46 - THE EFFECT OF CHRONIC ALCOHOLISM ON THE BONE DENSITY AND REPAIR IN TIBIAE OF RATS: IMMUNOHISTOCHEMICAL AND HISTOMETRIC STUDY

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INTRODUCTION

The alcohol consumption in the modern society is predominantly seen in a positive way, because it's a legal, psychoactive, and socially acceptable substance. According to data of Brazilian Ministry of Health (2010), this substance causes the addiction known as alcoholism, recognized as a disease since 1948, and represents one of the most serious socioeconomic problems to heath worldwide.

Epidemiologic studies have detected bigger and bigger numbers when it comes to alcohol consumption, in Brazil and other parts of the world, becoming pathologic and endemic mainly after the Industrial Revolution (MARQUES, 2001; MORAES et al., 2006; LARANJEIRA, et al. 2007).

When not led to death, people who drink this substance chronically suffer from problems that take place in different tissues in the human body, among these the bone tissue.

The bone homeostasis is affected by the inadequate alcohol consumption, which can destabilize the balance controlled by the system RANK/RANKL/OPG (BOYLE et al., 2003). In this system, when a link between RANK (Receptor Activator of Nuclear Factor Kappa-β) and RANKL (Receptor Activator of Nuclear Factor Kappa-β Lingand) occur there is the osteoclast formation and activity and, consequently, the bone tissue resorption (BOYLE et al., 2003). Osteoprotegerin (OPG) is able to regulate this resorption process because it joins to RANK, avoiding the link between RANK and RANKL. When it occurs, the bone resorption is interrupted (YASUDA et al., 1998).

OBJECTIVES

- Characterize the occasional morphologic and histochemical alterations in the osseous tissue of rats submitted to a progressive and controlled intake of alcohol.
- Quantify through histomorphometry the density and new bone formation on the tibiae;
- Analyze the expression of OPG and RANKL on the tibiae.

MATERIAL AND METHODS

All the experiments led in this study were previously approved by Comissão de Ética em Experimentação Animal da Faculdade de Medicina Veterinária da USP, Campus São Paulo (protocol number: 2476/2011).

Thirty (30) rats (Rattus norvegicus, albinus) of the Wistar breed, provided by the vivarium of Guarulhos University, with an average weight of 185 grams, not having any kind of pathology, kept in acrylic boxes padded with clean and appropriated shavings, fed with“ad libitum” standard ration for rodents, in environment with controlled temperature of about 23°C and 12-hour photoperiods of light and darkness.

The animals were divided randomly in groups determined like this: G1 (15 alcoholic rats submitted to a bone defect), G2 (15 non-alcoholic rats submitted to a bone defect).

The animals were randomly divided into groups so determined: G1 (15 rats alcoholics undergoing bone defect), G2 (15 nonalcoholic rats undergoing bone defect). The alcoholic group (G1) received as a liquid diet (method semi-voluntary) cane brandy (Pirassununga 51 ®, 39 GL, Muller Industries, Pirassununga, SP, Brazil) diluted in water with controlled concentration and progressive as table 1. Already the non alcoholic (G2) received only water as liquid diet "ad libitum".

Table1. Protocol to induce chronic alcoholism

Induction time (days)	*Concentration of brandy*
15 days	10º GL
15 days	15º GL
10 days	20º GL
10 days	25º GL
50 days	30º GL

RESULTS

The histometric analyses (figure 1) showed that there are significant differences between the bone density of the analyzed groups without defect. The tibiae of the control group showed bone density of approximately 144% bigger than the tibiae of the alcohol group (figure 2).

The static analyses of the renovated bone area expressed in mm² showed that the renovated bone area (figure 3) of the control group is 34% bigger than the renovated bone area of the alcohol group (figure 4).

In the photomicrography of the analyzed areas, it is possible to detect a bigger quantity of renovated tissue in the control group (Figure 5). We also verified that the amount of markings RANKL and OPG in the control group were similar and in greater quantity (Figure 6).

In photomicrographs of the areas analyzed is possible to note higher amount of newly formed tissue in the control group.

