Comparison of a New Medicinal Plant Extract and Triamcinolone Acetonide in Treatment of Recurrent Aphthous Stomatitis

SUMMARY

The aim of this study was to evaluate the effectiveness of a new medicinal plant extract (Ankaferd Blood Stopper®, ABS) on the healing process of aphthous ulcers. A total of 50 patients with recurrent aphthous stomatitis (RAS) who have had currently a single oral ulcer in the mouth were included in the study. Patients were randomly divided in 2 groups: ABS group and Kenacort group (triamcinolone acetonid). Data were collected by questionnaire regarding age, gender, RAS history including the number of ulcer episodes in 1 month, the number of ulcers in each episode, mean healing time of previous ulcers and the mean disease duration. Ulcer size, ulcer related pain, evaluated by VAS and effectiveness index (EI), was noted in each clinical examination.

In the present study, no significant differences were seen in demographic and RAS related data between the 2 groups. In ABS and Kenacort group, the mean ulcer size and VAS scores were statistically reduced at day 3 and day 7 compared to baseline values, yet no significant difference was observed between the 2 groups in these time points. The effects of ABS in RAS treatment were found similar to that of Kenacort-A orabase cream in this study. Since our study is a preliminary one, additional studies with larger number of subjects, investigating the antibacterial and immunologic effect of this unique medicinal product will help to understand the treatment mechanism of ABS and evaluate clinical benefits and any possible adverse effects of the application of ABS spray.

Keywords: Recurrent Aphthous Stomatitis; Triamcinolone Acetonide; Ankaferd Blood Stopper

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal diseases. It is characterized by painful recurrent single or multiple ulcerations of oral mucosa with a frequency of up to 5-25%³. Based on the size and number of ulceration, 3 subtypes have been classified: minor aphthous ulcers, major aphthous ulcers and herpetiform ulcers⁴.

Although RAS represents a very common oral lesion, its etiology is unknown. Many factors, such as local trauma, stress, immunodeficiency, infection, systemic diseases, haematopoietic deficiency, allergic agents and hormonal changes might play a role in the pathogenesis of RAS⁶. Since etiology is unknown, no curative therapy is available at present. Some medications including topical and systemic agents can be applied to relieve pain, alleviate inflammation, accelerate healing and prevent or reduce recurrence. Although most systemic medications are effective, they are limited due to their side effects.

Therefore topical agents, including glucocorticoids, antibiotics, analgesics, and laser therapy are prominent options in the treatment of RAS³,15.

Ankaferd Blood Stopper® (ABS) is a unique folkloric medicinal plant extract, which has been used as a haemostatic agent. ABS comprises a standardized
mixture of 5 different plants - Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica\textsuperscript{7,8}. Each of these plants has some effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators; however, the basic mechanism of action for the haemostatic effects of ABS is currently unknown\textsuperscript{14,20,22}. In a recent study it was mentioned that ABS might increase healing and decrease inflammation in bone healing in an animal model\textsuperscript{10}. Yet, there is no information about the effects of ABS on soft tissue in oral medicine.

Therefore, the aim of this study was to evaluate effectiveness of ABS on healing process of aphthous ulcers.

**Materials and Methods**

**Study Design**

Data were collected by questionnaire regarding age, gender, RAS history including the number of ulcer episodes in 1 month, the number of ulcers in each episode, the mean healing time of previous ulcers and the mean disease duration. Ulcer size and ulcer related pain, evaluated by VAS and effectiveness index (EI), was noted in each clinical examination.

