



IGFR-I expression and structural analysis of the hard palatine mucosa in an ethanol-drinking rat strain (UChA and UChB)

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ABSTRACT

The study analyzed the effects of chronic alcohol ingestion on the ultrastructure of the lining epithelium of the hard palatine mucosa of rats UChA and UChB (lines with voluntary alcohol consumption) in order to contribute to the understanding of the consequences of alcohol abuse for the morphology of the digestive system. Thirty female adult animals aged 120 days were divided into three experimental groups. (1) Ten UChA rats (genetically low ethanol consumer) with voluntary intake of 10% v/v (5.45 g/kg/day) ethanol solution and water. (2) Ten UChB (genetically high ethanol consumer) rats with voluntary intake of 10% v/v (7.16 g/kg/day) ethanol solution and water. (3) Ten Wistar rats with voluntary ad libitum water intake (control group). Both groups received Nuvital pellets ad libitum. The IGFR-I expression was intense in both experimental groups. The epithelial cells of the alcoholic rats UChA and UChB showed many alterations such as the presence of lipid droplets, altered nuclei, nuclei in corneum layer and disrupted mitochondria. It was concluded that ethanol intake induces ultrastructural lesions in the hard palatine mucosa.

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1. Introduction

Epidemiological studies have been demonstrated that oral mucosa may be affected by several oncogenesis disorders. Alcohol, tobacco, diabetes, dysregulation of oncogenes and tumor suppressor genes and mitochondrial mutations implicated in oral squamous cell carcinoma development (Vairaktaris et al., 2007; Bloching et al., 2008; Nagini, 2009). According with Burzlaff et al. (2007) the exposure to alcohol or tobacco affects the pattern of maturation in oral mucosal cells. Susceptibility to carcinogens and cell proliferation in the mucosa are increased with alcohol ingestion, resulting in genetic changes with the development of dysplasia, leukoplacia and carcinoma (Riedel et al., 2005; López-Blanc et al., 2009). Non-keratinized mucosae are prevalent to develop but keratinized mucosa also must be mentioned. Preferred sites in the oral mucosa for melanomas are hard palate and maxillary alveolus (Magremanne and Vervaet, 2008; Lourenço et al., 2010). Squamous cell carcinoma can be very aggressive (Morris et al., 2010).

UChA and UChB rat lines with voluntary alcohol consumption derived from original Wistar colony selected at the University of Chile (UCh) for about 70 generations (Quintanilla et al., 2007). These strains constitute rare models for studying the relationship among the genetic, biochemical, physiologic, nutritional and pharmacological factors from the effects of alcohol, with appetite and tolerance, which are important factors in human alcoholism (Pinheiro et al., 2007). The insulin-like growth factors (IGFs) are a family of mitogenic proteins involved in the regulation of cell growth and differentiation. The presence and role of the IGF system in oral mucosa is not clear but could influence the pathogenesis of oral cancer (Brady et al., 2007).

The objective of the present study was to determine the possible effects of chronic alcohol ingestion on the expression of IGFR-I and structure of the hard palate epithelium of UCh rats in order to contribute to the understanding of the consequences of alcohol abuse for the oral morphology.

2. Materials and methods

2.1. Animal's selection and ethanol consumption

Thirty adult female rats aged 120 days and weighing on average 380 g were used. Rats were housed individually under controlled

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temperature (22–28 °C) and day/night cycle (12 h/12 h) in a controlled room. All animals received Nuvital pellets ad libitum. The experimental protocol followed the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation. The animals were divided into three groups: (1) Ten UChA rats (genetically low ethanol consumer) with voluntary intake of 10% v/v (5.45 g/kg/day) ethanol solution and water. (2) Ten UChB (genetically high ethanol consumer) rats with voluntary intake of 10% v/v (7.16 g/kg/day) ethanol solution and water. (3)

Ten Wistar rats with voluntary ad libitum water intake (control group). From 21 days up to 59 days of age, the female rats of the UChA, UChB and Wistar strains received distilled water and food ad libitum. The female rats of the UChA and UChB strains had free access 10% (v/v) ethanol solution, distilled water and food from 60 days up to the 120 days of age, totalizing sixty days of chronic alcohol ingestion. Ethanol and water consumption were recorded every week. The selection and standardization of the UChA and UChB strains were performed according to [Mardones](#)