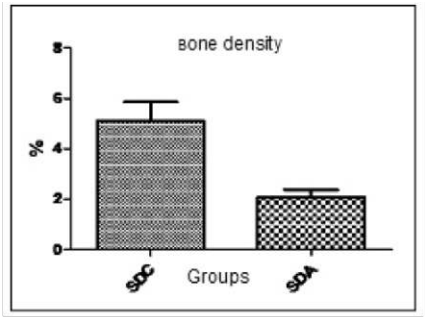

Figure 2. Statistical analysis of bone density in the study groups

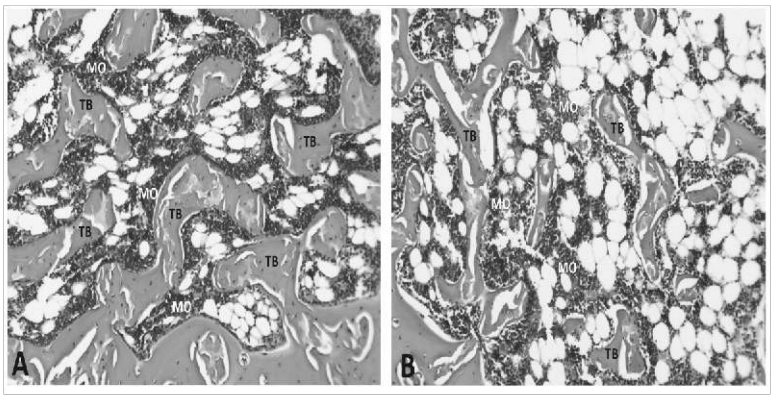

Figure 1. Histometric analysis cuts comparing the control group (A) and group (B) in relation to bone density.

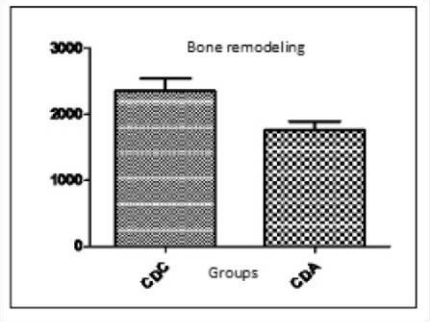

Figure 3. Statistical analysis of newly formed bone area in mm ²

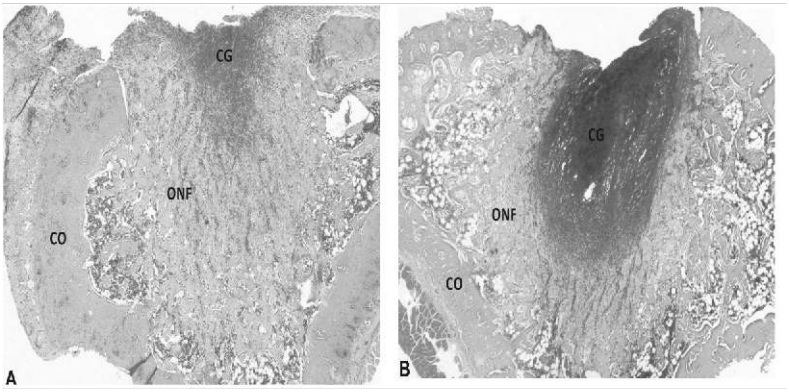

Figure 4. Histometric analysis of the area of new bone formation by comparing the control group (A) and group (B)

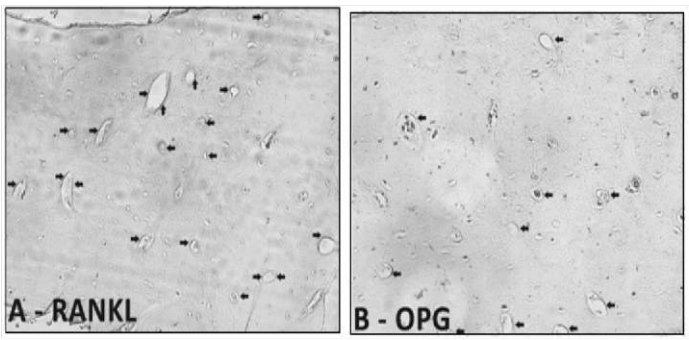

Figure 5. Markings of RANKL and OPG in the study group.