**Patient Selection**

A total of 50 patients with RAS (F/M: 27/23, mean age: 35.51±10.70/35.86±9.29 years) who have had currently a single oral ulcer were included to standardize the evaluation of RAS related factors in the study. All subjects were active during past 6 months. Patients were randomly divided in 2 groups: ABS group (folkloric medicinal plant extract study group; ABS® Pump Spray, Immun Gida Ilac Kozmetik, İstanbul, Turkey), and Kenacort group (triamcinolone acetonid; Kenacort-A Orabase® Pomad, 0,1% Triamcinolone acetonid, Bristol-Myers Squibb İlaçlar Inc. İstanbul, Turkey), which was the control group. In each group participated 25 in the study: the ABS group (n=25; M/F:11/14, mean age: 34.36±8.75/32.78±11.04 years) and the Kenacort group (n=25; M/F:12/13, mean age: 37.25±9.93/38.46±9.89 years).

The inclusion criteria for patients were to be over 18 years old and to have 1 well-demarcated, painful ulcer in easily accessible area of the mouth for less than 48 hours. The exclusion criteria were the presence of local trauma, infection and systemic disease regarding haematological, gastrointestinal or dermatological diseases in which RAS may be a part of their clinical presentation. In addition, patients who received any medication for RAS treatment including systemic steroids, vitamins, antibiotics, oral retinoids or immuno modulatory agents within 3 months, and alcohol and smoking users, were excluded from the study.

All the patients were instructed to rinse their mouth with tap water prior to administration of the agents and strictly warned not to use any other products for prevention or treatment of aphthous ulcers while participating in this study. In the ABS group, patients were instructed to apply ABS pump spray for 2 pumps to the appointed ulcer, 3 times a day for 7 days. In the Kenacort group, patients applied Kenacort-A orabase cream over the ulcer surface 3 times a day for 7 days. It was recommended not to eat or drink anything for 30 minutes after application of the agents in both groups.

Subjects were informed that there is no conventional scientific study supporting the ABS as a treatment, but clinical experiences suggest that it plays a role in tissue healing and reduction in pain. All patients were given an oral explanation and detailed informed consent form was signed by patient according to the Helsinki declaration.

**Evaluation of Ulcer Size**

Ulcer size was measured at maximum and minimum diameters of the ulcer by using a calibrated periodontal probe. The clinical examinations were carried out at the initial visit (day 0), day 3 and day 7, as described previously\textsuperscript{12}. The 2 measurements were multiplied to calculate surface area of the ulcer (mm\textsuperscript{2}).

**Evaluation of Pain**

To evaluate pain, a visual analogue scale (VAS) consisting of a 10-cm horizontal line between the poles of “no pain” to “unbearable pain” was used. Patients were told to mark the line with a vertical mark at the point that best represented the present pain level of the ulcer, at days 0, 3 and 7. At the end of therapy, all patients were also asked to self-report any adverse effects of ABS pump spray or Kenacort-A orabase cream.

**Evaluation of Effectiveness Index**

An effectiveness index (EI) of the ulcer was calculated with the following formula (with V3 and V7 referring to the values measured at days 3 and 7, and V1 referring to the baseline value measured at day 0):

\[
EI (\%) = \left[ (V1-V3 \text{ or } V7) - V1 \right] \times 100.
\]

Effectiveness indices were evaluated on a 4-rank scale:

1) Healed; EI ≥95%
2) Marked improvement; EI ≥70% to <95%
3) Moderate improvement; EI ≥30% to <70%
4) No improvement; EI <30%

**Marked improvement rate (MIR)** referred to EI 1+2 (EI ≥ 70%) and **improvement rate (IR)** referred to EI 1+2+3 (EI ≥ 30%)\textsuperscript{12}.

**Statistical Analysis**

Unpaired-t test as non-parametric test was performed to compare group differences in healing time of ulcer, ulcer size and pain level since the number of patients...
was less than 30. Paired-t test was used to compare the differences of ulcer size and pain level according to different time points in each group. Chi-square test was used to evaluate the effectiveness index. (SPSS 12.0 for Windows; SPSS, Chicago, IL). P value of <0.05 was considered to be statistically significant at 95% confidence interval.

