Ultra-structural alterations of the palatal mucosa in rats subject to a diet of alcohol

Alterações ultraestruturais da mucosa palatina de ratos submetidos a dieta alcoólica

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ABSTRACT

Objective
To evaluate the possible morphological alterations of the palatal mucosa in rats subject to a diet of alcohol.

Methods
Twelve adult male animals were used, divided into two groups: control and treated. The control group received food and water ad libitum, while the treated group received the same solid diet and a solution of water and ethanol diluted to 25%. After 160 days, the animals were sacrificed and the samples of palatal mucosa were submitted to methods of transmission electronic microscopy.

Results
In the macroscopic results, a coloring alteration was seen in the palatal mucosa in the alcoholic group. In addition, the control animals presented a greater gain in body mass in relation to the treated animals ($p<0.05$). However, the treated animals did not lose weight during the experiment; on the contrary, they gained body mass, despite gaining less when compared to the control. In the ultra-structure of the treated group, an increase in inter-cellular space, fusion of the secretory granules and the presence of cells in a degenerative state were observed.

Conclusion
It may be deduced that there were serious morphological alterations in the palatal mucosa of rats subject to an alcoholic diet.

***Indexing terms**: Alcoholism. Anatomy & histology. Mouth mucosa.*

RESUMO

Objetivo
Avaliar as possíveis alterações morfológicas da mucosa palatina de ratos submetidos a uma dieta alcoólica.

Métodos
Foram utilizados doze animais adultos, machos, divididos em dois grupos sendo um controle e outro tratado. O grupo controle recebeu ração e água ad libitum, enquanto o grupo tratado recebeu a mesma dieta sólida e uma solução de água e etanol diluído a 25%. Após 160 dias, os animais foram sacrificados e as amostras da mucosa palatina foram submetidas aos métodos de microscopia eletrônica de transmissão.

Resultados
Nos resultados macroscópicos, notou alteração da coloração da mucosa palatina do grupo alcoolizado. Além disso, os animais controle apresentaram maior ganho de massa corporal em relação aos animais tratados (p<0,05). Todavia, os animais tratados não perderam peso durante o período de experimento, ao contrário, os mesmos ganharam massa corporal, apesar de menor quando comparado com o controle. Na ultra-estrutura do grupo tratado, notou aumento dos espaços intercelulares, fusão dos grânulos secretores e presença de células em processo degenerativo.

Conclusão
Pode inferir que houve graves alterações morfológicas na mucosa palatina de ratos submetidos à dieta alcoólica.

Termos de indexação: Alcoolismo. Anatomia & histologia. Mucosa bucal.

INTRODUCTION

According to the World Health Organization, alcoholism is considered to be a serious worldwide public health problem as changes are observed in the socio-behavioral and physical relationships of individuals affected by alcohol dependence. Given these aggravating circumstances, several experimental studies have demonstrated the aggressive effect of a diet of alcohol on the oral mucous[1-12]. In recent decades, the relationship

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between alcoholism and oral cancer has been described in a number of experiments, however, the cancer-inducing mechanism still represents a paradigm that needs to be better explained[13]. Amongst the explanations for this pathogenesis is the cytotoxic and mitogenic effect of acetaldehyde, a metabolic product of alcohol which can result in atrophy of the oral epithelium, thereby increasing the vulnerability of the mucosa to physical-chemical attacks[14]. Other factors associated with the appearance of oral cancer include the action of alcohol in facilitating the passage of carcinogens via the cell membrane of the oral mucosa and the effect of alcohol on the heightened metabolic activity of the liver which could thereby activate carcinogenic substances[15-16].

Despite the numerous studies found in the literature concerning the effect of alcoholism on the health of the oral cavity, it is still important to produce new studies for a better understanding of the toxicity of alcoholism in the palatal mucosa. In view of this, the aim of the present study was to check for possible structural changes in the mucosa of the soft palate in Wistar rats subjected to a diet of alcohol.