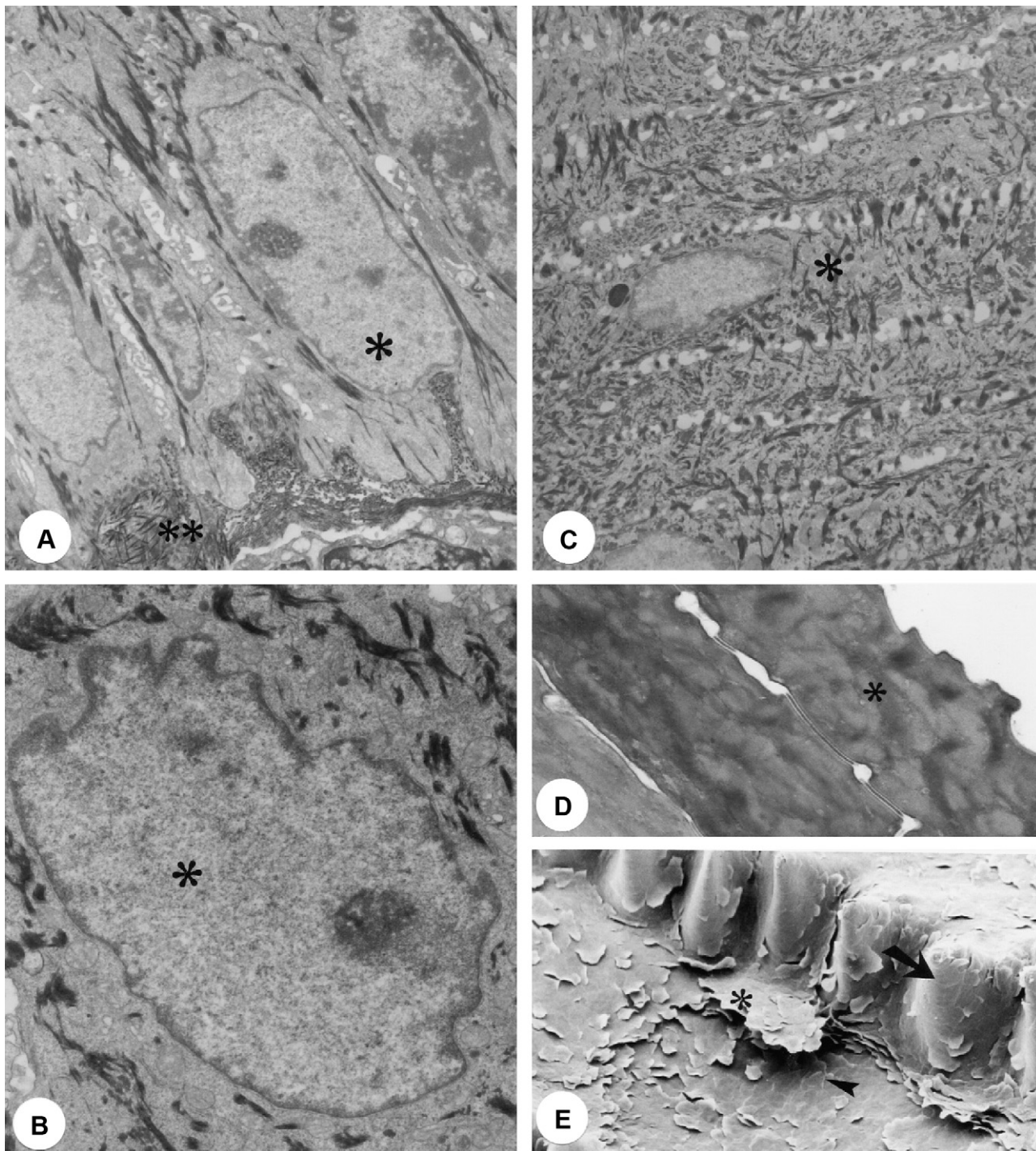


Fig. 1. TEM and SEM images from hard palatine mucosa of control rats. (A) Basal epithelial cells (*) and lamina propria (**). $\times 6500$. (B) Detail of spinosum cell with voluminous nuclei (*). $\times 13,000$. (C) Granulosum epithelial cells with elongated shape (*). $\times 6500$. (D) Cornueum epithelial cells with amorphous content (*). $\times 21,000$. (E) Transverse palatine plicae (arrow), intercellular limit (arrow head) and desquamating polygonal cells (*). $\times 80$.

and Segóvia-Riquelme (1983). For ethanol/acetaldehyde levels see Quintanilla et al. (2006, 2007). The rats of the Wistar strain received distilled water and food ad libitum until the 120 days of age.

