Spongy film of cellulosic polysaccharide as a dressing for aphthous stomatitis treatment in rabbits[1]

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ABSTRACT

PURPOSE: To develop an experimental model of acute inflammation, like aphthous ulcers, in oral cavity of rabbits, and also, to evaluate the results of the application of a polysaccharide spongy film of molasses from sugar cane as assist treatment in the healing process.

METHODS: Twenty adult rabbits weighting between 2.5 kg and 3.9 kg were divided into two groups: experimental and control infected ulcers were induced on the jugal mucosa by surgical excision. They were treated at the experimental group by curettage and dressing with spongy film of cellulosic polysaccharide film, whereas saline solution was used in the control group. Temporal evolution of the healing area, histopathology and bacteriological analysis were used to evaluate the healing process on the 3rd (D3), 7th (D7) and 11th days (D11).

RESULTS: The healing time and bacteriological study showed no statistical differences on the group means. Analyzing the histopathology of the experimental group we verified epithelial hyperplasia from D3 to D11, instead in the control group there was a greater clutter of the epithelial cells from the D3 to D11.

CONCLUSION: The experimental model used caused aphthous ulcers and the polysaccharide sponge film can be used as an aid in the symptomatic treatment and healing of the ulcerative lesions of the oral mucosa.

Key words: Polysaccharides. Biofilms. Saccharum. Wound Healing. Stomatitis. Animal Experimentation. Rabbits.

Introduction

Recurrent Aphthous Stomatitis (RAS) is a chronic disease, which is characterized by recurrent episodes of ulcerative lesions in any location of non-keratinized oral mucosa[1-3]. The clinical course of (RAS) is benign, causes pain and discomfort, difficulty chewing and lymph node alterations, compromising the quality of life. As it appears on several episodes throughout life, constantly become a source of anxiety to the patients[4-6].

The treatment of RAS is still controversial in the medical literature. Several drugs and substances have been used, although none of them have had real efficacy due to uncertainty about the ethiology of the disease. Success is achieved when an accurate diagnosis is done and the control of contributing factors is indentifield[1,5]. Symptomatic treatment aims to reduce the duration of the disease, preventing the patient from secondary infections, reducing soreness and aid in proper nutrition[3,7]. Products of the polysaccharide of molasses were applied experimentally in different models and satisfactory results were achieved, such as an abdominal wall mesh repair, in arteries and veins as well as replacement of temporalis fascia in myringoplasty in chinchillas[5,8-10]. The polysaccharide of molasses chemical composition, which is consisted by sugars and glucuronic acid[11], forms a pure, nontoxic and biocompatible product[12], being essential for the results' quality.

This experiment was planned with the purpose to develop an experimental model of acute inflammation, like aphthous ulcers, in oral cavity of rabbits, and also, of evaluating the results of the application of a polysaccharide spongy film of molasses from sugar cane as assist treatment in the healing process.

Methods

The project was approved by Ethics Committee on Animal Research, Federal University of Pernambuco (UFPE), nº 23076.020444/2008-49. This experimental study was accomplished at the Center for Experimental Surgery, UFPE, in the period from November, 2011 to February, 2012. The study population consisted of 20 healthy adult rabbits with mean age of 90 days, weighting between 2.5 kg and 3.9 kg.

The animals were allocated in two groups, with ten each, one as the control and another as experimental group. Induction of ulcers in the oral mucosa was performed using the same protocol in both groups. The anesthetic procedure was performed by the veterinarian of the Center of Experimental Surgery, UFPE. Ten minutes before the onset of anesthesia, atropine sulfate was applied intramuscularly at a dose of 0.44 mg / kg. To anaesthetize the animals we used a solution of 5 mg ketamine hydrochloride® and 2 mg of xylazine and applied 0.2 ml per 100g body weight IM.

After anaesthetized, all the animals were taken to the operating room and, under conditions of antisepsis, its oral mucosa was exposed with a long nasal speculum. Samples were collected for standard bacteriology of oral cavity by means of a sterile swab with swart. The incision area was 1 cm² to remove the mucosal and sub mucosal layer, after placing a cotton swab soaked in 10% hydrogen peroxide at the injury site, to stop bleeding. The lesions were not sutured and healing occurred by second intention, to simulate aphthous stomatitis (Figure 1). After induction of ulcers on the oral mucosa, the beginning of treatment was defined on D0, three days after its inductions followed by D3, D7 and D11 corresponding to the subsequent days of assessment of the ulcers in both groups. The spongy film of cellulosic polysaccharide used on the experiment was produced and provided by Polisa® of the Laboratory of the Research Group of Polymer of Sugarcane, of the Rural Federal University of Pernambuco (UFRPE).

