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Comparative Analysis of the Mast Cell Density in Normal Oral Mucosa, Actinic Cheilitis and Lip Squamous Cell Carcinoma

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Previous studies have shown that the number of mast cells is increased in ultraviolet (UV) irradiated skin and in neoplasias. Actinic cheilitis (AC) is a lesion caused by excessive exposure to sunlight that can transform into lip squamous cell carcinoma. The aim of this study was to compare the number of mast cells in 4 groups: NOM = normal oral mucosa (n=6); MDAC = mild dysplasia in actinic cheilitis (n=13); SDAC = severe dysplasia in actinic cheilitis (n=13); and LSCC = lip squamous cell carcinoma (n=15). The sections were stained by histochemical technique of blue toluidine and visual counting was performed with the aid of a reticulum coupled to the microscope ocular. A calibrated observer performed the count in 5 fields by case at ×400 magnification. The largest mean number of mast cells *per* group was observed in LSCC (40.1), followed by MDAC (30.5), SDAC (28.6) and NOM (12.2). There were significant differences between NOM and MDAC ($p<0.05$) and between NOM and LSCC ($p<0.05$). The increased density of mast cells observed in AC and in LSCC compared to NOM suggests a role for the mast cells in the development of these lesions.

Key Words: mast cell, actinic cheilitis, lip squamous cell carcinoma.

INTRODUCTION

Actinic cheilitis (AC) is a pathological condition affecting mainly the lower lip and is caused by chronic and excessive exposure of the lips to the ultraviolet radiation in sunlight (1). It affects mainly older and fair-skinned men (2). Mild to severe epithelial and connective tissue alterations are usually found. Epithelial changes include thickening of the epithelium and keratin layer, ulceration, acanthosis, and in more severe cases mild to severe dysplasia (3). In the connective tissue, solar elastosis and inflammation are found. Solar elastosis is characterized by the replacement of eosinophilic collagen by an amorphous basophilic granular material (1).

The early signs of sun damage to the lip are subtle, and the degree of clinical change is not necessarily related to the amount of either epithelial or connective tissue damage. Generally, the sun-damaged lip will have an asymptomatic white lesion of variable thickness with interspersed red areas and the lesion may be either well or ill defined (4).

AC is a pre-malignant lesion that can transform into squamous cell carcinoma (SCC) of the lip which is the most common form of oral cancer, corresponding to 95% of all oral malignant lesions. Studies have shown that mast cells are significantly increased in AC and SCC (3,5,6). As long-lived cells, they can have an enormous impact on the tissue microenvironment through the selective release of a wide variety of preformed and newly derived mediators including potent proteases, cytokines, chemokines and arachidonic acid metabolites (7).

Despite the evidence of an increased mast cell density in AC and SCC, the density of mast cells in different grades of dysplasia in AC compared to the density of mast cells in SCC has not yet been studied.

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Therefore, in order to assess if there are significant differences related to the histological grade of dysplasia or when a carcinoma is already established, 41 biopsies of cases of mild and severe dysplasia in AC and SCC were analyzed in comparison to normal lip tissue.

MATERIAL AND METHODS

Biopsies of 41 patients with AC and SCC were obtained from the archives of the Oral Pathology Laboratory of the Dental School of the Federal University of Pelotas. The cases were selected after approval by the Research Ethics Committee of the Federal University of Pelotas, and analysis of the sections stained with hematoxylin and eosin to confirm the diagnosis.

The control group was the normal oral mucosa (NOM, n=6). The experimental groups were mild dysplasia in AC (MDAC, n=13), severe dysplasia in AC (SDAC, n=13) and lip SCC (LSCC, n=15). All specimens were fixed in 10% buffered formalin and embedded in paraffin and 4-mm-thick sections were obtained from the tissue blocks. The solution used to stain mast cells contained 0.2 g of toluidine blue in 100 mL of distilled water and 2 mL of acetic acid. The sulfated proteoglycans in secretory granules of mast cells have a metachromatic property being stained by toluidine blue, so mast cells could be detected. After deparaffinization by immersion in xylene and descending grades of ethanol, the sections were rinsed in distilled water, stained with toluidine blue for 10 min, and then rinsed again in distilled water, dehydrated and mounted.