Results

In the present study, no significant differences were seen in age and gender between groups. Before study, baseline RAS data including the number of ulcer episodes in 1 month, the number of ulcers in each episode and the mean healing time of previous ulcers were similar in both groups (ABS: 1.5±0.5; 1.3±0.47; 7.63±1.87 day respectively, vs. Kenacort: 1.48±0.5; 1.36±0.48; 7.87±2.65 day) (p=0.58, p=0.77 p=0.85, respectively). The mean disease duration was 6.70±6.72 years in the ABS group and 6.30±6.11 years in the Kenacort group (p=0.82). In addition, the mean baseline ulcer size was similar in the ABS (10.89±3.12 mm²) and in the Kenacort group (10.14±2.88 mm²) (p=0.381). At day 3 and 7, no statistically significant difference in ulcer size was found between the groups (Tab. 1).

Table 1. Summary of ulcer size reduction

<table>
<thead>
<tr>
<th>Time point</th>
<th>ABS Group (mm²)</th>
<th>Kenacort Group (mm²)</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>10.89±3.12</td>
<td>10.14±2.88</td>
<td>0.381</td>
</tr>
<tr>
<td>Day 3</td>
<td>6.97±2.03</td>
<td>6.18±2.22</td>
<td>0.197</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.98±2.31</td>
<td>2.28±1.72</td>
<td>0.309</td>
</tr>
</tbody>
</table>

*Student-t test was used in the analysis

In the ABS group, the mean ulcer size was statistically reduced at day 3 (6.97±2.03 mm²) and day 7 (1.98±2.31 mm²) compared to baseline values (p=0.000 p=0.008, respectively). Similar results were seen in the Kenacort group (day 3: 6.18±2.22 mm² and day 7: 2.28±1.72 mm²) (p=0.000 p=0.021, respectively). Yet, no significant difference was observed between the groups in these time points (p=0.197 and p=0.309, respectively).

When the EI in ulcer size reduction was evaluated, in the ABS group, MIR and IR were 0% and 80% at day 3 and 80% and 100% at day 7, whereas MIR and IR were 0% and 76% at day 3 and 64% and 100% at day 7 in the Kenacort group. No significant difference was found between the groups (Tab. 2).

Table 2. Effectiveness index in ulcer size reduction

<table>
<thead>
<tr>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS Group</td>
<td>Kenacort Group</td>
</tr>
<tr>
<td>n:25</td>
<td>n:25</td>
</tr>
<tr>
<td>(1) Heal</td>
<td>0</td>
</tr>
<tr>
<td>(2) Marked Improvement</td>
<td>0</td>
</tr>
<tr>
<td>(3) Moderate Improvement</td>
<td>20</td>
</tr>
<tr>
<td>(4) No Improvement</td>
<td>5</td>
</tr>
<tr>
<td>p *</td>
<td>0.733</td>
</tr>
<tr>
<td>Marked Improvement Rate [(1)+(2) (EI ≥ 70%)]</td>
<td>0%</td>
</tr>
<tr>
<td>Improvement Rate [(1)+(2)+(3) (EI ≥ 30%)]</td>
<td>80%</td>
</tr>
</tbody>
</table>

*Chi-square test was used in the analysis

Table 3. Summary of ulcer pain reduction

<table>
<thead>
<tr>
<th>VAS</th>
<th>ABS Group</th>
<th>Kenacort Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.56±1.08</td>
<td>7.96±1.30</td>
<td>0.244</td>
</tr>
<tr>
<td>3</td>
<td>5.56±1.41</td>
<td>5.68±1.10</td>
<td>0.740</td>
</tr>
<tr>
<td>7</td>
<td>1.40±1.38</td>
<td>1.84±1.37</td>
<td>0.265</td>
</tr>
</tbody>
</table>

*Student-t test was used in the analysis

Mean baseline VAS scores of the ABS group (7.56±1.08) and the Kenakort group (7.96±1.30) were similar (p=0.244). At day 3 and 7, no statistically significant difference in VAS scores was found between the groups (Tab. 3).

