EFFECTS OF ALCOHOLISM AND ALCOHOLIC DETOXICATION ON THE REPAIR AND BIOMECHANICS OF BONE

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ABSTRACT

Objective: To evaluate the effects of chronic ethanol consumption and alcohol detoxication on the mechanical resistance of bone and bone neoformation around dense hydroxyapatite implants (DHA) in rats. Methods: Fifteen rats were separated into three groups: (1) control group (CT); (2) chronic alcoholic (CA), and (3) disintoxicated (DI). After four weeks, a DHA was implanted in the right tibia of the animals, and the CA group continued consuming ethanol, while the DI group started detoxication. The solid and liquid feeding of the animals was recorded, and a new alcohol dilution was effected every 48 hours. After 13 weeks, the animals were euthanized and their biological material was collected. Results: Bone tissue was found around DHA in all the animals. Group CA showed less bone neoformation, lower levels of ionic and total calcium when compared to the animals of the CT and DI groups. The DI animals showed higher values in all the variables in relation to the CA group. Conclusion: Ethanol consumption interfered in osteogenesis around the DHA implants, and in calcium levels and mechanical bone resistance. Alcohol detoxication was effective, as it increased osteogenesis, DHA osteointegration, calcemia, and mechanical resistance of the b one. Level of Evidence: Level I, therapeutic studies.

Keywords: Ethanol; Alcoholism; Hydroxyapatite; Biomechanics.

INTRODUCTION

The bones are essential rigid structures in the support of the body. However, they are subject to bone losses resulting from fractures, malformations or tumor resection. Although they present the capacity for spontaneous regeneration, major bone losses require interventions, such as bone grafts and biomaterial implants.[1] It was during the search for biocompatible, osteoconductive materials that would not promote immunogenicity reactions that Hydroxyapatite (HA)[2] appeared. This is a bioceramic resembling bone that exhibits strong chemical stability, and is used extensively by odontology and orthopedics.[3] It can be produced in the form of powder or blocks, either dense (DHA) or porous (PHA), with different granulations and associated with diverse materials such as collagen, demineralized bone matrix and osteoinductive proteins.[4,5]

Alcoholism is a chronic disease that affects 13% of the world population,[6] causing psychic, organic and socioeconomic disorders.[7] Ethanol, the main component of alcoholic beverages, acts as a toxic element to vital organs, acting destructively even in resistant tissues such as the bones.[4] Friday and Howard[8] showed that ethanol reduced cell proliferation, protein synthesis and the activity of alkaline phosphatase in human bone cells in vitro. Moreover, alcoholic patients exhibited predisposition to fractures, osteopenia and alterations in bone regeneration.[9] Histomorphometric studies on young rats under chronic ethanol ingestion evidenced a reduction in the speed of bone formation from the endosteum and periosteum, besides impairment of the mechanical properties of bone.[10]

Different methodologies have been used to assess the effects of alcohol on the bony tissue. Camilli et al.[11] used 20% ethanol for four weeks until the subperiosteal implantation of porous Hydroxyapatite (PHA) in the tibia and cranium of rats, maintaining the treatment for 140 days of experimentation. They concluded that the osteogenesis speed and the neoformed volume of the rats treated with alcohol were lower than those of the untreated rats. Soares et al.[4] offered the rats a liquid diet with ethanol (10%) associated with daily doses of nicotine (0.125 mg/100

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of the animal) administered subcutaneously over a period of 90 days, finding that the blood calcium level, the bone mechanical resistance and bone neoformation around DHA and PHA implants appeared reduced in comparison with the control group. The professional should be attentive to the patient's habits, such as alcoholism, smoking and the consumption of other drugs that may interfere in osteointegration and in bone repair after fractures. Considering the vast number of alcoholic individuals and the shortage of studies referring to the effects of alcohol detoxication, this study aimed to evaluate the effects of ethanol (15%) and of alcoholic detoxication on bone neoformation and on the structural properties of bone.

MATERIAL AND METHODS

Animal Protocol

After approval by the Committee of Ethics in Research of Universidade José do Rosário Vellano (UNIFENAS), Protocol no. 19A/2007, this study was conducted in the Phytotherapic Laboratory of UNIFENAS, in compliance with the Brazilian Legislation on Animals used for Experimentations, regulated by Federal Law 6638 (1979). The entire experiment observed the ethical principles in animal experimentation advocated by the Colégio Brasileiro de Experimentação Animal (COBEA).

