Fibroplasia in oral surgical wounds submitted to topical Brazilian Propolis

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Accepted 28 January, 2012

Studies have demonstrated that propolis is an effective substance for wound healing, however there is little evidence regarding its efficacy in the mechanisms of fibroplasias. The present investigation determined whether an ethanolic extract of propolis applied topically accelerates the healing process of oral surgical wounds. Forty-eight hamsters were distributed into the groups: I (n=16) received 30% ethanolic extract of propolis; Group II (n=16) received 0.01% dexamethasone; and Group III (n=16) received pure orabase cream. A localized incision was then made on the dorsum of the tongue and these wounds were submitted to the topical administration of the substances used in each group. Twelve animals were sacrificed at 3, 7, 14 and 28 days postoperative, for histopathological analysis was used Masson’s trichrome staining. Significant differences in fibroplasia were found among the groups on Days 7 and 14 (p=0.001), and the group I achieved the highest collagen deposition (p=0.040). Under the present conditions, the poropolis can accelerates the healing process of oral wounds in hamsters.

Keywords: Propolis, oral ulcer, wound healing and collagen.

INTRODUCTION

Healing is a dynamic, complex process through which an injured tissue is replaced with vascularized conjunctive tissue (Martin, 1997). Through a continuous, intricate sequence of tissue, cellular and subcellular events, the tissue undergoes inflammation, the formation of granulation tissue with the deposition of extracellular matrix and remodeling, thereby becoming intact once again (Park and Barbul, 2004; Efron and Moldawer, 2004; Werner and Grose, 2003). Studies in this field focus on the efficacy of topical and systemic medications on the injury repair process, especially in chronic injuries (Sehn et al., 2009).

Intrinsic factors, such as diabetes and malnutrition, and extrinsic factors, such as anti-inflammatory drugs of synthetic or natural origin, can impede, retard or accelerate the healing process (Fonder et al., 2008). Steroidal anti-inflammatory drug, are widely employed to modulate the inflammatory reaction. However, the continual use of these drugs can cause problems triggered by the suppression of the hypothalamic-hypophyseal-adrenal axis as well as other complications, such as hydroelectrolytic abnormalities, arterial hypertension, hyperglycemia, increased susceptibility to infection, osteoporosis, etc. (Ligon and Judson, 2011; Roquilly et al., 2011; Zhu et al., 2011).

Propolis has been used over the centuries for various purposes, such as an alternative to the use of glucocorticoids. A number of studies have demonstrated that propolis is an effective substance for wound healing (Sehn et al., 2003). This compound has antimicrobial (Cushnie and Lamb, 2005), antifungal (Sonmez et al., 2005) anti-inflammatory and healing properties (Ramos and Miranda, 2007). Besides increasing the contraction wounds and accelerating the tissue restoration process, propolis is also a natural, low-cost alternative for the treatment of ulcers, with a low degree of toxicity (Salomão et al., 2011). Propolis has also been studied with regard to its antioxidant, anti-mutagenic and diabetes-regulating actions (Teixeira et al., 2010; Senedese et al., 2011; Zhu et al., 2011).

While studies have been carried out on the aforementioned properties of propolis, there is little
evidence regarding its efficacy in the mechanisms of fibroplasia in ulcerated wounds due to the use of methods with inadequate specificity (Suemaru et al., 2008; Benderli et al., 2011). Thus, the aim of the present investigation was to determine whether an ethanolic extract of propolis applied topically interferes in the healing process of oral surgical wounds compared to dexamethasone in an *in vivo* experimental study.

**MATERIALS AND METHODS**

The present study used forty-eight golden Syrian hamsters (*Mesocricetus auratus*) with a mean age of 90 days maintained at room temperature with free access to a balanced chow (Nuvilab®, Nuvital Nutrientes SA, Brazil) and water *ad libitum*. A 12-hour day-night cycle was used throughout the experiment.

The animals were distributed into three groups based on the substances used: Group I (n=16) received 30% ethanolic extract of propolis (Apiário Mackllani Ltda, Santa Bárbara, Minas Gerais, Brazil); Group II (n=16) received 0.01% dexamethasone in orabase cream; and Group III (n=16) received pure orabase cream (Figure 1).

Total polyphenol contents in used ethanolic extract of propolis (EEP) were determined by the Folin-Ciocalteau colorimetric method (Singleton et al., 1999). Diluted commercial EEP solution (0.5 ml) was mixed with 0.5 ml of the Folin-Ciocalteau reagent and 0.5 ml of 10% Na₂CO₃, and the absorbance was measured at 760 nm after 1 h incubation at room temperature. Total polyphenol contents were calculated as gallic acid equivalents present in dried ethanolic extract (mg/g). Total flavonoid contents in the extracts were determined using a method described by Park et al. (Park et al., 1995), with minor modifications. For this, 0.5 ml of EEP solution, 4.3 ml of 80% methanol, 0.1 ml of 10% Al(NO₃)₃ and 0.1 ml of 1M potassium acetate was added. After 40 min at room temperature, the absorbance was measured at 415 nm. Total flavonoid contents were calculated as quercetin equivalent (mg/g) from a calibration curve.