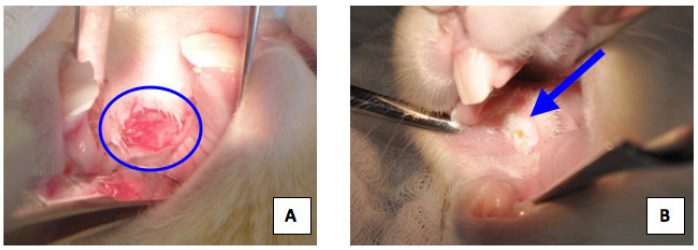

FIGURE 1 - A. Incision in the oral mucosa to induce ulcer. Final phase of the procedure. **B.** Spongy film of polysaccharide covering the lesion. Source: Compiled by author.

On the third day after the ulcer induction, D0, samples were collected for bacteriology by sterile swab medium and the lesions were photographed. Infiltration with local anesthetic and a biopsy from the center of the lesion margin was done for histopathologic diagnosis of the ulcer. Curettage of the lesions was done with scalpel blade nº15 in order to promote bleeding of the ulcers bed. On the experimental group a piece of spongy film of polysaccharide was placed to cover the entire space of the lesion by setting it by digital compression. The control group did not receive treatment; the healing of the lesion was by second intention.

In D7, samples were collected for bacteriology, using a sterile swab with swart and also photography was taken for control of lesions` healing areas. On D11 a photographic documentation was done and infiltration with local anesthetic with 0.2 ml of 2% xylocaine with vasoconstrictor for a biopsy to define the chronic pathological injuries. After clinical and laboratory examination

on D11. all the rabbits were donated to independent creators. For histological analysis, layers of healthy oral mucosa were included and the ulcer was stained with hematoxylin-eosin (HE). The systematic study was performed to evaluate qualitative and quantitative response of ulcers` healing. We evaluated the amount of inflammatory cells (poly and mononuclear infiltrates), the presence of interstitial edema, vascular congestion, the degree of granulation tissue formation and fibroplasia, micro abscesses and necrosis; classifying the data in strong, moderate, mild or absent, according to the intensity observed on the microscopic analysis. These data were transformed into quantitative variables by assigning indices and histological scores. Edema, congestion, polymorphonuclear exudate, neovascularization and fibrosis showed a chronic inflammatory process (Tables 1 and 2).

TABLE 1 - Quantitative analysis of the inflammatory parameters.

Inflammatory Parameters	Intensity			
	Accentuated	Moderate	Discrete	Absent
Neutrophils	-3	-2	-1	0
Edema	-3	-2	-1	0
Congestions	-3	-2	-1	0
Monomorfonuclear	+3	+2	+1	0
Neovascularization	+3	+2	+1	0
Fibroses	+3	+2	+1	0

Source: adapted from Vizotto *et al.*[18]

TABLE 2 - Scoring of the inflammatory process.

Phase of inflammatory	Process final score
Acute	-9 a -3
Subacute	- 3.1 a + 3
Chronic	+3.1 a +9

Source: adapted from Vizotto *et al.*[18]

We used the paired Student t test for comparison of parametric variables - Areas in differents stages of healing between the groups. For categorical variables - inflammatory parameters - was applied to the chi-square. The significance level of p used to reject the null hypothesis was 5% ($p<0.05$).

Results

There was no significant difference on the area (cm^2) means around the ulcer on the oral mucosa per group and time of treatment (days). Table 3 can be observed descriptive analysis area (cm^2) of the oral mucosa injury according to each study group and the treatment time (days).

TABLE 3 - Averages of the area (cm^2) around on the ulcer on the oral mucosa per group and time of treatment (in days).

Group	Area (cm^2)			Total
	D3	D7	D11	
Experimental	0.91±0.30	0.94±0.17	0.49±0.17	0.78±0.3
Control	0.98±0.36	0.82±0.31	0.42±0.14	0.74±0.4
p-value[1]	0.664	0.315	0.342	0.650

[1]p-value of Student's t test. Values represent mean ± standard deviation

Table 4 shows the degree of healing of the wound area (cm^2) according the times days D3, D7 and D11.

TABLE 4 - Distribution of the degree of healing of mucosal injuries in rabbits according to time and the treatment groups.

Degree of healing	D3 n (%)		D7 n (%)		D11 n (%)	
	Experimental	Control	Experimental	Control	Experimental	Control
Mild (area 0.9 to 0.6 cm^2)	9 (90)	8 (80)	10 (100)	7 (70)	2 (20)	2 (20)
Moderate (area 0.59 to 0.3cm^2)	1 (10)	2 (20)	0 (0)	3 (30)	8 (80)	6 (60)
Intense (less than 0.3 cm^2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (20)

Inflammatory parameters (neutrophils, edema, congestion, monomorfonuclear, neovascularization and fibrosis) were used as score on histological analysis between groups. Quantitative analysis of these inflammatory parameters was consistent with the sub acute phase (- 3 /+ 3.1) for all the groups.

