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MACROSCOPIC AND MICROSCOPIC STUDY OF TISSUE RESPONSE TO ORAL ANTISEPTICS AND ITS INFLUENCE ON CARCINOGENESIS

ESTUDO MACRO E MICROSCÓPICO DA RESPOSTA TECIDUAL FRENTE AO USO DE ANTI-SÉPTICOS BUCAIS E SUA INFLUÊNCIA NA CARCINOGÊNESE

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ABSTRACT

Studies have related the action of alcohol on the oral mucosa as a promoter of carcinogenesis, once most oral antiseptics contain alcohol. Its utilization for mouthrinses from 30 to 60 seconds, as indicated on the labels, yields a longer-lasting topical action when compared to the intake of alcoholic beverages. This study aimed at conducting a macroscopic and microscopic analysis of the tissue response of tongue mucosa of hamsters to daily topical applications of antiseptics (Anapyon, Listerine, Oral B) during 13 and 20 weeks, following the methodology for carcinogenesis investigation developed by the Discipline of Pathology of Bauru Dental School, University of São Paulo. After sacrificing the animals, their tongues were removed and fixed on 10% formalin. Macroscopic examination did not reveal significant alterations, and the specimens were processed by routine histotechnical procedures for HE staining. Three serial sections of each tongue were evaluated, and characteristics related to epithelial hyperkeratinization, atrophy, hyperplasia and dysplasia were organized in tables. Despite the observation for moderate dysplasia in one case in the Anapyon 20 week group**,** the further results were very similar to the control group (saline solution), eliminating the need of comparative statistical tests. By means of such methodology for testing the carcinogenesis-initiating action, it was concluded that oral antiseptics are unable to trigger the development of neoplasms.
Uniterms: Alcohol; Oral antiseptics; Carcinogenesis.

RESUMO

Estudos associam a ação do álcool na mucosa bucal como promotora da carcinogênese e a maioria dos anti-sépticos bucais contém álcool. Sua utilização com bochechos de 30 a 60 segundos indicados nos frascos possui ação tópica mais duradoura em comparação com a ingestão de bebidas alcoólicas. Este estudo objetivou analisar macro e microscopicamente a resposta tecidual da mucosa lingual de hamsters após aplicações tópicas diárias de anti-sépticos (Anapyon, Listerine, Oral B) durante o período de 13 e 20 semanas conforme metodologia de estudo da carcinogênese desenvolvida pela Disciplina de Patologia da Faculdade de Odontologia de Bauru da Universidade de São Paulo. Após a morte dos animais removeu-se a língua que foi fixada em formalina 10%. Durante a macroscopia não se observaram alterações significantes e as peças cirúrgicas foram processadas conforme os procedimentos histotécnicos de rotina para coloração com HE. Três cortes seriados de cada um dos terços linguais foram avaliados e características relacionadas a hiperqueratinização, atrofia, hiperplasia e displasia epiteliais foram organizados em tabelas. Apesar da observação de displasia moderada em um caso do grupo de 20 semanas do Anapyon, os demais resultados apresentaram-se muito semelhantes ao do grupo controle (soro fisiológico), eliminando a necessidade de testes estatísticos comparativos. Através de tal metodologia, testando a ação iniciadora da carcinogênese dos anti-sépticos bucais, concluímos que não são capazes de desencadear o desenvolvimento de uma neoplasia.
Unitermos: Álcool; Anti-sépticos bucais; Carcinogênese.

INTRODUCTION

Many substances are routinely in contact with the oral mucosa through feeding, intaking of drugs, oral hygiene and chewing, and such substances may lead to the initiation or promotion of oral chemical carcinogenesis[7]; thus some concern should be raised as to the effects caused by alcohol[20-24], tobacco[12,19,20], food preservers, synthetic foods, pesticides, transgenics, therapeutic drugs, tooth bleaching agents, and the topical action of a variety of products for oral hygiene as dentifrices and mouthrinses.