Visual counting of mast cells was performed with the aid of a reticulum (10 x 10 mm) coupled to the microscope ocular (Olympus® CX21, Melville, NY, USA) (Fig. 1). A calibrated observer performed the count in 5 fields by case in the connective tissue at ×400 magnification. Results were expressed as the mean value of mast cells *per* group. Data were tabulated and statistical tests were performed using SigmaStat 3.0 software (Systat Software, Inc., San Jose, CA, USA).

Statistical significance of the results was determined by one-way ANOVA. All pairwise multiple comparisons were done by Holm-Sidak method. Differences were considered statistically significant when $p<0.05$.

RESULTS

As illustrated in Figure 2, the largest mean number of mast cells *per* group was observed in LSCC (40.1), followed by MDAC (30.5), SDAC (28.6) and NOM (12.2). One-way ANOVA showed statistically significant differences in the mean number of mast cells among the groups ($p=0.014$). There were statistically significant differences ($p<0.05$) between LSCC and NOM and between MDAC and NOM (Table 1).

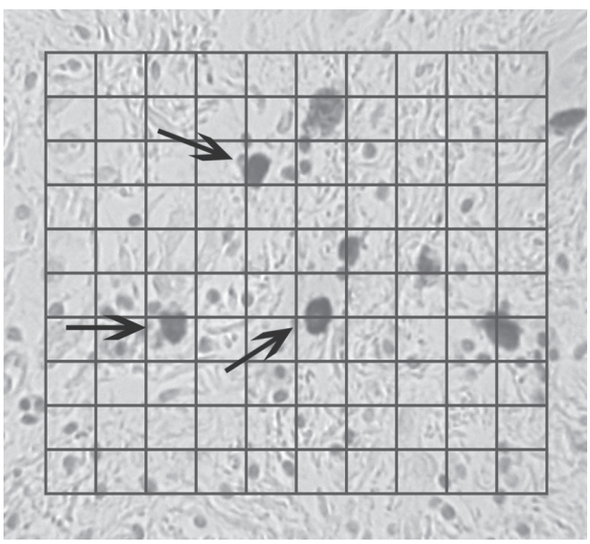

Figure 1. Schematic representation of the reticulum for counting of the fields with mast cells stained by histochemical technique of blue toluidine.

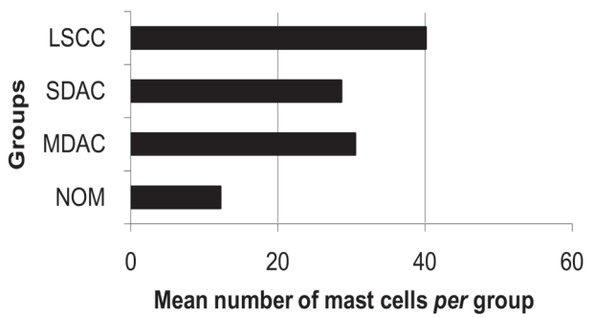

Figure 2. Mean number of mast cells *per* group. NOM = normal oral mucosa (n=6); MDAC = mild dysplasia in actinic cheilitis (n=13); SDAC = severe dysplasia in actinic cheilitis (n=13); and LSCC = lip squamous cell carcinoma (n=15).

DISCUSSION

Sunlight consists of radiation that varies in wavelength from 200 to 1800 nm. Ultraviolet (UV) B rays which range from 290 to 320 in wavelength, cause the superficial burning of the skin that leads to sunburn (4). According to Imayama et al. (8), chronic irradiation with a suberythematous dose of UVB (3 times/week for 12 weeks) produced a tortuous deformation of the superficial elastic fibers in rat sole skin, which are usually arranged linearly, that may correspond to the development of actinic elastosis.

Ultraviolet B rays are primarily responsible for sun-induced changes in the lip. Approximately 5 to 10% of UV radiation is reflected from the skin, but as much as 70% is absorbed. The lip has less protection than the skin because the epithelium is thinner, usually lacks the thicker keratin covering of skin, has less melanin and has less secretion from sebaceous and sweat glands (4).

Mast cells have been recognized as important effector cells of the deleterious effect of UV light on the skin (9,10). In the present study, mast cell density was increased in AC and LSCC compared to normal lip. The present results are in agreement with those of previous studies (3,5), in which metachromatic staining, immunohistochemical staining of tryptase-positive mast cells and enzymehistochemical staining of chymase-positive mast cells were performed.