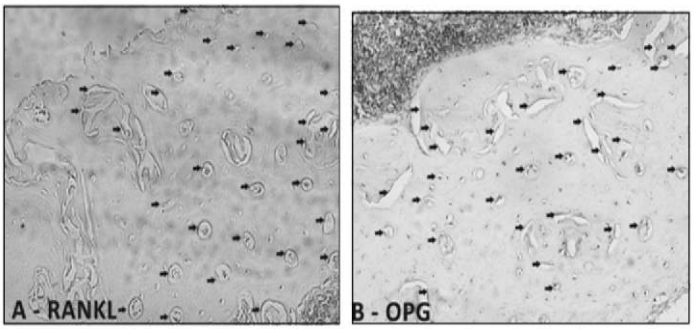

Figure 6. Markings of RANKL and OPG in the control group.

DISCUSSION

Regarding to the alterations that alcohol causes in the bone metabolism, several authors have described them. (SAVILLE, 1965; OPPENHEIM, 1977; KRISHNAMRA,1983; SCHUCKIT, 2009 e MAUREL, 2012).

Buchaim et al. (2004) have tested histologically the effects of alcohol in bone repair of Wistar rats' tibiae. The authors reported that in all animals of the experimental group there was a retardation concerning to the chronology of the bone repair process. In recent studies, Buchaim (2009) analyzed the effects of three alcoholic diets on bone repair in tibiae of rats. Different alcohol concentration were used for each group. The analyses showed that the different alcohol concentration used in the diets influenced the formation of new bone tissue in several ways. The repair and formation process was retarded in relation to the alcoholic concentration.

Histomorphometricly, our data showed significant differences related to bone density and repair between the groups alcohol and control. Our results agrees with Lima et al. (2011) showing retarded bone remodeling in rats from group alcohol. These results imply a possible negative effect on bone remodeling.

An important observation to be made about our experiment is the age of the rats. Our studies, which used young rats, comparing to other studies which used adult rats, did not show different results. Regarding bone density and remodeling, our results showed a smaller bone trabeculation in the alcohol group as well as a smaller bone regeneration capacity. Our results agree with the results of other studies (FREDERICK et al., 1999; HOGAN et al, 1999). The immunohistochemical results of our research agree with the results found in other studies that associate the imbalance of the system RANK/RANKL/OPG to bone diseases (HOFBAUER et al., 1999; GOLDRING, 2000; EGHBALI et al., 2003). Simonet et al. Simonet et al. (1997) did a series of experiments to understand the role of osteoprotegerin, and noticed that rats which showed the excess of this glycoprotein developed osteopetrosis, which is characterized by the increase of bone density and a reduction of the osteoclastic activity. These experiments supported the discovery that OPG is one powerful indirect inhibitor of the steoclastic activity. At a later time, in 1998, Yasuda et al. identified that other molecules were also involved in this process: RANK and its ligand RANKL, and they discovered that from the interaction of RANK/RANKL occurred the formation and activity of osteoclasts and consequently, the resorption of bone tissue. Our experiments showed that in the control group there is a bigger expression of OPG and RANKL than in the alcohol group, it leads us to believe that the system is in balance and that the bone renovation process is balanced. In the alcohol group we found a bigger expression of RANKL compared to OPG, showing a bigger osteoclastic activity and consequently a bigger bone resorption. The RANK and OPG activity is dependent on a series of other Immunoinflammatory factors that work as a net in favor or against tissue destruction. As there is little information in the literature about the role of RANKL and OPG in bone diseases related to alcoholism, caution is advisable when interpreting the expected results (SANTOS, 2009).

CONCLUSION

After the results analyses and according to the limitations of the used methods, we can conclude that:

Through morphometry, it was showed that in 100-day protocol of alcohol intake in a progressive and controlled concentration, there is a negative alteration in bone density and consolidation;

The immunohistochemical findings show in general terms that both control and alcohol groups show the presence of OPG and RANKL. The presence of these same proteins in different levels and quantities is noticeable in the alcohol group, thus emphasizing the bone tissue adaptations when exposed to alcohol

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THE EFFECT OF CHRONIC ALCOHOLISM ON THE BONE DENSITY AND REPAIR IN TIBIAE OF RATS: IMMUNOHISTOCHEMICAL AND HISTOMETRIC STUDY