The contact between the oral mucosa and these agents may promote cellular alterations, which in combination may lead to oral cancer[10,20,25-27]. Even though, alcohol is known by its action as a carcinogenic or cocarcinogenic or promoter of lesions, in association with other substances[11,16], several carcinogenic factors are not clearly understood yet. New products in the market have raised doubts on their biological safety to the tissues, and thus further investigation is wanted. Even though some addictions are considered voluntary and conscious, such as smoking and alcoholism, some others are not a matter of concern for most people, as the utilization of mouthrinses with antiseptic solutions in the search for a good breath or use of bleaching agents for esthetic purposes. In spite of being voluntary, the habit of utilization of mouthrinses is deemed unaware from a biological perspective, since there is a lack of knowledge on the components of the products and their actions on the oral mucosa[11, 23].

Since most mouthrinses contain alcohol, they are believed to trigger oral mucosal alterations, since alcohol was considered promoter of carcinogenesis[6,17,18]. Besides the investigations on tobacco, a harmful agent to the mucosa because of its several toxic substances that may initiate malignant lesions[12-14,20], alcohol has also been investigated and it is known that it has a very important combined effect on the epithelial alterations of the oral and pharyngeal mucosas[10]. High alcohol concentrations may yield considerable inflammation on the surface of the contacting oral mucosa, besides morphological and biochemical alterations in the cells by means of chronic ingestion[1,12,15]. Recent studies have considered the chronic ingestion of alcohol as a major risk factor for development of cancer of the upper airway-digestive tract, liver, rectum and breast, and there is some evidence that acetaldehyde (1st metabolite of oxidation of alcohol) is the main substance in charge of the alcohol-related carcinogenesis, since it interferes with the DNA synthesis and thus may lead to development of tumors[24].

Based on the concern that most oral antiseptics contain alcoholic solutions as vehicles, Pinera, et al.[23] (1996) conducted a study to establish the alcohol concentration of these solutions, since they may act on the mucosa due to the topical contact. The brands tested were Anapyon, Malvatricin, Listerine, Cepacol, Plax, Benzitrat and Flogoral, and the investigation employed a Gay-Lussac alcoholmeter and gaseous chromatography. The results were achieved in percentages: Anapyon=75; Malvatricin=29.9; Listerine=23.4; Cepacol=11.6; Flogoral=4.2; Plax=0.5; Benzitrat=0.3. For the purposes of comparison, the alcoholic content of some beverages consumed by the population were also established (Whisky, Vodka, Sugar Cane Spirit, Wine, Beer). The results were obtained in percentages: Whisky= 42.03; Vodka= 36.85; Sugar Cane Spirit= 36.49; Wine=10.51; Beer=4.59. Comparison of oral antiseptics and alcoholic beverages revealed that some antiseptics contain higher alcohol concentration than some beverages. Moreover, several authors observed an association between the development of oral lesions and oral hygiene with alcoholic antiseptics[3-5,16,20,22-23,26-28]. Factors as the frequency, means of utilization, alcoholic content, dilution and time of contact of the antiseptic solution with the mucosa may worsen the harmful effects of antiseptics on the tissues[23].

The labels of some products recommend mouthrinsing from 30 to 60 seconds, indicating a longer period of contact during mouthrinsing than in the intake of alcoholic beverages. This information leads to the concern with their frequent utilization and with the lack of investigation on this area, both on the biological effects and to increase the awareness of the risks. As a result of this, the study was conducted to test the action of mouthrinses with varying alcoholic contents as initiators of oral chemical carcinogenesis, and to compare the possible tissue alterations of each group.

MATERIAL AND METHODS

Before the beginning of the research, this study was submitted and approved by the Ethics Committee for Teaching and Investigation on Animals of Bauru Dental School, University of São Paulo. Forty young adult Golden Syrian hamsters (*Mesocricetus auratus*) were used, they were three months old and weighing nearly 300g, regardless of gender.

The substances applied were as follows. Group I: saline solution; Group II: Oral-B; Group III: Listerine; Group IV: Anapyon. All solutions were purchased in local markets. The solutions were applied with a camel hair brush n. 0 (Tigre brand) and each group had a different brush, which was properly labeled.

