Induction to etilic alcohol intake delays the healing process pos-fracture in rats

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ABSTRACT. The excessive ingestion of spirits cause metabolic and pathologic changes in different organic systems. In the bone it is known that alcohol causes osteoporosis, inhibits osteosynthesis and alters the metabolism of the bony cells. The present study aimed at verifying the influence of alcohol ingestion in the process of bone consolidation after fracture in rats. Twelve Wistar albino rats (*Rattus norvegicus*) were split in a control group (A), treated with commercial chow and water, and an experimental group (B), receiving commercial chow and sugar-cane brandy. Twenty-five days after fracture, the rats were killed and the collected material was subjected to light microscopy routine. It was observed that the bony callus was present in both groups of animals. In the regeneration region the animals from group A showed a smaller density of cartilage cells and thus a greater amount of newly-formed bone, while the animals from group B presented a larger density of cartilage cells, demonstrating a delay in the process of bone formation. According to the results obtained, we state that alcohol influences the fracture regeneration, delaying this process.

Key words: ethylic alcohol, fracture, bone regeneration.

RESUMO. Indução à ingestão de álcool etílico retarda o processo de regeneração pós-fratura em ratos. O consumo excessivo de bebidas alcoólicas provoca alterações metabólicas e patológicas em diferentes sistemas orgânicos. No tecido ósseo é sabido que o álcool provoca osteoporose, inibe a síntese de tecido ósseo e altera o metabolismo das células ósseas. O presente estudo teve por objetivo verificar a influência da ingestão alcoólica no processo de consolidação óssea pós-fratura de ratos. Para tanto, foram utilizados 12 ratos (*Rattus norvegicus*), da linhagem wistar e variedade albino, distribuídos em grupo A (controle) que foram tratados com ração comercial e água, e grupo B (experimental), que receberam tratamento com ração comercial e água ardente. Vinte e cinco dias pós-fratura, os ratos foram sacrificados e submetidos a tratamento de rotina para microscopia de luz. Quanto aos resultados, notou-se que o calo ósseo está presente tanto nos animais do grupo A quanto nos animais do grupo B. Na região de regeneração, verificou-se que os animais do grupo A apresentaram uma menor densidade de condrócitos e uma maior quantidade de tecido ósseo neo-formado, enquanto os animais do grupo B uma maior densidade de células cartilaginosas, demonstrando um retardamento no processo de formação do tecido ósseo. De acordo com os resultados obtidos, pode-se inferir que o álcool etílico influencia na regeneração de fraturas, retardando este processo.

Palavras-chave: álcool etílico, fratura, regeneração óssea.

Introduction

The intake of big amounts of etilic alcohol makes metabolic and pathological alterations in the most variated systems in the human organism. It alters the nervous system, provoking periferic neuropathies and alterations in the axon, glucose metabolism, lipids and proteins, and particularly, alters the nutritional aspects of organs such as: pancreas, stomach and intestine (Korsten and Lieber, 1979; Hirata and Hirata, 1991), early degeneration and atrophy of structures of the muscle esqueletal system (Torrejais *et al*., 2002*;* Pereira and Conegero, 2004).

Due to the effects of the etilic alcohol intake in human organism, several studies and experiments report about the alterations that the etilic alcohol provokes in the osseous tissue (Compston, 1992). The alcohol is considered an important risk factor to osseous fractures, osteopenia and osteoporosis (Laitinen and Valimaki, 1991; Chavassieux *et al.,* 1993; Garcia-Sanchez *et al.,* 1995; Dyer *et al.,* 1998; Molina-Perez *et al.*, 2000; Balzan *et al.,* 2001).

Klein (1997) describes the alcoholism effects over the osseous tissue, calling it osseous disease the provoked effects, which lead to a decrease in the osseous mass, where the mechanism of cellular control and osseous formation are significantly altered.

Huo *et al.* (1991) and Bikle (1993) state that the alcoholism predicts the subject to fractures due to the decreasing of the osseous density mainly in areas where there is an elevated percentage of trabecular bone as in femoral head and the distal part of the radio.