The study subjects were 15 male Wistar rats (*Rattus norvegicus*) at 40 days of age, weighing 180±2.5g, and kept in the post-graduate program vivarium of UNIFENAS with temperature control and 12-hour control in the light/dark cycle. Divided into three random groups (n=5): Control Group (CT): The animals received water *ad libitum*; Alcoholic Group (CA): These animals followed the determined chronic alcoholism model of "semi-voluntary", where alcohol was the only liquid food available. The animals first underwent a brief period of gradual adaptation to alcohol, receiving a 5% and 10% ethyl alcohol based liquid diet for two weeks, and in the third week 15% ethyl alcohol, continuing with this diet until the thirteenth week, following the protocol proposed by Cagnon *et al.*[12] Disintoxicated Group (DI): This group was submitted to the same protocol as the CA group. However, upon completion of the fourth week of 15% ethanol ingestion, the animals started the period of gradual alcohol de-adaptation, receiving a 10% and a 5% ethyl alcohol based liquid diet for one week each, and a liquid diet on a basis of water *ad libitum* up to the thirteenth week, when they were considered disintoxicated.

All the animals received the same solid diet (Nuvilab®), and water changes, further dilutions of the ethanol, weighing of the animals and measuring of solid and liquid consumption to calculate mean ingestion occurred every 48 hours throughout the experiment.

Surgical procedure

The animals were anesthetized with 1:1 solution of Ketamine (Francotar®) and Xylazine Chlorhydrate (Virbaxyl® 2%) in the dose of 0.10 ml/100g IM after six weeks of experimentation.[11] Trichotomy and longitudinal incision in the skin of the skullcap and of the left leg were performed and the periosteum was shifted to the side, exposing the cortical bone. A 5mm cavity was produced in the left parietal bone with the help of a trephine punch, and the defect was kept open to verify the neoformed bone. Another 3mm defect was produced in the proximal epiphysis of the left tibia, with the implantation of the dense hydroxyapatite (DHA) bioceramic. The periosteum in the parietal bone and tibia was repositioned through the suturing of its edges with 8.0 silk suture and the skin was sutured with 4.0 cotton thread. The DHA bioceramic was produced by the Chemistry Institute of Unicamp, measuring 3mm in diameter and 3mm in length. All the animals were treated with analgesic (sodium dipyrone 500 mg/mL) added to water *(ad libitum)* in the dose of 875 mg/kg. During the first 2 days of the postoperative period and 24 hours after surgery the animals walked without significant limitations. Upon completing 13 weeks of experiment the animals were anesthetized to draw blood through cardiac puncture[4], then euthanized with an overdose of anesthesia, administered intraperitoneally (IP), using xylazine/ketamine (Francotar®, Virbaxyl® 2%) in the concentration of 6 - 40 mg/Kg, respectively.

Mechanical Test of the Femurs

For the performance of the mechanical test, the animals' left femurs were collected, cleaned and packed in gauze soaked in saline solution (0.9%) then stored in a freezer (-20°C) until the day prior to the test.[4] The femurs were submitted to mechanical resistance test at the Laboratory of Mechanical Properties of the Faculty of Mechanical Engineering (FEM) of Unicamp. The mechanical test was conducted in a three-point flexion module, *MTS TestStar* II, using a load cell of 100 Kgf, at a speed of 1.3 mm/min. The distance between the two bone extremities was 50mm, and, to obtain the resistance value, a load was applied on the middle third of the bone (diaphysis) through one end, coupled to a universal testing machine.[4] The maximum force required for complete breakage of the femurs was obtained after a load cell recorded by the MTS Flexion Module in Newtons (N).

Histomorphometric Processing

The left parietal bone and the left tibia, once collected, were immersed in 10% buffered formalin for 48 hours and decalcified in a solution of formic acid, formaldehyde and sodium citrate for 15 days. Afterwards the bones were reduced and included in paraffin for histological cuts. Semi-serial cross sections were formed with a thickness of 6 micrometers, deposited on slides. They were then stained with hematoxylin/eosin (HE) for morphological analysis.[4] The volume of neoformed bone was obtained with the help of a quadrilateral reticule of 100 points coupled to the microscope eyepiece. After the points were counted, the formula $V_V = P_P/P_t$ (%), where V_V = density of volume or relative volume; P_P = quantity of points on the neoformed bone; P_T = total number of points of the system, was applied according to Delesse's principle proposed by Mandarim de Lacerda.[13]

Plasma calcium concentration

The animals' blood was drawn and the serum obtained and centrifuged at 3,000 rpm for calcium determination, following the method proposed by Labtest-Diagnóstica (Brazil), in which a stabilized aqueous reagent is used. The colorimetric method was used to determine total calcium. Measured in a spectrophotometer (Gehaka G3410, Brazil) at 570 nm of the color produced by the complex formed between ortho cresophthalein complexone and the calcium, in alkaline pH.[13]

Statistical Analysis

The data were expressed as mean±standard deviation. The Analysis of Variance (ANOVA) followed by the Tukey test was used to compare the mean values between different groups. We considered the differences significant when the p-value<0.05.