2.2. Microscopy

For histological analysis, hard palatine mucosa from 5 animals in each group were fixed in Bouin's solution for 12 h, dehydrated in a graded series of ethanol and embedded in paraffin routinely. The sagittal sections of five micrometers thickness were stained with haematoxylin and eosin. For transmission electron microscopy (TEM), fragments of the hard palatine mucosa were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 3 h and then postfixed in 1% osmium tetroxide

in the same buffer for 2 h. The fragments were dehydrated in alcohol solutions and embedded in Araldite; ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Philips EM 301 transmission electron microscope. For Scanning electron microscopy (SEM), the specimens were fixed with Karnovsky solution for 2 h and postfixed with 1% osmium tetroxide for 2 h. They were immersed in 2% tannic acid for 2 h, dehydrated in graded ethanol, replaced with isoamyl acetate and dried at critical point with CO₂ (Balzers CPD-010). The specimens were coated with gold (Balzers MED-010) and examined in a Philips FEM 515 scanning electron microscope. For IGF-IR expression was used rabbit antibodies (Santa Cruz Biotechnology, CA, USA) according with manufactures details in 5 animals in each group. Five slides of each hard palatine mucosa with 4 slices per slide were stained.

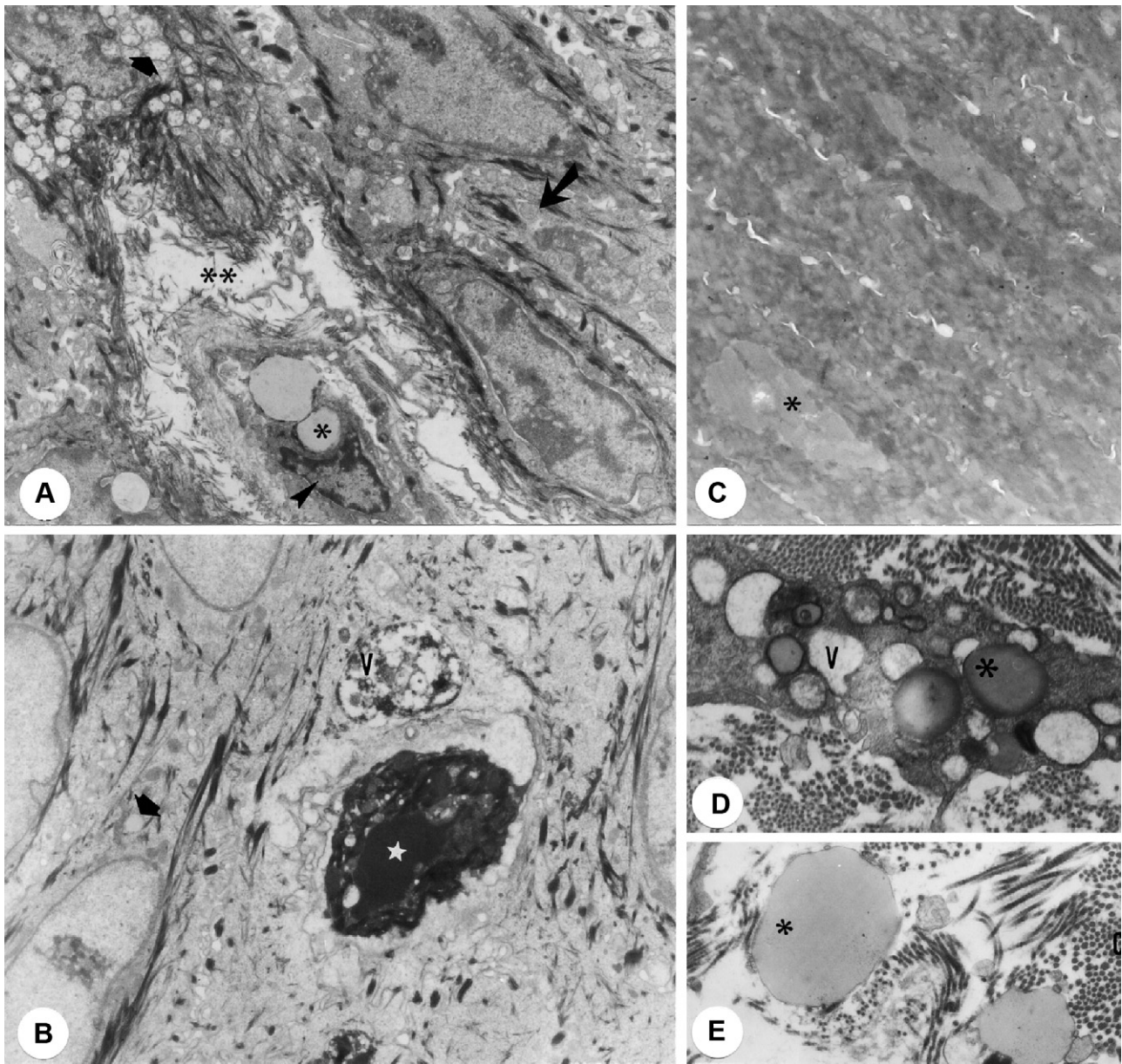


Fig. 2. TEM images of hard palatine mucosa of UChA rats. (A) General view of basal epithelial cells with altered mitochondria (small arrow), preserved mitochondria (big arrow). Note lamina propria with large spaces (**), fibroblast with irregular nuclei (arrow head) and large lipid droplet (*). $\times 5750$. (B) Basal epithelial cells. Note digestive vacuole (star), small lipid droplet (arrow) and large vacuolization (V). $\times 7750$. (C) Corneum epithelial cells with nuclei (*). $\times 7750$. (D) Fibroblast with several lipid droplets (*) and vacuolization (V). $\times 13,250$. (E) Magnification of lamina propria showing collagen fibers (C) and lipid droplet (*). $\times 13,250$.

