The Efficacy of the Bioadhesive Patches Containing Licorice Extract in the Management of Recurrent Aphthous Stomatitis

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This study evaluated the efficacy of licorice bioadhesive hydrogel patches to control the pain and reduce the healing time of recurrent aphthous ulcer.

This study was carried out in three episodes of ulcers: in the first episode of ulcer, all 15 patients were asked to record their baseline individual pain level by a visual analog scale. In the second and third episodes, comparative and consecutive subjective and objective evaluations of the bioadhesive were done. The effects of the following variables were investigated: (1) VAS pain score for 5 consecutive days, (2) profile of aphthous ulcers on days 3 and 5, (3) time to complete relief of pain and healing of the ulcers, (4) diameter of the lesions and necrotic zone.

A significant reduction in VAS was recorded following application of the licorice patches on days 2, 3, 4 and 5 compared with the no-treatment group ($p < 0.001$). Licorice patches caused a significant reduction in the diameter of the inflammatory halo and necrotic center compared with the placebo group ($p = 0.03$).

According to the results of this study, licorice bioadhesive can be effective in the reduction of pain and of the inflammatory halo and necrotic center of aphthous ulcers. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: recurrent aphthous stomatitis; licorice; mucoadhesive; biopatch; topical therapy.

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a common condition in which recurring ovoid or round ulcers affect the oral mucosa. It is one of the most painful oral inflammatory ulcerative conditions which cause pain during eating, swallowing and speaking (Miller and Ship, 1977; Sircus et al., 1967). Recurrent aphthous stomatitis is the most common oral condition diagnosed by dentists and physicians (Rogers, III, 1977; Woo and Sonis, 1996). The prevalence of the disease varies in different studies according to the investigated populations (Harries et al., 1987; Scully et al., 2003). An overall prevalence of 10–20% has been reported for the general population (Rennie et al., 1985; Scully et al., 2003), with a higher prevalence of RAS (56%) in selected groups, such as university students (Donatsky, 1973). Although hereditary (Ship, 1965; Shohat-Zabarski et al., 1992), dietary (Eversole, 1994), hematologic (Burgan et al., 2006), infectious (Barile et al., 1963; Sun et al., 1996), allergic (Boulinguez et al., 2003) and immunologic (Natala et al., 2000) factors have been implicated, their role as the main etiological factors in the pathogenesis remains to be elucidated. Furthermore, with the dramatic worldwide increase in patients with immuno-suppression caused by medical treatments, systemic diseases, or both, the prevalence of these conditions may be increasing. Pain is a common symptom of RAS (Mahdi et al., 1996).

There is no specific management for RAS, and therefore analgesic, antimicrobial and immuno-modulatory drugs have been used individually or simultaneously (Porter et al., 1998). Topical treatment is used to promote healing and pain relief (Scully et al., 2003) meanwhile, systemic treatment is reserved for severe cases and is not applicable in most situations (Katz et al., 1994). Antiinflammatory, antibiotic and some immunomodulatory agents have been used as topical and systemic treatments in RAS (Jurge et al., 2006) so far topical medications with mucosal adherence properties have been used with some success, including Orabase (Scully et al., 2003), Zirlactin (Rodu and Russell, 1988) and Cianoacrylate (Jasmin et al., 1993). In Chinese traditional medicine, licorice remains one of the most commonly prescribed herbs and has been used in the treatment of various ailments ranging from tuberculosis to peptic ulcers (Huang, 1993). Since licorice has been shown to have antiinflammatory properties in herbal medicine (Shibata, 2000), the present study undertook to assess the efficacy of the application of licorice bioadhesive hydrogel patches formulated from tragacanth gum in the pain control of RAS. As dental practitioners and researchers become better trained in oral medicine, it is anticipated that the physiopathology, prevention and treatment of RAS will improve in the future.