ABSTRACT

The osseous tissue has as characteristic the continuous cellular absorption and recomposition regulated by the interaction of RANKL and OPG. In case its continuity is lost, that is, any kind of bone injury, its remodeling leads to restoration and consequent skeletal integrity and its metabolism is influenced by hormonal, local, behavioral, environmental, and nutritional factors. The chronic consumption of alcohol may contribute to this imbalance, and, although significant correlations between the excessive alcohol intake and bone consolidation have been reported, new studies must be developed considering the great disparity between the time of exposure and quantity of alcohol intake comprising different protocols. The objective of this study was to verify quantitatively the effects of chronic alcohol consumption on bone healing and density on Wistar rats. The expression of OPG and RANKL was also observed. For this purpose, 30 Wistar rats were randomly divided into two groups: G1 (group 1) consisted of 15 rats which had, for 100 days, a liquid diet of liquor diluted in water with a progressive and controlled concentration; G2 (group 2) consisted in 15 rats on a liquid diet of only water and free of alcohol. After the 92nd day of the induction period of alcoholism, tibial defects of 3 mm in diameter were created in both groups and after 8 days from this surgery procedure the animals were euthanized and the tibiae were removed. The percentage of renovated bone and osseous density were submitted to histometric analysis. Through immunohistochemistry, the expression of OPG and RANKL were analyzed. As a result, it was verified that the osseous density and bone remodeling were smaller in the alcoholic group; it was also found a differentiation in the expression of OPG and RANKL. These results show that the proposed protocol of alcohol intake produces negative effects in the gain of bone quality when compared to the control group.

KEYWORDS: Alcoholism, Bone Remodeling, Bone Density

EFFET DE L'ALCOOLISME CHRONIQUE SUR LA DENSITÉ ET LA RÉPARATION OSSEUSE CHEZ LE RAT TIBIA : ÉTUDE HISTOMÉTRIQUE ET IMMUNOHISTOCHIMIQUE

RESUMÉ

Le tissu osseux est caractérisé par une absorption en permanence à plein et le réarrangement de la cellule, étant commandé par l'interaction de RANKL et OPG . En cas de perte de la continuité, son remodelage conduit à la restauration et à l'intégrité du squelette ultérieure, et votre métabolisme influencée par des facteurs hormonaux , des lieux, comportementaux , environnementaux et nutritionnels . L'ingestion chronique d'alcool peut contribuer à ce déséquilibre, et bien que des corrélations significatives seront signalées entre la consommation excessive d'alcool et la guérison de l'os , de nouvelles études devraient être développés compte tenu de la grande disparité entre le moment de la soumission et le montant de la consommation d'alcool englobant les différents protocoles . Les objectifs de cette étude étaient d'évaluer quantitativement les effets de la consommation chronique d'alcool sur la densité osseuse et la réparation osseuse chez le rat, et d'observer l'expression de l'OPG et de RANKL . Séparé 30 rats , et (G1) 15 rats solution de consommer des boissons alcooliques diluées dans l'eau pendant 100 jours avec l'augmentation de concentration et contrôlés et 15 rats non alcoolisées consommer uniquement de l'eau comme une diète liquide (G2). Après la période d' induction de 92 jours de l'alcoolisme , les deux groupes ont été soumis à un défaut osseux tibial a été réalisée . 8 jours après la chirurgie, les animaux ont été euthanasiés et les tibias enlevés . Pourcentage de la formation osseuse et la densité osseuse a été évaluée histometrically . Observé par immunohistochimie l'expression d' OPG et de RANKL . En conséquence, nous avons observé que la densité et le remodelage osseux étaient plus faibles dans le groupe alcoolique. Nous avons également constaté une différenciation dans l'expression de RANKL et OPG en différents groupes. Nos résultats démontrent que l'ingestion de protocole proposé d'alcool a des effets négatifs sur la qualité de l'os par rapport au groupe témoin.
MOTS-CLÉS: alcoolisme,. La densité osseuse . Le remodelage osseux

EFECTOS DEL ALCOHOLISMO CRONICO SOBRE SOBRE LA DENSIDAD Y REPARACION OSEA EM TIBIA DE RATONES: ESTUDIO HISTOMETRICO Y IMUNOHISTOQUIMICO