METHODS

A total of twelve adult male rats (Rattus norvegicus), aged 12 weeks, from the vivarium of the State University of Campinas (Campinas, São Paulo) and the Jundiaí Faculty of Medicine (Jundiaí, São Paulo) were used. The animals were divided into two experimental groups, the control group (C) and the treated group (TG). The control group (C) received food and water ad libitum, while the treated group (TG) received the same solid diet and a solution of water and ethanol diluted to 25%.Throughout the experiment, the quantification of water and food consumption and the gain in body mass was performed, standardizing 70, 100 and 160 days as the references for evaluating the evolution of these parameters. The values were analyzed using the statistical Student t-test ($p \leq 0.05$).

160 days after the experiment began, the animals were sacrificed via a subperitoneal injection of chloral hydrate (0.3ml/100g). The samples were evaluated macroscopically and photo documented using a Nikon digital camera and then subjected to the procedure of transmission electron microscopy. For the analysis of the ultra-structure, a PHILIPS EM Transmission Electron Microscope, belonging to the Institute of Biology's Electron Microscopy Laboratory at the State University of Campinas (UNICAMP), was used.

We confirm that the present study was developed in compliance with ethical principles and was approved by the Ethics in Research Committee at the Jundiaí Faculty of Medicine (FMJ), under file reference no. 97/2010.

RESULTS

Macroscopy

The palatal mucosa of the animals in the control group (C) was highly vascularized, having a reddish appearance, different from the alcoholized animals (TG) which had a chalky color. It is important to emphasize that pathological changes, such as ulcerations and nodules, were not checked (Figures 1A and 1B).

Figure 1. Palatal mucosa of the control group and treated group, respectively. Note the normal reddish color of the soft palate (Pm) in the control group, different from the treated group. 5x.

Variance in the liquid and solid diet (animal food)

The control group consumed a greater amount of liquid and food than the treated group (Figures 2 and 3).

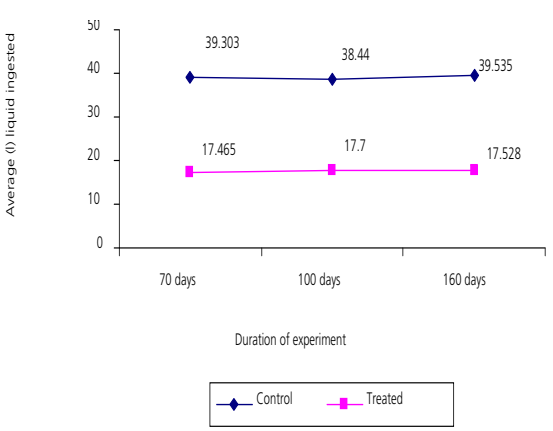

Figure 2. Variance in consumption of liquid.

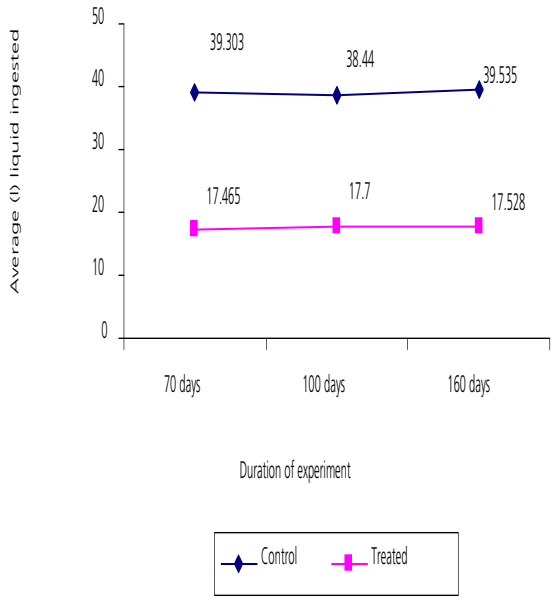

Figure 3. Variance in consumption of solids.