The solutions were applied on the middle third of the lateral edge on the left side of the tongue, after the removal of any liquids in excess by pressing the brush against the opening of the flasks. The animals received application of the solution four consecutive times, on a daily basis. An effort was made to apply the solution always on the same area of the lingual mucosa. The four groups were subdivided into two experimental periods of thirteen and twenty weeks.

After the study periods of thirteen and twenty weeks, the animals were killed by anesthetic injection into the heart. After that their tongues were removed, and fixed on 10% formalin and embedded in paraffin. Three 4-ìm thick serial sections were achieved for each lingual third in a microtome LEIKA RM 2045 and stained with Hematoxylin and Eosin.

RESULTS

Macroscopic alterations such as white spots, red spots, exophytic lesions and ulcers were not observed in the study, with normal aspect of the oral mucosa in all cases. Two animals did not resist through the whole period of the study and died before the end of the research. One belonged to Group I and the other to Group IV, both died on the 20th week. The cause of death was unknown and was regarded as systemic.

Normality aspects found on the epithelial superficial layers were considered, such as: the presence of hyperorthokeratinization, hypergranulosis and the number of epithelial layers varying from six to ten. Considering the findings of some dysplasia, it was considered normal the absence of hyperparakeratinization, dyskeratoses, nuclear polymorphism, loss of normal stratification, loss of basal polarity, loss of relationship nucleus-nucleolus and nucleus-cytoplasm relationship and the presence of several mitoses, hypercromatism, drop-shaped epithelial ridges and integrity of basal membrane. At last there was an analysis regarding the mononuclear inflammatory infiltrate considering normal the absence of any type of juxtaepitelial, diffuse or focal mononuclear inflammatory infiltrate. These aspects of normality were observed in almost all specimens, except for two specimens in the Listerine group and one specimen in the Anapyon group.

Analysis of Group I (13 and 20 weeks) revealed all aspects of normality of the oral mucosa on the lateral edge of the tongue used as criteria for calibration of the two examiners (CLC, RFP), with alteration of epithelial morphology according to the tongue area observed (Figure 1).

Group II did not present any alteration and maintained the normal aspects similarly to Group I with hyperorthokeratinization, high number of mitoses, gustatory receptors, variations in epithelial thickness, hypergranulosis, and presence of filiform lingual papillae (Figure 2).

Group III did not exhibit any significant abnormality, yet two specimens on the 20th week presented hyperparakeratinization on some epithelial areas. Moreover, this area revealed loss of cellular cohesion on the basal layer and an underlying focal mononuclear inflammatory infiltrate (Figure 3).

In Group IV, on the 13th week specimens did not present any alteration. One specimen on the 20th week exhibited moderate dysplasia with nuclear polymorphism on a localized epithelial area, loss of nucleus/cytoplasm relationship, loss of cellular cohesion, disorganization of the basal layer and hyperparakeratinization (Figure 4).

DISCUSSION

This study aimed at investigating oral antiseptics with different alcohol concentrations as to their ability to initiate carcinogenesis and the possible tissue responses of the oral mucosa to these substances, as well as comparing them after a certain experimental period with a control group without alcohol. Carcinogenesis consists of two stages: initiation, caused by a carcinogenic agent, and promotion, which is an exacerbation of carcinogenesis by a carcinogenic agent[2,9].

The selection of the three antiseptics employed in this study was based on their popularity and consequently widespread utilization. The inclusion of a group receiving applications of an alcohol-free antiseptic aimed at providing a control group for the other substances included in the composition of antiseptics for later comparison of a control group receiving daily applications of saline solution, the