RESUMEN

El tejido óseo tiene como característica estar en constante absorción y recomposición celular, controlado por la interacción de RANKL e OPG. Em caso de perdida de su continuidad su remodelacion lleva a la restauraciòn integral del esqueleto, donde su metabolismo es influenciado por factores hormonale, locales, funcionales, ambientales y nutricionales. La ingestion cronica de alcohol contribuye para esse desequilibrio, apesar de que correlaciones significativas han sido relatadas entre el consumo excesivo de alcohol y la consolidacion osea, nuevos estudios deben ser llevadas acabo devido a la gran diferencia entre el tiempo de sumision y la cantidad de ingestion de alcohol reuniendo los diferentes protocolos.Los objetivos deste estudio fueron evaluados cuantitativamente los efectos del consumo cronico de alcohol em la reconstruccion osea y densidad osea de ratones Wistar ademas de observar la expresion de OPG y RANKL.Separamos 30 ratones Wistar, siendo (G 1) 15 ratones consumiendo aguardiente diluido en agua por 100 días en concentraciones progresivas controladas y 15 ratones no alcohólicos consumiendo dieta líquida a base de agua (G2).Después de 92 días del período de inducción con alcohol, ambos grupos fueron sometidos a un defecto óseo en la tibia. 8 días después de la cirugía, fue practicado la eutanasia y posteriormente se procede al retiro de la tibia. El porcentaje de hueso neo formado y la densidad ósea formada fueron evaluados histometricamente. A través de la inmunohistoquimica observamos la expresión de OPG e RANKL. Como resultado podemos observar que la densidad y la remodelación ósea fueron menores en el grupo de ratones alcohólicos. Encontramos también diferenciación en la expresión de RANKLK y OPG en los diferentes grupos. Nuestros resultados demuestran que el protocolo propuesto de consumo de alcohol, produce efectos negativos en la cualidad ósea comparados con el grupo de control.

PALABRA CLAVE: Alcoholismo, Recuento de Células, Remodelación Ósea

EFEITO DO ALCOOLISMO CRÔNICO SOBRE A DENSIDADE E O REPARO ÓSSEO EM TIBIAS DE RATOS: ESTUDO HISTOMÉTRICO E IMUNOHISTOQUÍMICO

RESUMO

O tecido ósseo tem como característica estar constantemente em plena absorção e recomposição celular, sendo controlado pela interação de RANKL e OPG. No caso da perda de sua continuidade, sua remodelação leva à restauração e consequente integridade do esqueleto, sendo o seu metabolismo influenciado por fatores hormonais, locais, comportamentais, ambientais e nutricionais. A ingestão crônica de álcool pode contribuir para esse desequilíbrio, e embora correlações significativas venham sendo relatadas entre o consumo excessivo de álcool e a consolidação óssea, novos estudos devem ser desenvolvidos haja vista a grande disparidade entre o tempo de submissão e a quantidade de ingestão do álcool englobando os diferentes protocolos. Os objetivos desse estudo foram avaliar quantitativamente os efeitos do consumo crônico de álcool no reparo ósseo e densidade óssea em ratos Wistar, além de observarmos a expressão de OPG e RANKL. Separamos 30 ratos Wistar , sendo (G1) 15 ratos consumindo solução de aguardente diluída em água por 100 dias com concentração progressiva e controlada e 15 ratos não alcoólatras consumindo como dieta líquida somente água (G2). Após o 92º dia do período de indução do alcoolismo, ambos os grupos foram submetidos a um defeito ósseo realizado na tíbia. 8 dias após o procedimento cirúrgico os animais foram eutanasiados e as tíbias removidas .A porcentagem de osso neoformado e a densidade óssea foram avaliadas histometricamente. Através da imunohistoquimica observamos a expressão de OPG e RANKL. Como resultados, pudemos observar que a densidade e remodelação ósseas foram menores no grupo alcoólico. Encontramos também diferenciação na expressão de RANKL e OPG nos diferentes grupos. Nossos resultados demonstram que o protocolo proposto de ingestão de álcool exerce efeitos negativos na qualidade óssea quando comparado ao grupo controle.

PALAVRAS CHAVE: Alcoolismo, Densidade óssea, Remodelação óssea.