Variance in gain in body mass

The animals in the control group had a larger gain in body mass than the treated animals ($p<0.05$). However the treated animals did not lose any weight during the period of the experiment (Figure 4).

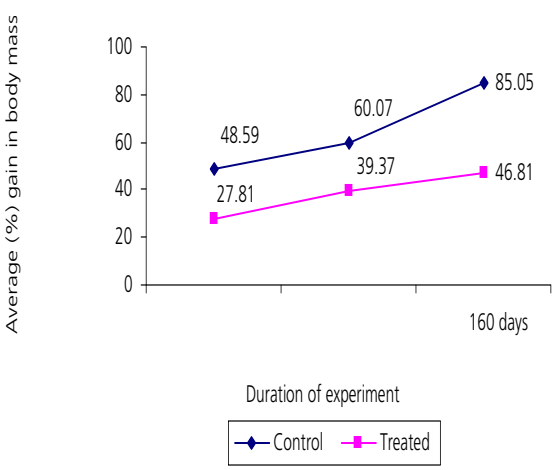

Figure 4. Variance in gain in body mass.

Transmission Electron Microscopy

Control group

In the control group (C), a nucleus of epithelial cells was observed with normal contours and homogeneous chromatin condensed around the edges. The intercellular spaces and the basal membrane remained within normal with defined contours. Also observed were several desmosomes, secretory vacuoles and clear organelles such as granular endoplasmic reticulum, Golgi complex and mitochondria (Figure 5).

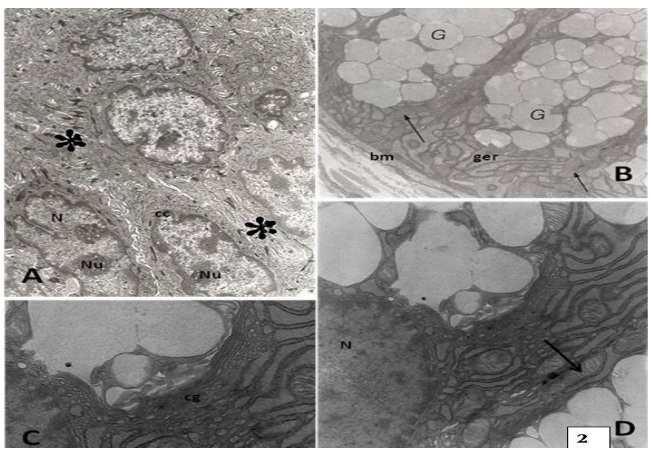

Figure 5. Electron micrograph of the epithelium of the palatal mucosa in the control group. A) nuclei (N) and nucleoli (Nu) of the epithelial cells with defined contours and condensed chromatin around the edges (cc), normal intercellular space with desmosomes(*) between the epithelial cells. B) basal membrane intact (bm), normal granular endoplasmic reticulum (ger) and secretory vacuoles (G) of low electron density. C) Well developed cisternae of the Golgi complex (cg). D) intact mitochondria with clear crests (→). Nucleus (N).

Treated group

In the TG group, an increase was observed in the space between the epithelial cells, the nucleoli were difficult to locate, there were monocytes between the epithelial cells, a smaller number of secretory granules of different sizes, fusion between granules, an increase in the space between the glandular cells, mitochondria concentrated in the basal region, myoepithelial cells with abnormal contours, lipid inclusions and neutrophils close to the glandular acini. A process of cell degeneration was also witnessed (Figure 6).