Lorenz and Lorenz (1984), radiologically analyzing the fracture healing in 40 alcoholic rats and 5 non - alcoholic, observed a delay in the development of the osseous callus and considered necessary additional experiments to prove the obtained results.

Friday and Howard (1991) report on about the direct effects of ethanol over the proliferation of human osseous cells in vitro. It was found that the protein synthesis, the fosfatase alkaline activity and the cellular proliferation diminished after ethanol treatment. These finds agree with the results obtained by Grilly and Anderson (1988) and Diamont *et al.* (1989), which evidenced through histomorphomeric analysis a reduction in osseous formation with deficit in number of osteoblast per osseous surface in alcohol addicted patients.

In view of the issues presented, and considering the lack of specific investigations concerning the action of alcohol on the process of fracture regeneration, the present study has the purpose of analyzing the influence of ethyl alcohol ingestion on the process of post-fracture bone regeneration in rats.

Material and methods

Twelve laboratory animals were used, *Rattus norvegicus* of the albinus variety and Wistar strain, supplied by the Central Biotery from the State University of Maringá, State of Paraná. The animals were maintained in room with controlled temperature (22 ± 2ºC), air renewal, chow (Nuvilab^{TM}) *ad libitum,* cycle of 12h light/12h dark and were divided in two groups:

- Group A (control group), composed of six male rats, which were kept in two cages with 3 animals each.
- Group B (experimental group), composed of six male rats, which were kept in two cages with 3 animals each.

To group A, it was offered Nuvilab commercial chow (recommended by the National Research Council and National Institute of Health - USA) with normal protein level (22%) and drinking water, both *ad libitum*. Water was supplied in specific drinking bottles. For group B, it was offered Nuvilab commercial chow with normal protein level (22%) *ad libitum*, but the water was replaced by sugar-cane brandy (commercial mark "51", 39º GL, Muller Industries, Pirassununga, State of Sao Paulo, Brazil). At the age of 21 days, after weaning, the rats had a period of adaptation to sugar-cane brandy in increasing concentrations: 10º for 10 days, 15º for 11 days, 20º for 12 days e 25º for 12 days and diluted to 30º from the 66th day. The alcoholism model adopted was the "semi-voluntary" one, in which sugar-cane brandy was the only source of liquid available to the animal. This model was utilized by Pereira *et al.* (2002) in his studies about the ultra-structure in epithelial cells in the ependimal duct, Martinez *et al.* (1993) and Cagnon *et al.* (1996), in their studies about accessory glands in the male reproductive tract, and Pereira and Conegero (2004), on the muscle-tendineous interface, who submitted the rats to the same concentration and evidenced significant structural alterations in animals of the experimental group.

When they completed 100 days of age, each rat was anesthetized i.p. with thiopental (40% mg kg^{-1} body weight). The humerus of the right anterior limb was fractured; next, the limb was immobilized with adhesive band next to the lateral aspect of the thorax, directed to the head, as described by (Bohler, 1954; Campbell, 1966).

Twenty-five days after the fracture, the rats were immobilized, anesthetized through intraperitoneal injection of pentobarbital sodium (Hypnol - 40% mg kg^{-1} of body weight), where the humerus of the right posterior limb was removed. To follow, the animals were sacrificed with overdoses through an intraperitoneal injection of pentobarbital sodium.

After fixation, the bone material was reduced to small blocks and subjected to a decalcifying solution, dehydrated in ascending series of alcohol, diaphanized in xylene and included in paraffin for histological sections of 5 μm-thickness. These sections were stained with Hematoxylin-Eosin.

After that, the material was analyzed in an Olympus microscope, and the selected laminae were documented in an Olympus BX-40 photomicroscope. The images were measured employing the software Image Pro Plus 4.5 (Media Cyber Technique). A BX-40 microscope coupled to a digital camera was used to measure the area of the region of fracture regeneration (in mm^2) in the animals from the control and experimental groups, according to Figure 1.