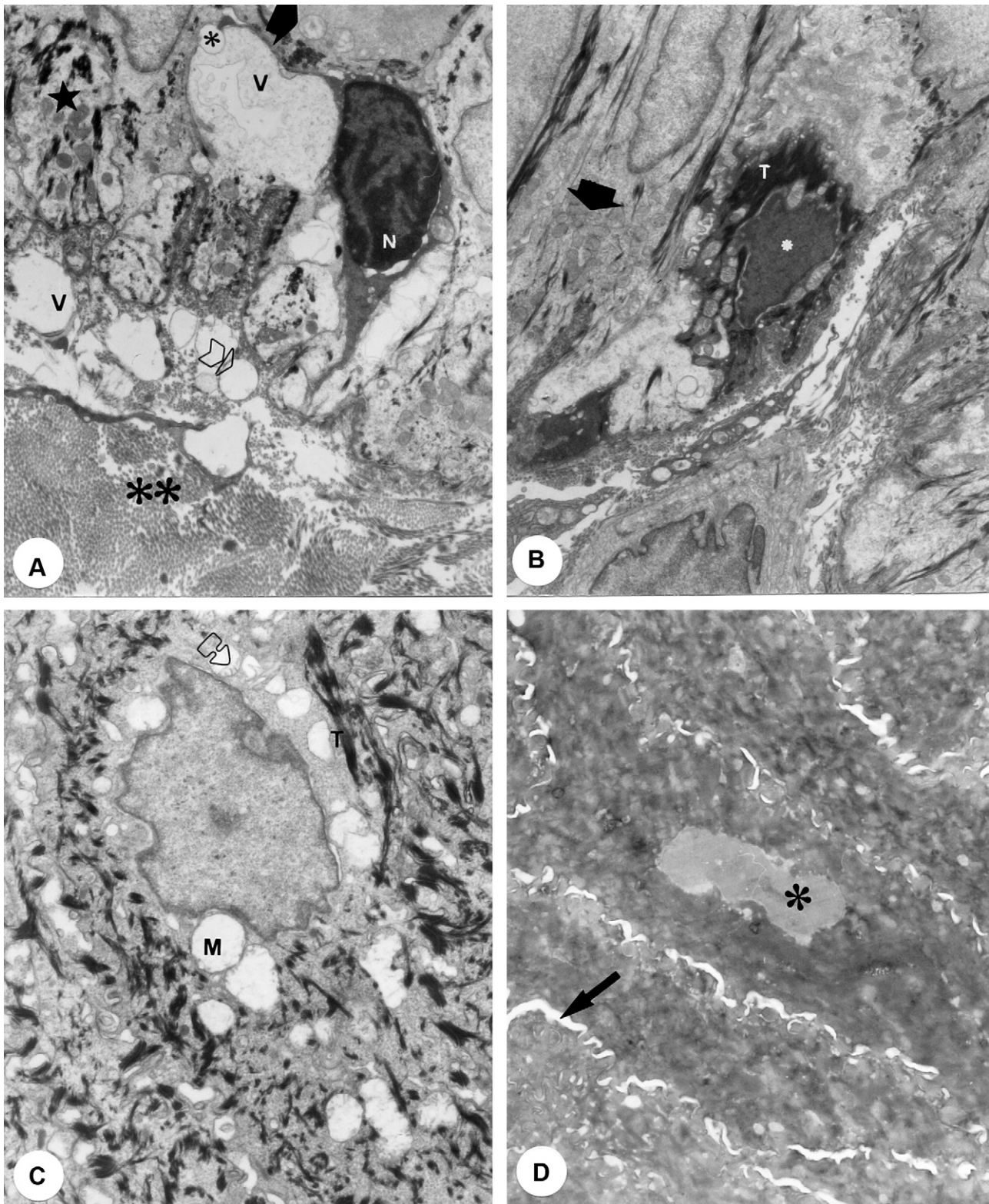


Fig. 3. TEM images of hard palatine mucosa of UChB rats. (A) Panoramic view of basal cells and lamina propria (**). Note altered nuclei (N), enormous vacuole (V), lipid droplet (*), altered mitochondria (arrows) and normal mitochondria (star). $\times 7750$. (B) Basal epithelial cell with altered nuclei (*), increased tonofilaments (T) and mitochondria (arrow). $\times 7750$. (C) Granulosa cell with altered mitochondria (M), tonofilaments (T) and mitochondria cristae (arrow). $\times 9750$. (D) Corneum cell showing nuclei (*) and intercellular spaces (arrow). $\times 7750$.

3. Results

3.1. Macroscopy and light microscopy

Macroscopic study did not reveal differences in the morphology of the hard palatine mucosa of control and UCh animals. No signs of ulcerations were detected on the alcoholic palatine mucosae. Both groups presented the hard palatine mucosa composed by keratinized squamous stratified epithelium and lamina propria.

3.2. Transmission electron microscopy

3.2.1. Control group

The keratinized squamous stratified epithelium of the hard palatine mucosa presented basal, spinosum, granulosum and corneum layers. Basal cells located predominantly vertically to the basal lamina showed columnar shape and voluminous basal nucleus with distinct nucleolus. Their cytoplasm cells contained granular endoplasmic reticulum, ribosomes, mitochondrias and filaments. Reduced intercellular spaces could be seen. The spinosum cells exhibited polygonal shapes and central oval nucleus with distinct nucleolus. Their cytoplasm showed ribosomes, desmosomes, mitochondria, granular endoplasmic reticulum and 10 nm filaments. The granular flattened