euthanised with an overdose of sodium thiopental (Tiopental sódico®) and their femurs were collected for histological processing. The experimental protocol was approved by the Unifenas Committee of Ethics in Research under no. 19a/2004.

2.3. Histological and morphometrical analysis

The femurs were fixed in 10% buffered formalin for 48 hours, decalcified and submitted to routine histological processing (Soares et al., 2010). Transversal histological 6-µm thick sections were cut from the implantation sites of the femur. The samples were routinely processed and stained with hematoxylin and eosin (Camilli et al., 2004).

The volume of newly formed bone close to the hydroxyapatite implants was obtained using a 100-point quadrilateral grid system coupled to the ocular micrometer of a light microscope. Newly formed bone was quantified using the formula Vv = Pp/Pt (%), where Vv is the volume,

Pp the number of points on the newly formed bone, and Pt the total number of the system points, according to the principle of Delesse mentioned by Mandarim-de-Lacerda (1999).

2.4. Statistical analysis

The data were expressed as mean ± SEM. ANOVA followed by the Tukey test were used to compare the means of different groups. The value of $p < 0.05$ indicated significant differences.

3. Results

Liquid and solid consumption was ideal in all the groups. But group AA exhibited lower liquid ingestion when compared with groups CT and A (Table 1). Group AA also showed lower solid ingestion in comparison with group CT (Table 1).

The morphometric results showed that group AA had a lower volume of neoformed bone around the HA implants when compared with the other groups (Table 1), while group A had less bone neoformation around implants than group CT (Table 1).

The histological analysis revealed bone defect. The neoformed bone, continuous with the cortical lamina in all the groups, covered the HA granules and filled its pores (Figure 1).

The periosteum covered the implantation site in all the groups, but was thicker, showed more intense osteogenic activity and also small regions of fibrous tissue in the bone/implant interface in groups A and AA when compared with group CT. These findings were more intense in group AA.

4. Discussion

The present study showed that ethanol reduced osteogenesis around PHA osseointegrated implants. This phenomenon in groups A and AA are attributed solely to the chemical effects of ethanol, since the daily consumption was above 25 g of ration and 15 mL of water, with no signs of malnutrition and dehydration (Svendsen and Hau, 1984). The control of liquid and solid ingest was fundamental to avoid a negative influence on the results, since protein undernourishment may be caused by a low solid ingestion, and dehydration, by a low liquid ingestion (Holbrook and Connor, 1993).

Different methods, with the variables alcoholic concentrations and time of ethanol exposure, have been used to evaluate the effects of alcohol on the bone tissue and its repair. Studies on alcoholism have used ethanol concentrations ranging from 10% to 30% in periods of 4 to 12 weeks (Soares et al., 2010; Nyquist et al., 1999; Keiver et al., 2005; Baran et al., 1980).

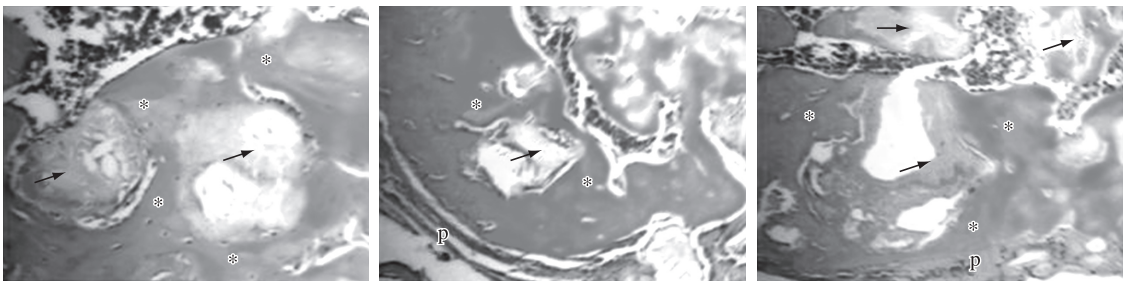
The chronic ingestion of 10%-20% ethanol by animals submitted to implants of dense and porous hydroxyapatite restricts the volume of neoformed bone (Soares et al. 2010; Camilli et al., 2004). The chronic consumption of 10% ethanol was the lowest concentration observed in the literature and is considered harmful to osteogenesis and osseointegration around implants of biomaterial.

The animals of the present study, treated with 5% ethanol, showed a lower volume of neoformed bone tissue around PHA implants when compared with the control group, thus demonstrating that even at such low concentration, ethanol had a deleterious effect on the bone tissue. These conditions have revealed alterations

Table 1. Daily consumption of liquid (mL) and solid diets (g), weight gain, and neoformed bone volume around HAP implants (%) in groups CT, A, and AA. No significant difference was found between weight gain and final weight.

	CT	A	AA
Liquid (mL)	50 ± 1.9	45 ± 2.8	30,3 ± 1.5$^{*,\#}$
Solid (g)	29.5 ± 1.1	27 ± 0.4	26.5 ± 0.6*
Weight gain (g)	146.7 ± 3.3	133.9 ± 4.8	132.2 ± 3.2
Final weight (g)	333.5 ± 23.4	300.5 ± 16.15	299.5 ± 20.8
Neoformed bone volume	44.6 ± 0.6	35 ± 0.7*	28.6 ± 1.1$^{*\#}$

*$p < 0.05$ compared with CT; $^{\#}p < 0.05$ compared with A. Data: mean ± SEM.



CT A AA

Figure 1. Cross section of the femur of groups CT (control), A (5% alcohol) and AA (15% alcohol) – Neoformed bone (*) around PHA (arrow) connecting one granule to another and continuous with the cortical bone. Periosteum (p) in AA is thick and shows osteogenic activity (115× – H.E.).

in bone repair with regard to migration, proliferation and differentiation of osteogenic cells into osteoblasts (Marks and Popoff, 1988; Klein et al., 1996).

Alcohol consumption reduced osteogenesis around PHA implants in A and AA animals. Although more damaging at the 15% dilution, ethanol was not an overdose and did not induce death. PHA osseointegration occurred in alcoholic rats, but with a lower peri-implant bone volume when compared with nonalcoholic animals. Ethanol interferes with the levels of hormones which are essential in bone remodelling (Baran et al., 1980) and in the bone repair process, thus either delaying or preventing the osseointegration of implants (Diez et al., 1997). In addition, it inhibits cellular proliferation, accelerates osteoblastic apoptosis and reduces the levels of blood ionised calcium, thus hindering bone mineralisation (Soares et al., 2010; Keiver et al., 2005), which increases the risk of fractures and obstructs the bone repair process (Chakkalakal et al., 2002). To determine whether alcohol has a direct effect on osteoblasts, researchers measured levels of osteocalcin, a protein secreted by osteoblasts and thought to be a measure of osteoblast function. Using in vitro preparations of osteoblasts from rats, most investigators reported a decrease in osteocalcin levels in response to alcohol administration, suggesting that alcohol decreases osteoblastic activity (Peng et al. 1991). Microscopic studies of bone tissue from rats demonstrated decreased trabecular bone volume, decreased numbers of osteoblasts, and decreased rates of bone formation, indicating impaired bone formation and mineralisation, along with other characteristics indicative of osteoporosis (Sampson et al.,1998).

It was concluded that ethanol consumption at the 5% and 15% concentrations interfered with osteogenesis around PHA implants. Considering the large number of alcoholics, it is important to verify the drinking habits of the patients in order not to compromise the stability and maintenance of osseointegrated implants.

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