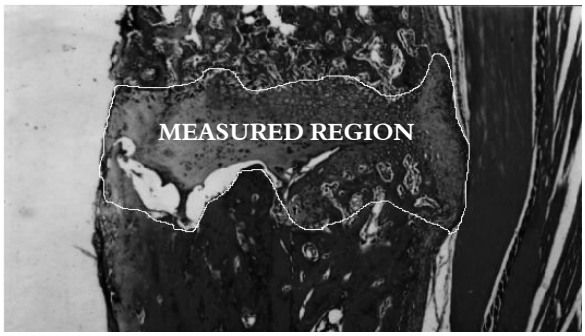

Figure 1. Longitudinal section evidencing the measured region of fracture.

The analyses of the data were made through descriptive statistics, where the results were verified through Student's T test with a significance level between samples of $p<0.01$.

Results

According to the methodology employed, it was observed that the bone callus was present both in the control and in the experimental animals, as demonstrated in Figures 2 and 3.

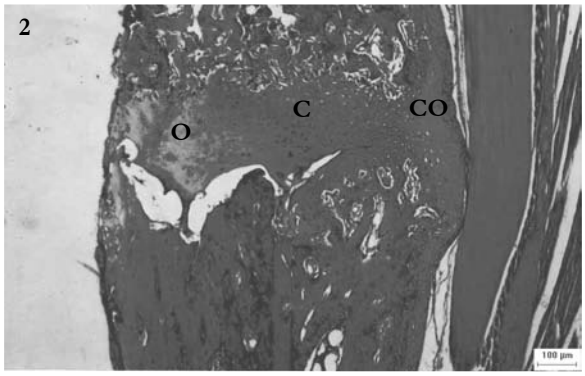

Figure 2. Longitudinal section in the region of fracture regeneration evidencing the bone callus (CO), cartilage cells (C), and newly-formed bone tissue (O). Control animal, HE, Bar: 100 μ.

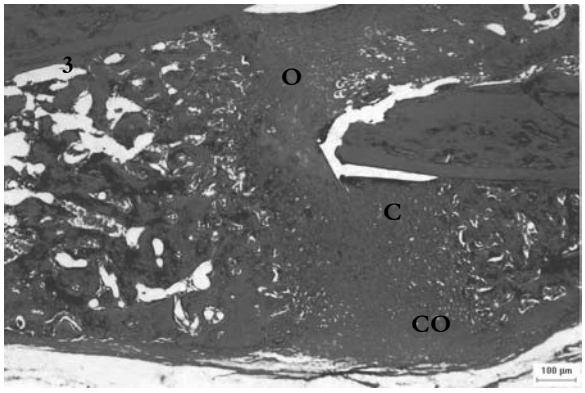

Figure 3. Longitudinal section in the region of fracture regeneration evidencing the bone callus (CO), cartilage cells (C), and newly-formed bone tissue (O). Experimental animal, HE, Bar: 100 μ.

In the region of fracture regeneration, it was verified that in the animals of group A presented a smaller density of chondrocytes and thus a greater amount of newly-formed bone tissue (Figure 4), while the animals of group B showed a larger density of cartilage cells (Figure 5).

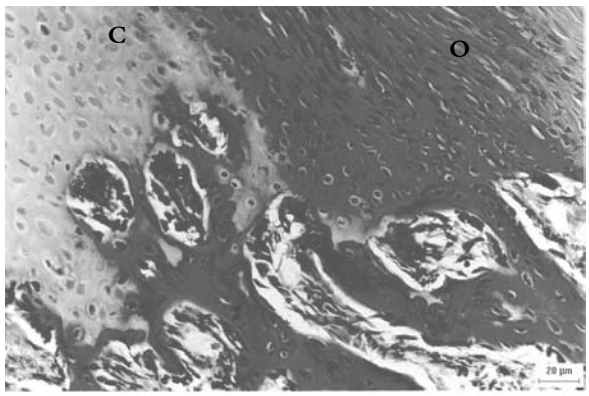

Figure 4. Longitudinal section in the region of fracture regeneration evidencing cartilage cells (C), and newly-formed bone tissue (O). Control animal, HE, Bar: 20 μ.