cells with elongated central nuclei contained ribosomes, mitochondria, 10 nm filaments and keratohyalin granules. The corneum layer showed flattened cells with amorphous cytoplasm and absence of nuclei. SEM images demonstrated their polygonal superficial shape, intercellular borders and the micror-

idges disposed in several directions. Desquamating corneum cells were observed. The lamina propria is composed by bundles of collagen fibers arranged in several directions (Fig. 1).

3.3. Alcoholic group

UChA and UChB hard palatine mucosae were also lined by keratinized squamous stratified epithelium. However, it could be seen increased intercellular spaces between basal and spinosum cells. The epithelial cells and lamina propria of both alcoholic groups presented some important alterations compared to control group. Lipid droplets dispersed through the cytoplasm were observed in all layers with oval shape. Many nuclei exhibit high electron density with dispersed chromatin. Epithelial cells and fibroblasts showed altered mitochondria with ruptured cristae and also pyknotic nucleus like autolysins cells. Another distinct change was the presence of nucleus in the corneum layer. Differences between alcoholic groups were the presence of intense vacuolization and tonofilaments in epithelial cells of animals UChB. Lamina propria also presented lipid droplets dispersed among collagen fibers and fibroblasts with altered nuclei (Figs. 2 and 3).

3.4. IGF-IR expression

The IGF-IR expression was not detectable in the epithelial layers of both groups. On the other hand, the connective tissue presented intense positive reaction on the blood vessels of control, UChA and UChB groups (Fig. 4).