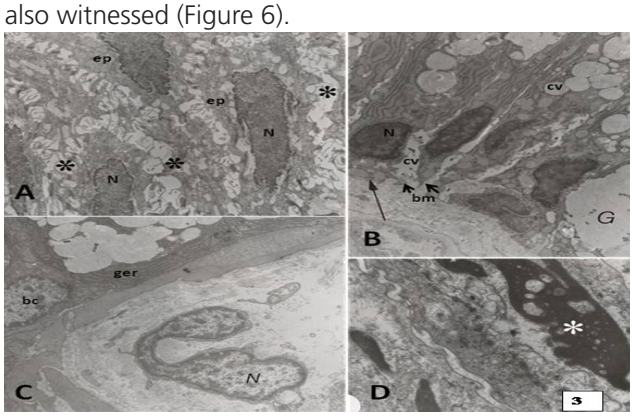

Figure 6. Electron micrograph of the epithelium of the palatal mucosa in the treated group. A) epithelial cells (ep) separated by ample spaces (*) and uncharacteristic desmosomes, nuclei (N) with pleated sheet. B) Projection of the basal membrane between the epithelial cells (bm and arrowhead), cytoplasm vacuolization (cv), nuclei with fully condensed chromatins (N), reduction in quality of secretory vacuoles (sv), mitochondria concentration and fusion of the secretory granules (G). C) Atrophy of the cisternae of the granular endoplasmic reticulum (ger), amorphous nucleus with condensed chromatin (N), basal cell (bc). D) Cell degeneration with nuclear condensation (*).

DISCUSSION

Issues related to certain human diseases can be positively studied in laboratory animals, including those diseases stemming from alcohol abuse, such as pathological changes in the liver, pancreas, muscles and oral mucosa.

Several types of alcoholic beverage have been studied for their respective harmful effects on the oral mucosa. Whisky has been described as a drink which carries a high risk of predisposition to oral cancer, which is more accentuated when compared to beer or wine[17]. Kabat & Wynder[18] concluded that the main carcinogenic agent in alcoholic beverages is the ethanol. Ethanol was used in this study on account of the following characteristics: it is the principal constituent of many alcoholic beverages; it is absorbed more quickly by the gastroduodenal mucosa; acetaldehyde is a metabolic product of ethanol and has mutagenic properties and, lastly, it is characteristically a more popular drink, unlike whisky which is practically exclusive to the upper socio-economic classes.

Some authors have suggested that the quantity of alcohol and the length of exposure appear to be the drivers of the appearance of neoplasias and not the type of alcoholic beverage[19]. In this study, ethanol diluted to 25% was used, the Wistar rats being exposed to treatment for 160 days, sufficient time to provoke changes in the mucosa of the soft palate, as these parameters were close to those found in the literature. Zorzetto[20] found cellular changes in the cheeks of rats subjected to an intake of alcohol for a period of 240 days of treatment. On the other hand, Martinez et al.[21] reported significant ultra-structural changes in the epithelial cells of the hard palate mucosa of rats subjected to chronic intake of 30% alcohol for 90, 180 and 270 days of treatment.

An adequate nutritional diet is necessary for the animals to grow normally and stay healthy. The variation in the level of concentrations of nutrients such as vitamins, minerals and protein from the food could modify biological response in many experiments. Water is also a crucial nutrient and to limit it could have disastrous effects on the rat over the course of its life[22]. It is therefore essential to monitor the state of health of each animal during the studies on alcoholism, as poor nutrition contributes to the development of pathologies[23]. In our study, there was a weekly check on the quantity of daily consumption of food and liquids by the animals in the control group and treated (alcoholized) group.

The results demonstrated that the treated rats consumed a smaller quantity of liquid and food than those in the control group, however during the entire experiment, the treated animals maintained a constant consumption of liquids and food, i.e. there was no reduction in the quantity of liquid and food consumed over the course of the experiment. The daily average consumption of liquid by the control group animals throughout the experiment varied between 39.3 ml and 39.53 ml, while in the treated group it varied between 18.16 ml and 17.7 ml, i.e. approximately 2.5 times lower than the control group. Food consumption varied between 22.47 and 22.94 g in the control group and 13.79 and 15.29g in the treated group, being approximately 1.5 times lower than consumption in the control group.

Martinez[24] studied the effect of alcohol on the endometrium of the Wistar rats and also found that alcoholized rats consumed smaller quantities of liquids and food than those in the control group, however this outcome did not demonstrate that the alcoholized animals entered a state of malnutrition.