Macroscopically, at the experimental group after three days of treatment, on D3, there was presence of the Spongy film of polysaccharide fixed in all ulcers. On D7, we could notice in all the animals that Spongy film of polysaccharide remained fixed on wound healing process without signs of infection. Also, on D11 we observer that lesions were only one bottom epithelized with raised edges.

In the control group on D3, we observed the presence of fibrin only, in wet injury healing process. On D7 of the same group, in all animals the evolution of the ulcers presented a centripetal healing process without signs of infection. On D11 the lesion was still with the bottom of ulcers with granulation tissue and the raised edges but not fully epithelized.

On D7 of treatment at the experimental group all animals showed hyperplastic stratified epithelium containing crusts of cellular debris on reactive tissue (neovascularization, fibrosis), muscle bundles, mucous glands, serous gland and papillae with

necrotic area with many macrophages. On D11, there was a predominance of mild epithelial hyperplasia. On D7 there was mild epithelial hyperplasia, spongiosis (intercellular edema epithelial), exocytosis, necrosis epithelial (ulceration area) and dyskeratosis in all animals of the control group. On D11 a predominance of intense epithelial hyperplasia, dyskeratosis, spongiosis, hyperkeratosis and exocytosis were observed. On the bacteriological study, a reduced trend of microorganisms was noted in the ulcer on D7 of the experimental group.

Discussion

The treatment of aphthous stomatitis is still a controversial chapter on medical literature. Several drugs and substances have been used, but none had real efficacy due to uncertainty about the exact etiologic mechanism of the disease.

This study sought to examine the wound healing process of ulcers of rabbit's oral mucosa treated with mechanical barrier and cells conductions of the spongy film of polysaccharide. Several studies about symptomatic treatment of oral ulcer showed that there was improvement in pain[1,2,5].

The aim of this study was to use Spongy film of polysaccharide as a symptomatic treatment for pain relief, acting as a biological dressing for protecting the injury. Weight gain on D11 at the experimental group was not significant (p=0.854).

Aguiar *et al.*[13] and Martins, *et al.*[14] reported that among treatments with film of cellulosic polysaccharide there were no cases of infection and the tolerance to the material was satisfactory and there were no adverse reactions.

Furthermore, pharmaceutical preparations containing the extract of mallow, also have relevance in the treatment of oral ulcers. When used for a period of seven days, with 2-3 applications per day. Gazel[6] refers that the application of these substances must be accompanied by adherence facilitators, since these medications are easily removed, making difficult to establish an effective availability on the mucosal surface. Tubaro *et al.*[15] and Miller[16] reported that chamomile helps the lesions healing, showing no contraindication as carcinogenic or allergic. Ximenes[1] recalls that mucosal protective agents such as milk of magnesia and cyanoacrylates have been used to reduce pain. Another protective lining that has been tested in a study conducted in Italy was the sucralfate, with good results. Mouthwash chlorhexidine 2% appears to prevent secondary infections. The mechanism of action of these substances is due to the formation of a protective film on the bottom of the ulcer, avoiding trauma and increased pain. Mouthwash containing aqueous 1% Novocain for pain relief has been also described in the literature.

The surgical technique was adequate and easy to induce ulcer of the oral mucosa in rabbits. However, as rabbits are ruminants, they have keratinized papillae that assist the mechanical chewing function, but there was no impairment on the final result of this study, allowing characterizing the evolution of the model of Incision of the oral mucosa to induce aphthous stomatitis.

The isolation and identification of the oral cavity bacteria of rabbits showed no microbial pattern in neither group, probably due to the diversified bacterial flora of the rabbits oral mucosa. However, there was a tendency to decrease the number of microorganisms at the experimental group, rabbits treated with the Spongy film of polysaccharide. Raising the possibility that the product could have worked out as a mechanical barrier of protection. The polysaccharide degradation by bacterial action can occur and thereby releasing sugar and providing a bacteriostatic effect as observed by Coelho[17]. This effect is attributed to the polysaccharide and is attributed as an aid in infection control.

In order to analyze the temporal evolution of healing area of oral mucosa ulcers, photographic documentation and measurement of the same area was carried out. The percentage of the experimental group of rabbits which had been closed about the same light phases D3 and D7 of the study (90% and 100% respectively). In phase D11 the scar closing with moderate degree of healing was (80%). In the control group, the percentage of closure with mild degree of healing was in the first two phases - D3 and D7 - 80% and 70%, respectively whilst in the third phase (D11) the healing with moderate degree was (60% of cases) with 20% showed intense degree of healing.