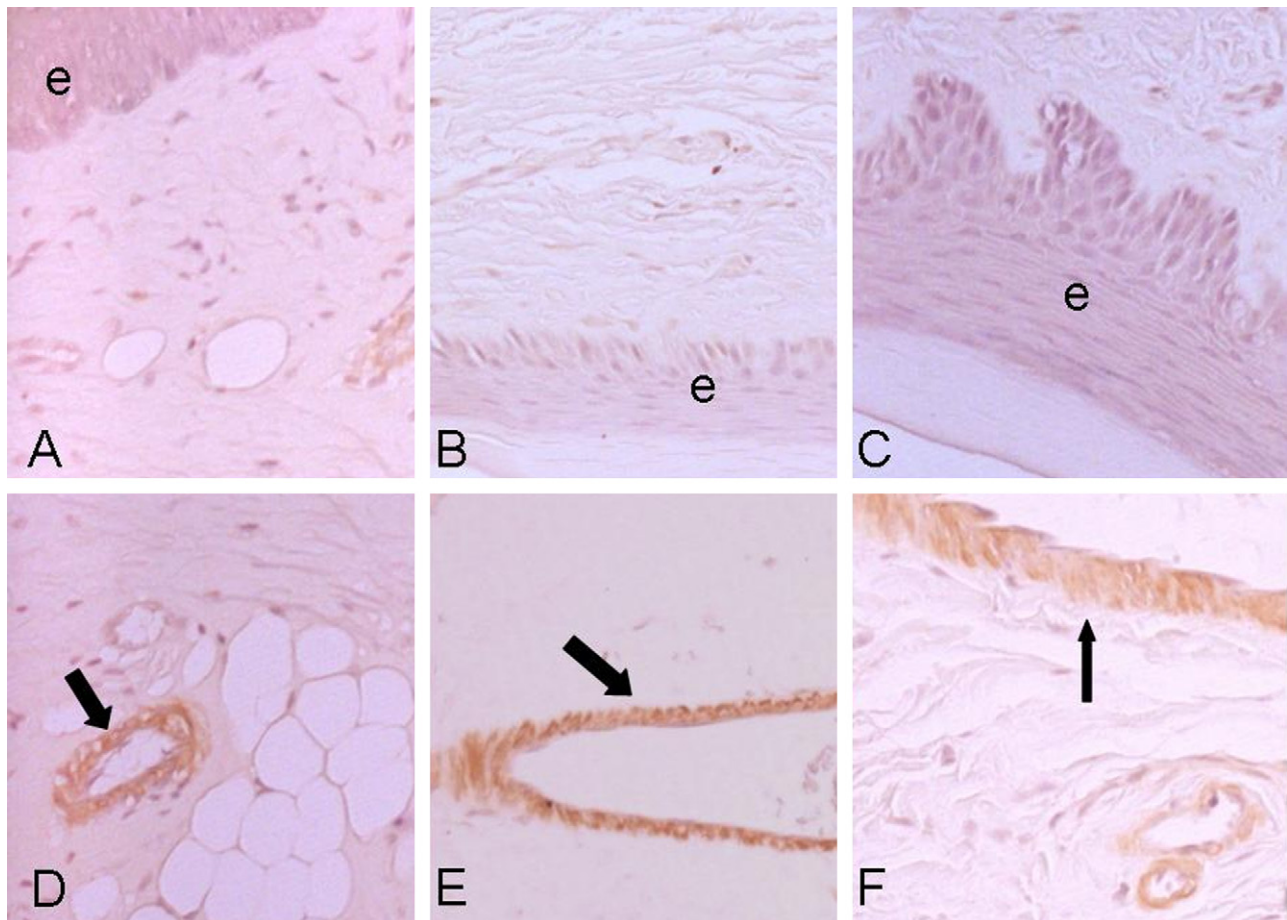


Fig. 4. Immunolocalization of the IGF-IR expression on the palatine mucosa of rats. (A) Control group. 10 \times . (B) UChA group. 10 \times . (C) UChB group. 20 \times . Epithelium with no expression (*). (D) Control group. 10 \times . (E) UChA group. 10 \times . (F) UChB. group. 20 \times . Note positive expression on blood vessels of both groups (arrows).

4. Discussion

Macroscopic investigation did not reveal differences in the hard palatine mucosa of control and UCh animals agreeing with findings described by Oksala and Schein (1971) in the oral mucosa of rats. On the other hand, Müller et al. (1983) described ulcerations in the rabbit oral mucosa after 48 h of 40% alcohol ingestion. The authors mentioned that there are two types of alcohol-toxic tissue and organ damage: the direct effect of ethanol by the contact with the mucous membrane and the indirect action by the absorption of the ethanol in the blood and subsequently by all tissues. The toxic effects are proportional to the degree of ethanol concentration.