RESULTS

The liquid and solid consumption was satisfactory among the groups, yet the animals from the CA and DI groups ingested a smaller amount of the liquid diet than the animals from the CT groups. (Table 1) Solid diet consumption was lower in the animals from the DI group than in the animals from the CT and CA groups. (Table 1) During the experiment the animals gained weight, and there were no significant differences among the experimental groups. (Table 1)

The morphometric results demonstrated that the animals from the CA and DI groups presented a lower volume of neoformed bone around the DHA implants than those from the CT group, while the animals from the CA group where those that least osteointegrated DHA. (Figure 1 and Table 1) In the bone defect produced in the parietal bone, a lower volume of neoformed bone was also observed in the animals from the CA group. (Figure 2 and Table 1)

The maximum force (N) required to break the femurs of the animals from the CA group was lower than and statistically different from the force used to break the femurs of the animals from the CT and DI groups. (Table 1) Displacement at maximum force was the same among the three experimental groups. (Table 1) The total plasma calcium levels of the animals from the CA group were lower than and statistically different from the findings for the CT and DI groups. (Table 1) The ionic calcium levels of the animals from the CA and DI groups presented values that were lower than and statistically different from the CT group. (Table 1)

DISCUSSION

Our results showed that 15% ethanol decreased osteogenesis around the osseointegrable DHA implants, while alcohol detoxication aids in bone neoformation and the animal's liquid and solid diet consumption in the light of chronic ethanol consumption did not characterize protein malnutrition or dehydration. Thus the limitation in the osteogenesis process in the animals from groups CA and DI are attributed only to the chemical effects of ethanol.

Different methodologies, with the variables, alcohol concentrations and ethanol exposure time are used to evaluate the effects of ethanol on the bone tissue and on its repair. Studies referring to experimental alcoholism used ethanol concentrations ranging between 10 and 30% in periods of four to twelve weeks.[4,11] Under these conditions alterations were evidenced in bone repair as refers to migration, proliferation and differentiation of the osteogenic cells into osteoblasts.[14] The animal protocol used in this study was sufficient to cause bone alterations in the animals, neither characterizing overdose nor inducing the animals' death. Little mention is made of the effects of alcoholic detoxication and bone repair in scientific literature. However, in clinical practice it is often recommended that the patient avoid ethanol consumption in the postoperative period.

The animals from the CA group consumed a smaller amount of

Table 1. *Comparison of weight gain (Δ); consumption of liquid and solid diet; volume of newly formed bone; maximum force for femoral breakage; concentration of ionic and total calcium in the control (CT), alcoholic (CA) and detoxicated (DI) groups*

	CT	CA	DI
ΔP (g)	267 ± 20[a]	264 ± 5.7[a]	263 ± 20[a]
Liquid (mL)	45 ± 1.5[a]	36.2 ± 2.0[b]	38 ± 0.5[b]
Solid (g)	49.4 ± 2.1[a]	40.5 ± 7.6[a]	38.4 ± 1.0[b]
Newly formed bone (%) around DHA (tibia)	40.2 ± 0.5[a]	32.6 ± 0.5[b]	36.4 ± 0.9[c]
Newly formed bone (%) in the bone defect (parietal)	90 ± 3.4[a]	53 ± 1.4[b]	71 ± 5.5[c]
Maximum Force (N)	165 ± 36.5[a]	100 ± 14[b]	140.5 ± 16.5[c]
Displacement (mm)	0.58 ± 0.35[a]	0.52 ± 0.35[a]	0.55 ± 0.31[a]
Total Calcium (mEq/L)	17.2 ± 1.4a	12.3 ± 1.3b	17.7 ± 1.2c
Ionic Calcium (mmol/L)	1.1 ± 0.05[a]	1.2 ± 0.06[b]	0.7 ± 0.02[c]