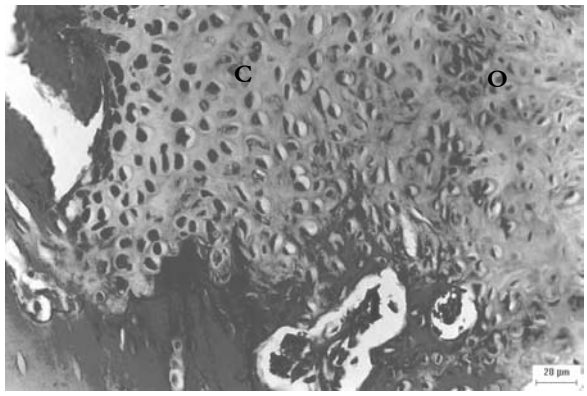

Figure 5. Longitudinal section in the region of fracture regeneration evidencing cartilage cells (C), and newly-formed bone tissue (O). Experimental animal, HE, Bar: 20 μ.

On the other hand, in the animals of group A, it was observed newly-formed bone tissue at full length with some cartilage cells (Figure 6), while in the animals of group B, it was observed only cartilage cells at full length (Figure 7), demonstrating a delay in the process of formation of bone tissue.

In the regeneration region of both the control and experimental groups, it was verified that in the control group the mean was of 2.43 $mm^2 \pm 0.16$ mm^2 (standard deviation, SD), while in the experimental group the mean was 3.00 $mm^2 \pm 0.19$ mm^2 (SD). A significance level of $p=0.0002$ was obtained, as presented in Table 1.

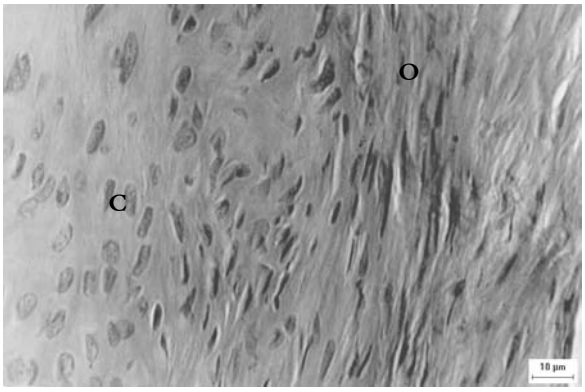

Figure 6. Longitudinal section in the region of fracture regeneration evidencing cartilage cells (C), and newly-formed bone tissue (O). Control animal, HE, Bar: 10 μ.

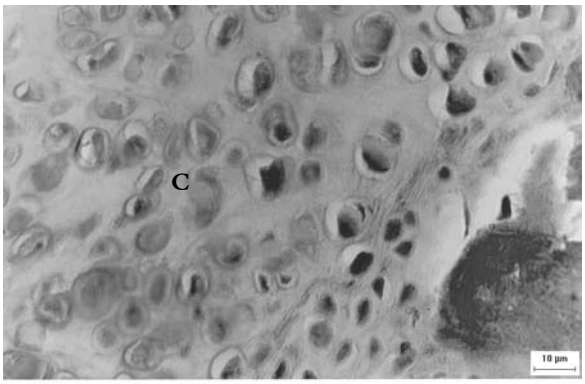

Figure 7. Longitudinal section in the region of fracture regeneration evidencing cartilage cells (C). Experimental animal, HE, Bar: 10 μ.

Table 1. demonstrated mean and standard deviation of regenerated area of the humerus of rats in the control and experimental groups.