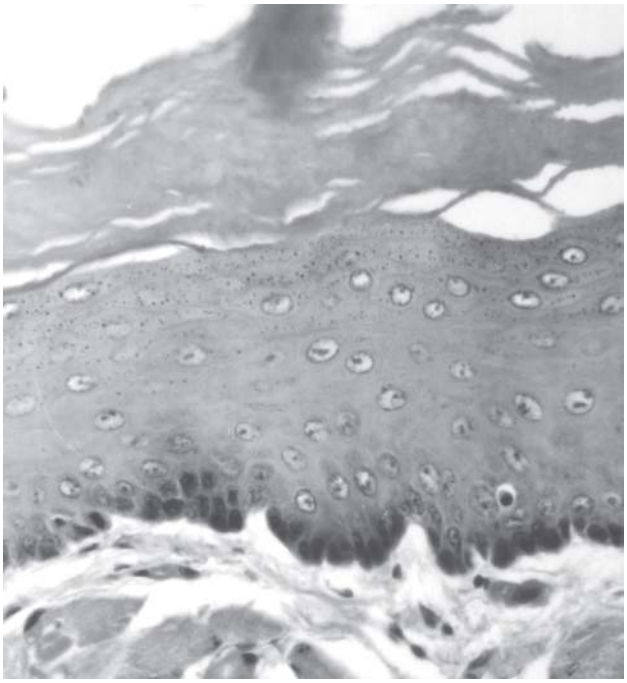

FIGURE 1- Microscopic section of a specimen in Group I demonstrating hyperorthokeratinization, some mitoses, preservation of epithelial stratification and normal hypercromatism of the basal layer, besides integrity of the basal membrane

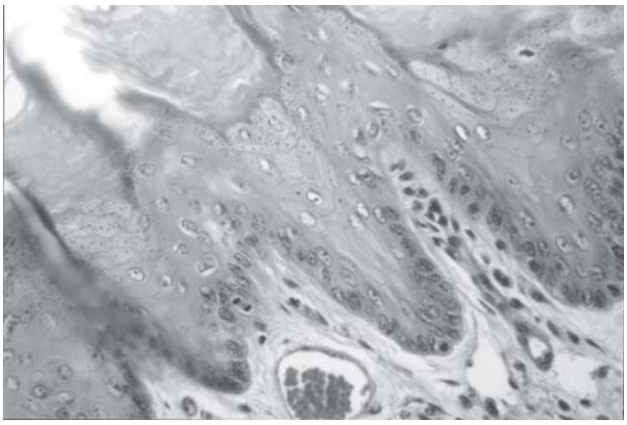

FIGURE 2- Microscopic section of a specimen in Group II revealing presence of lingual and epithelial papillae, hyperorthokeratinization, hypergranulosis, preservation of epithelial stratification, normal hypercromatism of the basal layer, intact basal membrane, and blood vessels in the fibrous connective tissue

negative control.

Besides the alcohol concentration, other aspects considered included dilution of the antiseptics in water, time of contact with the mouth and frequency of its use. The manufacturers' instructions for utilization of these products indicated mouthrinsing for 60 seconds; however, application of the substances in non-anesthetized animals did not allow direct contact of the antiseptic with the oral mucosa for such time.

Comparison of the methodologies employed in the studies of Fassoni[7] (1992) and Lima[18] (1997), the experimental period was similar in this study, yet the frequency of application was different. Whereas the frequency of application in the aforementioned studies was three times a week, the present study comprised daily applications. This frequency was considered as closer to the reality of the population consuming oral antiseptics, whose habits may range from daily mouthrinses, mouthrinsing several times a day, or some times per week. Also, the antiseptics were not diluted, since the instructions did not mention this need.

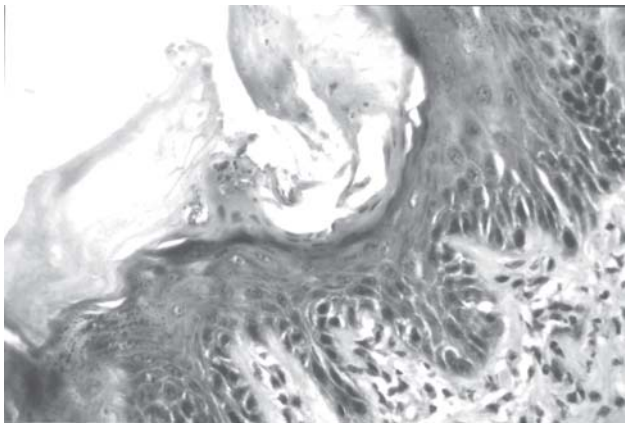

FIGURE 3- Microscopic section of a specimen in Group III exhibiting focal area of hyperparakeratinization associated to microbial biofilms in an apparently traumatized region, with some disorganization of epithelial stratification and focal mononuclear inflammatory infiltrate immediately below the connective tissue