MATERIAL AND METHODS

Patients. A total of 15 patients, 5 women and 10 men (age 22–35 years, mean ± SD, 26.27 ± 4.28 years) with...
a history of recurrent aphthous stomatitis and currently suffering from ulceration located in the anterior region of the mouth were selected from patients referred to the School of Dentistry in Babol (North of Iran). Excluding criteria were: iron deficiency, inflammatory and allergic conditions, history of medication, smoking, pregnancy, wearing denture, receiving antibiotics for RAS and those who were unable to apply patches. The clinical diagnosis of minor RAS was made by the presence of a well-demarcated, painful ulcer on the non-keratinized oral mucosa. The previous history of their RAS for the duration and periods between episodes were recorded. The design of study was approved by the Ethics Committee of Babol Medical University and all patients signed a well-explained written informed consent form.

The bioadhesive patches. Two series of patches, a base and patches containing licorice were used in this study. The patches were made from a pharmaceuticals grade tragant gum and were prepared in the Department of Pharmacology of Babol Medical University. Licorice extracts were isolated by maceration of *Glycyrrhiza glabra* with chloroform in 48 h. After filtering and drying the extract, patches containing licorice 1% were made. The patches were sealed in foil sachets. They were non-tacky and non-adherent in the dry environment and are easily applied to the site. Contact with saliva at the site, hydrated the gel, forming an adhesive hydrogel which attaches to the mucosa.

Clinical procedure. This was a placebo-controlled, observer-blind, consecutive-group clinical trial. The study was performed over three clinical visits along three episodes of RAS. The first episode of RAS was used to gather some baseline data of the subjects. The subjects completed this step and were designated the no-treatment group. The second and third episodes were assigned to bioadhesive without licorice and bioadhesive with licorice, respectively. At the first visit the following steps were undertaken: (a) Written signed informed consent was obtained from each patient willing to participate in a protocol approved by Research Review Board, Babol University of Medical Sciences, Babol, Iran. (b) Complete medical and dental histories were taken. (c) A routine clinical examination was made, with special attention to the current ulcer. (d) A patch was applied to the ulcer by the examiner and was explained to the patient. (e) A detailed personal and ulcer assessment questionnaire was filled out including: site of the ulcer, size of the ulcer, duration, frequency, pain, previous treatment and received medications and the healing time of the ulcer episodes. (f) A package that contained five sets of four biopatches was given to each patient (q.i.d.) for 5 days of treatment.

Then the patients were asked to moisten the patches with saliva for better adherence and apply them to the ulcers, with a cut-off time of up to 20 min. After these steps the patients recorded the parameters and the pain scores again.

Daily ulcer parameter records. The daily ulcer parameter recorded a 10 cm visual analogue scale (VAS). The patient was requested to record the level of pain of the ulcers, four times a day before meals (in the morning, in the evening, at night) and 20 min before bedtime. On the daily sheet, patients should note the time they put on the patch, the time the patch was detached from the ulcer and the time they felt the pain returned. The size of ulcer and the necrosis diameter was recorded by the examiner on days 1, 3, 5 of the study. The final improvement of pain was recorded by the patient.

**Statistical analysis.** Wilcoxon U-test, paired t-test and repeated measures were used to test for significant associations observed between, before and after pain scores, and the VAS scores of the 3 days, and also ANOVA or Kruskal-Wallis H-test were used to test differences in pain experience with and without the oral patches. The 5% level of significance was used.

**RESULTS**

All 15 patients (5 female and 10 male mean ± SD age 26.27 ± 4.28) recorded three consecutive RAS episodes. The first episodes of RAS were investigated to obtain some baseline information on the subjects. The second and the third episodes were assigned to bioadhesive with or without licorice.

**Comparison of daily pain intensity in different treatments**

The Kruskal-Wallis H-test showed that the patients experienced significantly greater baseline pain when the ulcers were not covered by biopatches than when covered by patches with or without licorice (*p* < 0.01). The mean VAS of covered ulcers were significantly decreased compared with the baseline episode on day 3 (*p* = 0.018) and day 4 (*p* = 0.033) of treatment. The test showed a significant VAS reduction of covered ulcers by licorice containing patches compared with the uncovered ulcers on day 2 (*p* = 0.031), day 3 (*p* = 0.008), day 4 (*p* = 0.02) and day 5 (*p* < 0.01).