For the execution of the surgical wound, the hamsters were submitted to general anesthesia through an intraperitoneal injection of sodium thiopental® (Cristália, Brazil; 20mg/Kg). A localized incision was then made on the middle portion of the dorsum of the tongue with a punch 4 mm in diameter and 1 mm in depth. The oral surgical wounds were submitted to the topical administration of the substances used in each group, applied with the aid of a camel-hair brush every 12 hours for up to seven days.

Four animals from each group were sacrificed at each of the pre-established postoperative periods (3, 7, 14 and 28 days) with a lethal dose of anesthesia through an intraperitoneal injection of sodium thiopental® (Cristália, Brazil; 100mg/Kg). The tongue tissue specimens were dissected, dehydrated, dehydrated in alcohol, clarified in xylol and embedded in paraffin. Histopathological cuts measuring 5μm in thickness were made on a microtome (Spencer 820) and submitted to Masson's trichrome staining.

This study received approval from the Ethics Committe
for Animal Experimentation of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (Brazil). The entire experimental part followed the guidelines for the teaching-scientific practice of animal vivisection as well as the ethical principles of animal experimentation in compliance with the Brazilian constitution.

**Histopathological analysis**

For the semi-quantitative analysis of collagen deposition at the different postoperative evaluation times, the slides were examined under an optical microscope (Olympus BX 41, Japan) (magnification: 400X). A score was attributed to each microscopic field of the surgical wound. In ulcerated wounds, one field on the edge and one in the deep portion were considered (Figure 2A). On completely re-epithelialized wounds, one field on the surface and one in the deep portion were considered (Figure 2B). Semi-quantification was performed based on the following criteria (Figure 3):

1. Score 1 (+), when collagen deposition corresponded to 5 to 25% of the total microscopic field under analysis;
2. Score 2 (++), when collagen deposition corresponded to 26 to 50% of the total microscopic field under analysis;
3. Score 3 (+++), when collagen deposition...
Table 1. Distribution of collagen deposition scores at different postoperative evaluation times in Groups I, II and III

<table>
<thead>
<tr>
<th>Time and Score</th>
<th>Group I Propolis n(%)</th>
<th>Group II Dexamethasone n(%)</th>
<th>Group III Orabase cream n(%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3 Score 1</td>
<td>8(33.3)</td>
<td>8(33.3)</td>
<td>8(33.3)</td>
<td>G1 x G2 = 1.00  *</td>
</tr>
<tr>
<td>Day 7 Score 1</td>
<td>0(0.0)</td>
<td>7(29.1)</td>
<td>4(16.1)</td>
<td>G1 x G2 = 0.001 **</td>
</tr>
<tr>
<td>Score 2</td>
<td>8(33.3)</td>
<td>1(4.1)</td>
<td>4(16.1)</td>
<td>G1 x G3 = 0.030</td>
</tr>
<tr>
<td>Day 14 Score 2</td>
<td>1(4.1)</td>
<td>8(33.3)</td>
<td>4(16.1)</td>
<td>G1 x G3 = 0.140</td>
</tr>
<tr>
<td>Score 3</td>
<td>7(29.1)</td>
<td>0(0.0)</td>
<td>4(16.1)</td>
<td>G2 x G3 = 0.030</td>
</tr>
<tr>
<td>Day 28 Score 2</td>
<td>0(0.0)</td>
<td>3(12.5)</td>
<td>0(0.0)</td>
<td>G1 x G2 = 0.090 *</td>
</tr>
<tr>
<td>Score 3</td>
<td>4(16.1)</td>
<td>4(16.1)</td>
<td>4(16.1)</td>
<td>G1 x G3 = 1.000</td>
</tr>
<tr>
<td>Score 4</td>
<td>4(16.1)</td>
<td>1(4.1)</td>
<td>4(16.1)</td>
<td>G2 x G3 = 0.090</td>
</tr>
</tbody>
</table>

*Chi-square test  
** Fisher’s exact test

Table 2. Comparison of scores among groups having received propolis, dexamethasone and orabase cream

<table>
<thead>
<tr>
<th></th>
<th>Median (Q1 – Q3)</th>
<th>p*</th>
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<tbody>
<tr>
<td>Group I</td>
<td>2.00 (1.25 – 3.00)</td>
<td>0.040</td>
</tr>
<tr>
<td>Group II</td>
<td>2.00 (1.00 – 2.00)</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>2.00 (1.00 – 3.00)</td>
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</table>

* Kruskal-Wallis test

corresponded to 51 to 75% of the total microscopic field under analysis;
4. Score 4 (+++), when collagen deposition corresponded to more than 75% of the total microscopic field under analysis.

Cases in which collagen deposition was less than 5% were not considered due to the difficulty in histopathological analysis, which may generate false positive or negative.