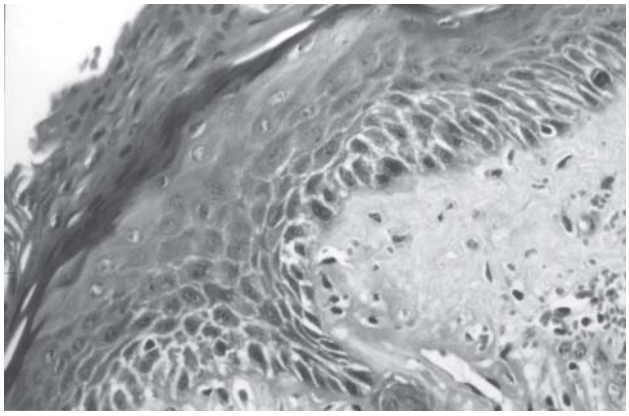

FIGURE 4- Microscopic section of a specimen in Group IV presenting extensive area of hyperkeratinization, loss of normal epithelial stratification, loss of polarity and disorganization of the basal layer, nuclear pleomorphism and increased number of mitoses

Macroscopic analysis of the specimens did not reveal formation of tumors, even though hamsters are susceptible to the development of cancer[2,8]. The mild alterations observed were restricted to the careful observation of microscopic characteristics. An interesting outcome was the establishment of extremely similar microscopic characteristics between the groups receiving application of Oral B antiseptic and the control group, i.e. without tissue alterations; this was the only alcohol-free antiseptic. This suggests that the alterations observed in the Listerine and Anapyon groups may be related to the presence of alcohol.

Jawdet and Damouk[10] (1993) believe that consumption of alcohol may increase the mucosal susceptibility, and Elzay[6] (1966) and Lima[17] (1999) concluded that alcohol does not act as an initiator of oral chemical carcinogenesis, but it is a promoter, since the association between DMBA and alcohol led to earlier and greater development of tumors. This study investigated only the initiator action of antiseptics and concluded that they are not initiators of carcinogenesis. Other authors[8,15] were unable to demonstrate a correlation between alcohol and experimental chemical carcinogenesis.

Epidemiological studies[5,20,26-28] consider that excessive utilization of oral antiseptics with high alcohol concentrations may contribute to the development of cancer, acting in combination with other factors, since alcohol is considered a promoter of oral cancer in many experimental studies[6].

Listerine was investigated by Bernstein and Carlish [3] (1979), who observed that application of oral antiseptics yielded atypical tissue reactions. This study observed focal areas of alteration of the superficial epithelial layer with areas of parakeratinization and underlying epithelial disorganization, besides a subepithelial focal mononuclear inflammatory infiltrate, in two animals. However, these focal areas might be a result of biting trauma as well.

The dysplastic lesion observed in one animal in the group receiving application of Anapyon for 20 weeks should be carefully discussed, due to its isolated occurrence in the study and to the susceptibility of hamsters to the development of neoplasms[2,8], however, it should not be neglected, considering the relevance of the fact that an antiseptic available in supermarkets, drugstores and other places may have yielded a premalignant lesion. Thus, investigations addressing these promoters or cocarcinogenic potentials are warranted, following a methodology of DMBA-induced oral chemical carcinogenesis with a view to achieve scientific bases to inform the population as to the risks and consequences of utilization of these substances without proper instruction and/or indication by the dentist.

CONCLUSION

The oral antiseptics used in this study did not trigger the development of neoplasms, and thus they did not

demonstrate to be initiators of carcinogenesis. The observation of dysplasia in one of the animals in the group receiving application of Anapyon, with a high alcoholic content, combined to the absence of tissue alterations in the group receiving application of alcohol-free antiseptic Oral B, raised a concern as to the biological action of these antiseptics on the tissues.

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