To develop a new treatment of the aphthous stomatitis, we tried to analyze the histological qualitative and quantitative parameters of healing of ulcers in the oral mucosa of rabbits treated with spongy film of polysaccharide through the quantification of angiogenesis, edema, congestion, neutrophils and fibrosi[18,20].

The data were classified as severe, moderate, mild or absent, according to the intensity in which they were found, and transformed into quantitative variables by assigning index and histological features. Edema, congestion and neutrophilic exudate were indicative of acute inflammation, and granulation tissue, fibrosis and exudate monomorfonuclear were indicative of chronic inflammation. Indices of the acute inflammatory process were assigned with negative sign, whilst the chronic inflammatory process, a positive sign was marked. After the assignment of the indexes, we proceeded to the sum of these; thereby each group of animals had a final score allowing the classification of three phases of the inflammatory process.

The rabbits in the control group showed a higher intensity of neutrophils, edema and congestion than rabbits treated group at all stages of the study, except for the D11 that the mean score of edema in the control group was higher than the experimental group. Also, the presence of exudate monomorfonuclear and fibrosis were more intense in the experimental group than the control group in the first two phases of the study, D3 and D07, but it wasn't found in D11. The quantitative analysis of these inflammatory parameters was consistent with the subacute phase (-3/3.1) in the control and experimental groups.

With respect to neovascularization, the average score of the experimental group was higher than the group treated at all stages of study indicating that the control group had the highest intensity in this parameter. Regarding to neovascularization, the average score of the experimental group was higher than the group treated at all stages of study indicating that the control group had the highest intensity in this parameter.

The inflammatory reaction is the contribution of the cells in the process of tissue repair[20]. There were signs of inflammation in the animals receiving the spongy film of polysaccharide higher placement than those not receiving in the control group. At the experimental group we observed the formation of an inflammatory process at the expense of polymorphonuclear cells with the presence of epithelial hyperplasia in D3 from the treatment, observing a clutter cellular from D3 to the last day of follow-up (D11) in control group. Regarding to the macroscopic observation, it was found that the experimental group, in an initial phases the opened wound, peripheral vascular irrigation with fibrin and presence of polysaccharide film.

In D7, the lesion tended to present less humid, closing centripetally with the presence of polysaccharide film. In D11, there was a complete healing of the lesion with raised edges of the ulcer bed and complete reepitheliezation.

In the control group on D3, macroscopically the lesion resembles macroscopically the experimental group. However, in D7 is slightly more moist and in D11 also showed a tendency of complete healing, but the presence of tissue with a little bleeding at the bottom of the ulcer could be observed, a fact which corroborates the findings of histopathology. This result can be attributed to the mechanical effect of the polysaccharide film.

This product may also contribute as a biological dressing, accelerating the healing process, since it decreases contact with the oral cavity. The RAS is permanently exposed to mechanical trauma, as by mastication of food, also as by brushing the teeth in the case of humans. Coelho[17] related that the polysaccharide of the sugar cane forms a stable film which, when in contact with wound fluids, releases an amount of sugar which can raise the osmolarity and reduce the number of bacterial, a positive factor in the healing process.

Coelho[17] noted that the biopolymer sugar cane forms a stable film which, when in contact with wound fluids, releases an amount of sugar that can lead to concentrate and medium hyperosmolarity, which is considered irritant and a positive factor in the healing process by stimulating granulation. One concern was to study the material, how long remain fixed at the injury site and exert its biological function in ulcer site as a curative.

The spongy polysaccharide film used in this study contained no medication and it worked just as mechanical barrier. This study is important because it proved the permanence of the polysaccharide film in ulcer at D3 and D7, justifying the dressing as a mechanical barrier. As there is the possibility to add drugs in the polysaccharide sponges it can also be used as a drug-releasing system. Relating to this study we also must consider that rabbits are ruminants, which increases the mechanical effect of mastication. This fact is extremely important considering that a major problem for the symptomatic treatment of the aphthous stomatitis is to fix the drug long enough. Dilution with saliva, the chewing movements and the speech are factors which turn very difficult the use of an effective substance for long enough in the oral mucosa. Another advantage of the spongy polysaccharide film is that it allows the incorporation of many drugs in their composition which can further accelerate the healing and pain relief, considering that this is what most affects the quality of life of patients.

Conclusion

The experimental model used caused aphthous ulcers and the polysaccharide sponge film was effective as a mechanical barrier and can be used as an aid in the symptomatic treatment and healing of the ulcerative lesions of the oral mucosa, associated with its low cost, compatibility and easiness of application.

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