The repeated-measures test showed that the average VAS in the treatment period with licorice patches was decreased compared with biopatches as the placebo (Fig. 1), but the inter-group changes were decreased significantly (*p* < 0.0001) (Fig. 2).

![Figure 1. Mean (± SEM) comparative pain intensity based on VAS during day 0–5 of study in three different periods of the treatment (*n* = 15 subjects in each period). Complete pain relief on day 5 was achieved by licorice biopatch compared with the no-biopatch group, using one-way ANOVA, post-hoc test (*p* < 0.001).](DOI: 10.1002/ptr)
Figure 2. Trend of pain intensity by day of study in three different periods of treatment. Inter- and intra-group changes are shown in the curves ($p = 0.001, n = 15$).

The diameter of inflammatory halo

This diameter was measured on days 3 and 5 of treatment. The one-way ANOVA test showed a significant decrease in the diameter of the halo of inflammation of the ulcers only on day 5 ($p = 0.043$) (Table 1, Fig. 3). Within-group analysis by paired $t$-test showed a significant decrease in the diameter of the halo of the ulcers only on day 5 ($p < 0.001$) (Fig. 3, Table 1).

The diameter of necrotic zone of the ulcer

This diameter was measured on day 3 and day 5 of treatment. The one-way ANOVA test showed a significant decrease in the diameter of the necrotic zone of the ulcers only on day 5 ($p < 0.001$) (Table 1). Within-group analysis by paired $t$-test showed a significant decrease in the diameter of ulcers in the licorice biopatch treatment periods on days 3 and 5 ($p = 0.003$) (Fig. 4, Table 2).

Complete pain relief time

Within the period of treatment, the patients claimed a decrease in the number of days to complete relief of the pain from a mean for the study group of 6.86

Table 1. Mean ± SD diameter of halo of the aphthous ulcer (mm) on days 3 and 5 of treatment

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Day 1 of treatment</th>
<th>Day 3 of treatment</th>
<th>Day 5 of treatment</th>
<th>$p$ value (Friedman test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-treatment</td>
<td>4.33 ± 2.12</td>
<td>3.63 ± 1.38</td>
<td>3.53 ± 1.48</td>
<td>0.337</td>
</tr>
<tr>
<td>Vehicle biopatch</td>
<td>5.15 ± 2.13</td>
<td>3.53 ± 1.09</td>
<td>2.90 ± 1.10</td>
<td>0.042</td>
</tr>
<tr>
<td>Licorice biopatch</td>
<td>5.06 ± 2.1</td>
<td>3.56 ± 1.16</td>
<td>2.53 ± 1.00</td>
<td>0.0001</td>
</tr>
<tr>
<td>$p$ value (one-way ANOVA)</td>
<td>0.512</td>
<td>0.996</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

Post-hoc Tukey test showed a significant difference in aphthous ulcer diameter between no-treatment (without biopatch period of study) and licorice containing biopatch only on day 5 ($p = 0.037$).

Table 2. Mean (± SD) diameter of aphthous necrotic zone (mm) on days 3 and 5 of treatment

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Day 1 of treatment</th>
<th>Day 3 of treatment</th>
<th>Day 5 of treatment</th>
<th>$p$ value (Friedman test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-treatment</td>
<td>2.13 ± 0.81</td>
<td>2.83 ± 1.42</td>
<td>2.26 ± 1.03</td>
<td>0.052</td>
</tr>
<tr>
<td>Vehicle biopatch</td>
<td>2.75 ± 0.83</td>
<td>2.33 ± 0.69</td>
<td>1.96 ± 1.06</td>
<td>0.101</td>
</tr>
<tr>
<td>Licorice biopatch</td>
<td>2.77 ± 0.82</td>
<td>2.26 ± 0.86</td>
<td>1.60 ± 1.08</td>
<td>0.015</td>
</tr>
<tr>
<td>$p$ value (one-way ANOVA)</td>
<td>0.066</td>
<td>0.971</td>
<td>0.022</td>
<td></td>
</tr>
</tbody>
</table>