Descriptive statistics	Control group	Experimental group
Mean	2.43 mm^2	3.00 mm^2
Standard deviation	0.16	0.19
Maximum	2.73 mm^2	3.15 mm^2
Minimum	2.3 mm^2	2.64 mm^2
Amplitude	0.43	0.51
$p=$	0.0002	

Discussion

This work had the purpose of studying the fracture regeneration in bones of rats subjected to 30° ethyl alcohol ingestion. This concentration was adopted by other authors (Martinez *et al.*, 1993; Cagnon *et al.*, 1996; Pereira *et al.,* 2002; Pereira and Conegero, 2004), who studied the effects of alcoholism in other animal systems.

The influence of alcoholism in the different systems was also described by other authors (Grilly and Anderson, 1988; Dyer *et al.,* 1998). This investigation verified that the effects of alcoholism on the bone tissue are related to several osteopathologies, as described by other authors (Baran *et al.,* 1980; Lorenz and Lorenz, 1984; Crilly *et al.,* 1988*;* Lieber, 1988; Friday e Howard, 1991; Compston, 1992; Felson, 1995; Garcia-Sanchez *et al.*, 1995; Klein, 1997), also leading to an increased propensity to fractures, as stated by other authors (Erhart, 1962; Johnell *et al.,* 1982; Diamont *et al.,* 1989*;* Fortes and Cardo, 1991; Levy, 1991; Bikle, 1993; Chavassieux, 1993; Balzan, 2001). However, they make no mention about the effects of alcoholism on the process of fracture regeneration.

Peris *et al.* (1995) studied vertebral fractures in 75 chronic alcoholic men, but did not mention the regeneration process, and limited to report the influence of alcohol in the process that triggered the osteoporosis which would make these patients more prone to fractures. In this study, in contrast with the authors mentioned above, we carried out the analysis of alcoholism in the process of fracture regeneration, and we could figure out that the data of those authors sustain these findings, once they represent collateral effects that have an indirect action on fracture regeneration.

When the region of the fracture regeneration was analyzed, it was verified to be larger in the experiment group than in the control group, demonstrating the influence of alcoholism in the process of fracture repair. This results meet the findings of Dyer *et al.* (1989) and Balzan *et al*. (2001), who found a diminished osteoblastic activity and a greater activation of osteoclasts due to alcoholism.

The smaller area found in the animals of the control group is due to the normal osteoblastic activity, where the chondrocytes are replaced by bone tissue, an event that depends on the osteoblasts. In the experimental group, it was verified a significantly larger area, including a greater concentration of chondrocytes, which lead to infer that the osteoblastic activity is impaired by the ingested alcohol, and consequently, the process of fracture repair is delayed. The decreased activity of the osteoblasts as a consequence of alcoholism was also described by other authors (Johnell *et al.,* 1982; Klein, 1997 and Dyer *et al.,* 1998).

When analyzing the statistical data concerning the regeneration region of animals from the same group, we verified that in the control group the mean was 2.43 $mm^2 \pm 0.16$ mm^2 (standard deviation, SD), while in the experimental group the mean was 3.00 $mm^2 \pm 0.19$ mm^2 (SD), confirming that ethyl alcohol significantly affects the size of the region of fracture regeneration. When the T test was applied, a significance level of $p=0.0002$ was obtained, corresponding to a highly significant result, once the

significance level adopted was of $p<0.01$.

According to the results obtained, we can add, in addition to the effects mentioned above, that alcohol influences the fracture regeneration, delaying this process.

Conclusion

According to the employed methodology and the obtained results, we can infer that:

- the osseous callus is presented not only in animals from the control group but also in the experimental group;

- there was a statistically significant difference, meaning that the regeneration area of the experimental group showed itself bigger than in the control group, demonstrating that the alcohol intake delayed the osseous regeneration process;

- in animals from the experimental group there was a higher contingent of cartilaginous cells, and in animals from the control group a diminished contingent of these elements was observed, characterizing a higher speed in the healing process of fractures in animals from the control group.

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Received on September 01, 2006.
Accepted on March 21, 2007.