Concerning electron microscopy, structural alterations were detected in the palatine epithelium of the alcoholic animals such as accumulation of lipid droplets, intense vacuolization, altered nucleus morphology, presence of nucleus in corneum cells, disrupted mitochondrias and intercellular spaces. Increased intercellular spaces and lipid droplets were described by Mascrès and Joly (1981) and Zorzetto et al. (2002). Martinez et al. (2005) also reported toxic effects of ethanol ingestion on the hard palatine mucosa of *Calomys callosus* as vacuolization, altered mitochondria, picnotic nucleus and nucleus in corneum cells. Other digestive system organs show ultrastructural alterations due ethanol ingestion. Kamlesh et al. (2006) showed perinuclear space, edema, presence of apoptotic bodies and disintegration, and/or dilatation of endoplasmic reticulum in the pancreata of ethanol-fed ADH⁻ deer mice. Yan et al. (2007) described severe ethanol mitochondria injury in liver. Bhonchal et al. (2008) revealed ultrastructural changes in small intestine like widened intercellular junction, distorted microvilli, increased rough endoplasmic reticulum, and increased and dilated mitochondria.

Ethanol metabolism results the formation of reduced purine nucleotides (NADH), which breaks the equilibrium of the NADH/NAD ratio, possibly being responsible for the acute metabolic consequences of excessive alcohol ingestion (Lieber, 1984). An important factor in ethanol metabolism by alcohol dehydrogenase is the ability of the liver to re-oxidize NADH to NAD especially through an action of the mitochondria. A damaging effect of alcohol on the liver is the production of defective mitochondria (Arai et al., 1984). Ethanol metabolism produces active oxidants inducing mitochondrial membrane depolarization. The mitochondrial permeability has been identified as a key step to apoptosis (Adachi and Ishii, 2002). Alcohol consumption has been shown to severely compromise mitochondrial protein synthesis (Cahill and Sykora, 2008).

Alcohol intake may cause cellular unbalanced and cellular death. According to Lluís et al. (2003) and Lieber et al. (2007) alcohol ingestion resulted in lower mitochondrial GSH levels. Through control of mitochondrial electron transport chain-generated oxidants, mitochondrial GSH modulates cell death and hence its regulation may be a key target to influence disease progression and drug-induced cell death (Fernandes-Checa and Kaplowitz, 2005). Direct DNA damage results from acetaldehyde, which can bind to DNA, inhibit DNA repairs systems and lead to the formation of carcinogenic exocyclic DNA etheno adducts. Chronic alcohol abuse interferes with methyl group transfer and may alter gene expression (Seitz and Sticke, 2006). The capacity of mitochondria to oxidize acetaldehyde is significantly reduced in the presence of NAD-dehydrogenase substrates, with consequent high levels of acetaldehyde (Hasumura et al., 1975). Alcohol ingestion provokes metabolic modifications in hepatocytes, such as reductions of fatty acid oxidation, glycogenesis and albumin (Thompson, 1978). The increase in acetate modifies fatty acid metabolism by inhibiting lipolysis, causing hepatic steatosis. Acetate is later released into blood plasma where it may be degraded, with the release of energy, or accumulated

as fatty acids and cholesterol in extrahepatic tissues (Hirata and Hirata, 1991; Mcgarry, 1992).

In UCh rats the expression pattern of IGFR-I as the same of control rats. The literature related few works about IGFR-I and palatine mucosa. Ferguson et al. (1992) described the differential expression of insulin-like growth factors I and II during mouse palate development. Brady et al. (2007) characterized the expression and function of IGF-I and IGF-II in oral squamous carcinoma and normal cell lines. Conflicting data are related about IGF-I and alcoholism in different tissues. It can be seen reduction on this growth protein (De La Monte et al., 2005) or increased expression of IGF-I and IGF-I receptors (Longato et al., 2008).

No signs of metaplasia were observed agreeing with Bofetta et al. (1992), Summerlin et al. (1992) and Martinez et al. (2005) that mentioned that longer periods of alcohol ingestion may provokes such damages. Therefore, chronic ethanol ingestion altered the hard palate epithelium structure of rats UCh.

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