Post-hoc Tukey test showed a significant difference in aphthous necrotic zone diameter between no-treatment (without biopatch period of study) and licorice containing biopatch only on day 5 ($p = 0.020$).
The aim of this study was to investigate whether the effect of licorice bioadhesive patches, applied four times a day until complete healing, would significantly reduce the pain associated with RAS and also promote healing and pain relief. The bioadhesive administered in this study, consistently provided significant symptomatic pain relief experienced by patients ($p < 0.01$). Despite the effect of licorice biopatches on pain relief being considerably more than the no biopatch treatment period, the profile was almost equal to the results of treatment with biopatches without licorice. This was particularly evident during the early days of the ulcers, when the ulcers were covered by biopatches. These findings of a benefit of patch therapy are in agreement with those of Mahdi et al. (1996) and Rodu and Russell (1988), who reported a significant reduction in the severity of aphthous ulceration following application of hydrogel patches.

Another study administering hydroxypropyl cellulose mucosal adhesive film containing local analgesic for treating RAS in two patients reported a clinical improvement in both cases (Yotsuyanagi et al., 1985). The first mechanism of action of the patches is probably mechanical protection. The dry patches in contact with mucin-coated epithelium form a swollen bioadhesive hydrogel layer that directly adheres to the ulcerated site. Some products such as Orabase (an oleaginous ointment base of sodium carboxymethyl cellulose) with or without corticosteroids for treatment of RAS and other oral inflammatory conditions have been shown to have an equal protective and curative effect (Jasmin et al., 1993; Kutcher et al., 2001; Mahdi et al., 1996; Scully et al., 2003).

Those findings confirm the results of the present study that biopatches containing licorice were almost equally effective as the preparation of patches without licorice, suggesting that the mechanical mucosal protection alone was important in reducing pain and promoting healing. In this study the diameter of the inflammation zone was significantly reduced with biopatches containing licorice on day 5 (Table 1, Fig. 2). Based on a previous study, this effect could be due to the anti-inflammatory effect of licorice (Kobayashi et al., 1993) and the anti-inflammatory effect of licorice is proven (Shibata, 2000). Licorice has been administered in traditional medicine for the treatment of acid-based gastrointestinal disorders. The main reason for this indication has been focused on the anti-inflammatory properties (Barfod et al., 2002; Fujisawa et al., 2000; Kobayashi et al., 1993; Roehr, 1998).

The anti-inflammatory property of licorice in the present study created an increasing role in the healing process compared with other periods of the study. On the other hand, in the licorice group, pain was reduced more than in the other groups. This effect could be exerted by the anti-inflammatory property of licorice (Roehr, 1998), but the mechanical protection of biopatches with or without licorice is a very considerable factor for reducing symptoms of RAS (Scully et al., 2003). Also the mechanical protection effect of biopatches could exert an overlapping action on the effect of licorice in this investigation; it may have been possible to show a more significant effect if the doses of licorice had been increased, i.e. the effect of licorice could be dose-dependent. Although a previous study used a mouthwash form of deglycerinized licorice and showed a significant antiaphthous effect (Das et al., 1989), an investigation should be carried out with biopatches containing different concentrations of licorice in further studies.

In conclusion, according to the results it may be suggested that licorice biopatches can exert an extra improving effect on aphthous ulcers in comparison with the no-treatment group. Also the protective effect of biopatches has an important role in healing processes.
of the ulcers; it is recommended that the clinical effects of licorice should be investigated. Some new studies could be designed with different higher concentrations of licorice in the patches.

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REFERENCES