It is hard to specifically establish the ideal quantity of food and liquid consumption by the animal due to variations to which they are subjected vis-à-vis nutritional and physiological factors resulting from the consumption of alcohol[23]. Svendsen & Hau[25] suggested that normal rats, in the growth phase, would consume between 8 and 25 grams of food a day and have a daily intake of water of between 5 and 80 ml. In the present study, young rats were used and it was found that the quantity of food and liquid consumed by the treated animals is within the established patterns for a normal diet.

The influence of poor nutrition on pathological changes induced by chronic alcoholism is contradictory. Many studies are inconclusive because they have been performed on chronic alcoholics who became malnourished as a consequence of alcoholism and any changes are accordingly due to malnutrition and not the direct effect of alcoholism[26].

One of the symptoms of the combination of alcoholism and malnutrition is weight loss26. Campana et al.[27] also stated that malnutrition in rodents is characterized by reduced body weight and they point out other symptoms such as change in behavior, hair loss and diarrhea. As far as weight loss is concerned, it was proved through statistical analysis that the rats in the control group gained significantly more body mass than the treated animals The animals in the control group, within a period of 70, 100 and 160 days of the experiment, gained 48.59%, 60.07% and 85.05% body mass, respectively, while the animals in the treated group gained 27.81%, 39.37% and 46.81%. In contrast to these results, Martinez[24] found that alcoholized female rats showed the same variance in body weight as those female rats not subjected to consumption of alcohol. On the other hand, Sampson et al.[28] found that young rats that ingested 35%

alcohol suffered a delay in gaining body mass. Martinez[21] observed a lower daily increase in body mass in the rats treated with 30% sugarcane rum than in rats in the control group. Despite the animals in this study having gained less weight than those in the control group, this does not explain why they entered a process of malnutrition; on the contrary, the treated animals gained weight in spite of being a lower gain than in the control group.

As for the macroscopic anatomy, changes were observed in the coloring of the soft palate, appearing reddish in the control group animals and chalky in the alcoholized animal. Esquep et al.[29] witnessed pigmented lesions in the palate of alcoholic patients. Muller et al.[30] described the presence of ulcerations in the oral mucosa of rabbits 48 hours after they ingested alcohol diluted to 40%. On the other hand, Zorzetto[20] found no macroscopic changes such as ulcerations and change in the pigmentation in the oral mucosa of alcoholized rats. Similarly, Martinez21 studied the effect of sugarcane rum diluted to 30% on the hard palate mucosa of rats and did not observe any macroscopic differences between the rats of the control group and those that were alcoholized. The present study did not come across any ulcerative lesions or erosions in the rats subjected to chronic alcoholism.

With transmission electron microscopy, an increase was noted, in the treated group, of intercellular space between the basal epithelial cells, a result which is similar to that of Muller et al.[30], in which it was found that the epithelial cells of the oral mucosa in rabbits lost mutual contact. Zorzeto[20] observed pyknotic nuclei with abnormal contours and reentrances and also lipid droplets in the oral mucosa of alcoholized rats. The changes that occurred in the glandular layer of the alcoholic animals comprised the apparent reduction in secretory granules, different sizes of granules, fusion between these granules as well as having hard-to-identify contours, increase in the intercellular spaces, lipid inclusions, mitochondria concentrated in the basal region and abnormalities in the contour of the myoepithelial cells.

CONCLUSION

The treated (alcoholized) animals were not malnourished and suffered serious alterations to the ultra-structure of the palatal mucosa suggesting cell apoptosis.

Collaborators

ET PALOMARI participated in the preparation of the research planning, the data evaluation methodology, as well as in the analysis of the macroscopic and ultra-structural results and the composition of the article. CAF CARVALHO was responsible for the ultrastructural photographs as well as their analysis and the composition of the article. MR CUNHA was responsible for the review of the bibliography, the data analyses and the description of the results, discussion and the composition of the article.

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