Two means, followed by the same small letter, are not different from one another (P> 0.05) in the Tukey test. The results are presented as mean ± standard deviation.
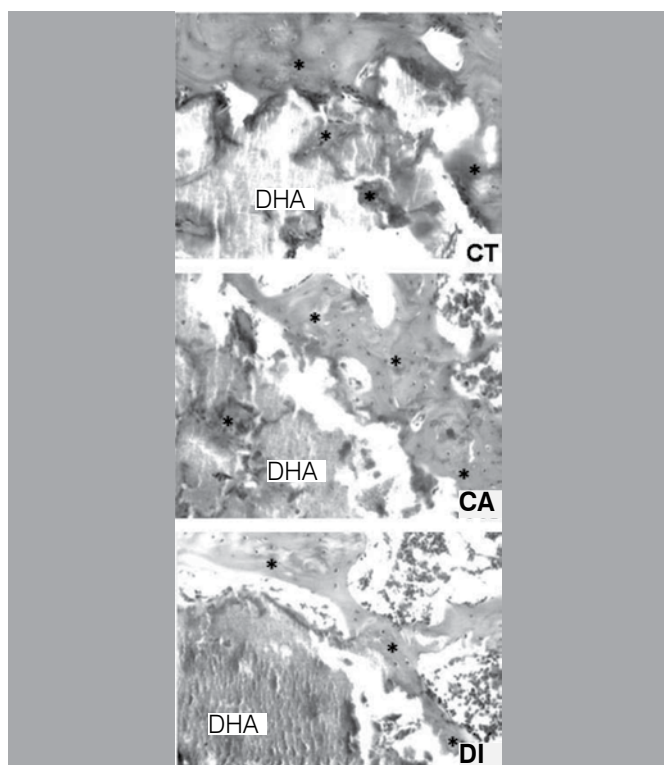
Figure 1. *Photomicrography of cross-section of the tibia of groups CT, CA and DI. The newly formed bone (*) around the DHA implant performed on the left tibias of the animals (115x – H.E.).*

feed and water than the CT and DI animals. However, the daily consumptions were above 25g of feed and 15 mL of water per day, not characterizing malnutrition and dehydration.[4,15] Control over liquid and solid ingestion during the experiment was essential, since low solid ingestion can cause protein
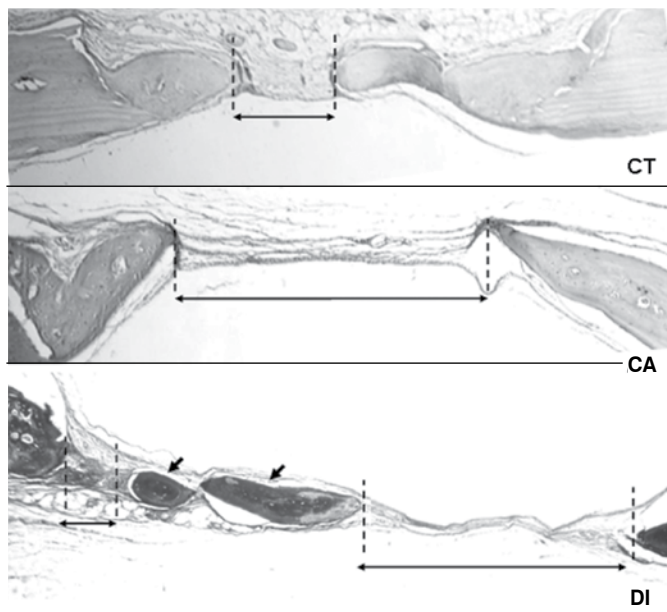
Figure 2. *Frontal photomicrography of the parietal bone displaying less neoformation in the bone defect (↔) among the animals from groups CA and DI than among those from the CT group. Observe intermediate bone formation at the center of the defect in DI (↓) (200x – H.E.).*

malnutrition and low liquid ingestion dehydration, negatively influencing the results.[16]

Ethanol consumption decreased osteogenesis around the DHA implants in the CA and DI animals. Yet DHA osseointegration was observed in alcoholic rats, with lower peri-implant bone volume than in the non-alcoholic and detoxicated animals. Although the volume of neoformed bone in the detoxicated ani-

mals was lower than in the control group animals, it is possible to observe that the elimination of ethanol consumption has a positive effect on bone repair. Ethanol interferes in the bone healing process, limiting osseointegrable implants or rendering them unviable, inhibiting cell proliferation and accelerating osteoblast apoptosis in humans,[17] decreasing the levels of blood ionized calcium and jeopardizing the bone mineralization process.[18]

Chronic ethanol consumption induces a decrease in calcemia.[10] The ionic calcium and total calcium levels of the animals from the CA group were lower than the values observed in the animals from the CT and DI groups, since the calcemia levels of the animals from the DI group, although lower than those of the CT group, are higher than those of the animals from the CA group, demonstrating that alcohol detoxication allows improvements in blood calcium levels.

Ethanol alters the mineral composition and the mechanical properties of bones, making them more fragile and prone to fractures.[4] The mechanical tests conducted on the femurs evidence the bone fragility of the femurs of the animals from the CA group, as these were broken with less force than with the animals from the CT and DI groups. Even though the detoxicated animals require less force to break than the CT animals, they still demonstrate greater bone resistance than the chronic alcoholic animals.

CONCLUSION

Ethanol consumption in the concentration of 15% interfered negatively in osteogenesis around the DHA implant, in the calcium levels and in bone mechanical resistance. Alcohol detoxication proved effective, as it increased osteogenesis and osseointegration of DHA, calcemia and bone mechanical resistance.

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