Statistical analysis

All data collected during the histopathological analysis were compiled in a data bank using the SPSS program (version 17). When appropriate, either the chi-square test or Fisher’s exact test was used to determine significant differences between groups in the comparison of frequencies. The non-parametric Kruskal-Wallis test was used for the comparison of scores among the three groups. The level of significance was set at 5% (p<0.05).

RESULTS

All microscope fields showed collagen deposition more than 5%, and then all cases were considered for histopathological analysis. The histopathological analysis demonstrated that the deposition of collagen fibers increased progressively from Day 3 to Day 28 (Table 1). Significant differences in fibroplasia were found among the groups on Day 7 [Group I vs. Group II (p=0.001) and Group I vs. Group III (p=0.020)] as well as on Day 14 [Group I vs. Group III (p=0.001) and Group II vs. Group III (p=0.020)]. In the comparison of median scores regardless of postoperative evaluation time, Group I achieved the highest collagen deposition scores and Group II achieved the lowest scores (Table 2).

Analysis of dry extract in evaluated commercial ethanolic extract of propolis results in a concentration of 16.66% (±0.13). According to Brazilian law, this value is higher than the minimum concentration of solids that must be present in extracts of propolis (tinctures). The total polyphenol and flavonoid contents quantified in the
EEP, expressed as gallic acid and quercetin equivalents, were 92.19 mg/g and 35.99 mg/g, respectively.

DISCUSSION

In this study, surgical wounds were made by mechanical trauma by a punch. The wounds were induced to mimic the development and course of traumatic and aphthous ulcers which are common complaints in the dental office. This methodology differs from previous work that evaluated the effect of propolis on oral mucosal damage induced by chemical and radiotherapy agents (Suemaru et al., 2008; Benderli et al., 2011).

The present study employed Masson’s trichrome staining, which is specific to collagen and allowed a better understanding of the effect of ethanol extract of propolis on fibroplasia. EEP was found to enhance the tissue repair process through the rapid deposition of interstitial collagen. The components responsible for this acceleration are not yet clearly defined. The findings of the present study corroborate those reported in the literature demonstrating the propolis enhances the healing process in mucous and cutaneous tissues (Ozturk et al., 1999; Ozturk et al., 2000; Ozturk et al., 2000b; Gregory et al., 2002).

Propolis is reported to be an anti-inflammatory and healing agent (Sehn et al., 2009; Salomao et al., 2011). Unlike the results reported herein, Suemaru et al. (2008) assessed the topical action of chemically induced oral wounds in hamsters and found no significant difference with regard to the healing process. The species and low concentration of propolis used may account for these non-expressive results.

In the present study, ethanolic extract of propolis proved capable of modulating the inflammatory response, likely by promoting angiogenesis and cell proliferation, which are essential to the process of fibroplasia. The anti-inflammatory activity of the EEP may be explained by the presence of polyphenols and flavonoids, which have antioxidant properties. It is likely that the action of these components on free radicals leads to the moderation of inflammatory infiltration. Moreover, Banskota (2001) found that the alcohol extract of propolis suppresses the production of prostaglandins, leukotrienes and other mediators derived from arachidonic acid generated by the lipoygenase pathway.

The results of the present study demonstrate that the corticoid used in Group II altered the deposition of the collagen extracellular matrix, slowing down the process of fibroplasia when compared to EEP. Wound healing consists of a cascade of cellular and molecular events that interact in the remodeling of the tissue. This dynamic event involves biochemical and physiological phenomena. The efficiency of this process depends on the concomitant formation of new blood vessels and the intensive migration of fibroblasts. With the increase in the number of fibroblasts activated for collagen production at the site, the granulation tissue is replaced with stronger, more elastic conjunctive tissue (Martin, 1997; Hartlapp et al., 2001).

Glucocorticoid-based medications have been used for the treatment of ulcerated oral wounds (Porter et al., 2000; Scully et al., 2008). As demonstrated in the present study, compared to EEP these drugs slow down the healing process and, according to the literature, can affect cell functions and structures, causing negative side effects (Ship et al., 2000; Rodriguez et al., 2007; Nieman, 2011).

The findings of this study corroborate the results of some in vivo studies which evaluated the effect of corticoids on inflammatory function in surgical wounds on rats through different experimental models (Hashimoto et al., 2002; Luo et al., 2004; Yazici et al., 2009; Miranda et al., 2012). These researches support the evidence that the administration of corticosteroid delayed the healing process.

Propolis, which is a natural, low-cost product, proved more efficient at inducing fibroplasia in comparison to dexamethasone and orabase cream. Further studies are needed to clarify the biomolecular mechanisms of propolis and determine the possible clinical application of this substance as a tissue healing agent in inflammatory oral ulcers.

CONCLUSION

The increase in collagen deposition in wounds treated with ethanolic extract of propolis demonstrates that this substance accelerates the healing process of oral wounds in hamsters when compared to dexamethasone cream.

REFERENCES