The mean number of mast cells was significantly different ($p<0.05$) between SCC (40.1) and normal lip (12.167), and between MDAC (30.462) and normal lip (12.167). SDAC (28.6) showed a mean number of mast cells very close to that of MDAC (30.462) and very different from that of normal lip (12.167), but without statistical significance ($p>0.05$).

Table 1. Pairwise multiple comparisons performed by the Holm-Sidak method.

Pairwise comparisons	Difference of means	p value*
LSCC vs. NOM	27.93	<0.05
MDAC vs. NOM	18.30	<0.05
SDAC vs. NOM	16.37	>0.05
LSCC vs. SDAC	11.56	>0.05
LSCC vs. MDAC	9.63	>0.05
MDAC vs. SDAC	1.92	>0.05

NOM = normal oral mucosa; MDAC = mild dysplasia in actinic cheilitis; SDAC= severe dysplasia in actinic cheilitis; LSCC= lip squamous cell carcinoma; *Differences were considered statistically significant when $p<0.05$.

AC is an inflammatory reaction and can transform into SCC. As mast cell degranulation predominantly occurs during inflammatory condition (12), these cells may play a role in the regulation of inflammatory responses. Mast cell contains potent mediators, including histamine, heparin, proteinases, leukotrienes, and multifunctional cytokines that contributes to processes of inflammation, angiogenesis and matrix degradation (10). These effects may alter the microenvironment around the UV damaged epithelium, which may contribute to malignization and spreading of the AC lesion (3) and may contribute to lip SCC progression (5).

Jandinski et al. (10) found no significant difference in mast cell number between normal tissue and benign hyperkeratotic and dyskeratotic tissues. An increase in number of mast cells was observed in low-grade carcinoma suggesting an immunological cause for this increase. The medium-high grade carcinomas showed a decrease in mast cell number attributed to an unfavorable cellular environment. They stipulated that the transition from an increased to a decreased number of mast cells in oral mucosa represents a competition between the immunologic system and the tumor cellular environment.

Important progress has been made in understanding mast-cell regulation by the discovery of several inhibitory mechanisms that might balance the agonistic activities of the mediators discussed previously. The inhibitors include ligands of immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors (such as FcγRIIb and CD300a) 93-98, the anti-inflammatory cytokines IL-10 and TGFβ (REFS 99,100), CD200 (REFS 101,102), and intracellular signaling molecules 70, 96, 103, 104 that modulate FcεRI-mediated mast-cell activation. Additional molecules such as retinol, β2-adrenoceptor agonists and ECM proteins binding to CD63 have been reported to inhibit mast-cell proliferation and functions 105-108. The inhibition data derive mostly from experiments in the murine system. Therefore, the *in vivo* relevance of such findings for humans in health and disease cannot be determined at present. However, it seems possible that some of the findings could be extended to the human system (12). The development of new mast-

cell-specific drugs might modulate mast-cell function.

The increased density of mast cells observed in AC and LSCC compared to NOM suggests a role for the mast cells in the development of these lesions, which have the excessive exposure to UV sunlight radiation as the etiologic factor.

RESUMO

Estudos prévios mostram que os mastócitos estão significantemente aumentados na pele irradiada por ultra-violeta e neoplasias. A queilite actínica (QA) é uma lesão causada por excessiva exposição solar, que pode transformar-se em carcinoma espinocelular de lábio. O objetivo deste estudo foi comparar o número de mastócitos em 4 grupos: MON = mucosa oral normal (n=6); QADL = queilite actínica com displasia leve (n=13); QADS = queilite actínica com displasia severa (n=13); e CECL = carcinoma espinocelular de lábio (n=15). Os cortes foram corados pela técnica histoquímica do azul de toluidina e a contagem visual foi realizada utilizando um retículo acoplado à ocular do microscópio. Um observador calibrado realizou a contagem em 5 campos por caso em magnificação de ×400. A média de mastócitos por grupo foi maior no CECL (40,1), seguida da QADL (30,5), QADS (28,6) e MON (12,2). Houve diferença estatisticamente significante entre MON e QADL ($p<0,05$) e entre MON e CECL ($p<0,05$). A maior densidade de mastócitos na QA e no CECL em relação à MON sugere um papel para os mastócitos no desenvolvimento dessas lesões.

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