In the ABS group, the mean VAS score was statistically reduced at day 3 (5.56±1.41) and day 7 (1.40±1.38) compared to baseline values (p=0.000 and
In the ABS group, MIR and IR were 0% and 28% at day 3 and 84% and 100% at day 7, whereas MIR and IR were 0% and 44% at day 3 and 64% and 100% in the Kenacort group. No significant difference was found between the groups (Tab. 4).

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ABS Group</td>
<td>Kenacort Group</td>
</tr>
<tr>
<td></td>
<td>n:25</td>
<td>n:25</td>
</tr>
<tr>
<td>(1) Heal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(2) Marked</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Improvement Rate</td>
<td>(1)+(2) (EI ≥ 70%)</td>
<td>0%</td>
</tr>
<tr>
<td>p*</td>
<td>0.189</td>
<td>0.273</td>
</tr>
<tr>
<td>(3) Moderate</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Improvement Rate</td>
<td>(1)+(2)+(3) (EI ≥ 30%)</td>
<td>28%</td>
</tr>
<tr>
<td>(4) No Improvement</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Improvement Rate</td>
<td>(1)+(2)+(3) (EI ≥ 30%)</td>
<td>100%</td>
</tr>
</tbody>
</table>

* Chi-square test was used in the analysis

According to the patients’ remarks no side effects were seen in both groups. Yet, some difficulties were seen in the usage of agents. Patients had an unpleasant bitter taste and extrinsic staining of the teeth in ABS group while the adherence of Kenacort-A orabase cream to ulcer surface was the main problem during the application in Kenacort group.

**Discussion**

The present study was designed to evaluate the effects of ABS on tissue healing of RAS. Control group was composed of Kenacort-A orabase cream (a topical steroid cream; 0,1% Triaminsolon asetonid) which is an extensively used topical steroid agent for RAS treatment. Placebo group was not used in this study design as it should be unethical. Evaluation of ulcer size as an objective index and pain level as a subjective index in RAS were used in the study. Since no significant difference was observed between groups according to 3 different time points, similar effects were obtained by 2 different treatment modalities.

ABS is a standardized extract from the following plants: *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, *Urtica dioica* and the antibacterial activity of these plants has been investigated previously7,23. Thus *Thymus vulgaris* has shown bacteriostatic activity for Gram positive and Gram negative bacteria13 as well as *Glycyrrhiza glabra*, *Vitis vinifera* and *Alpinia officinarum* have been shown to be antibacterial8, with *Urtica dioica* being endowed with noticeable antibacterial activity against Streptococcus pyogenes, Staphylococcus aureus and Staphylococcus epidermidis11. Each of these plants has some effects on cellular proliferation, blood cells, endothelium and vascular dynamics20.

ABS as a medicinal product has been approved in the management of external haemorrhage and dental surgery bleedings based on the safety and efficacy reports indicating its sterility and non-toxicity9. ABS successfully control bleeding related to dental treatment, can give the dental profession more time and confidence during surgery. It is useful not only for local hemostasis in periodontal surgery and dental extractions, but also for wound healing and prevention of infections5.

Isler et al10 investigated the effects of ABS on early bone healing in a rat model. The conclusion was reached that defects treated with ABS showed more intense new bone formation and less occurrence of necrosis, which may be related to the increased speed of healing and decreased inflammation, associated with the antioxidant activity of the components of the ABS. Akbal et al1 designed an experimental study to show the effectiveness of ABS in enhancing mucosal healing and suppressing stricture formation caused by caustic oesophageal injuries. The results of that experimental study showed that oral ABS application prevents inflammation, scar formation, weight loss and mortality in oesophageal caustic injuries.

RAS is one of the most common oral mucosal disease, has been studied for many years by numerous
investigators and, unfortunately, no curative management is available at present. Most clinical trials seem to suggest that topical or systemic steroids provide the best long-term management and the least number of significant side effects if used properly\(^1\). The use of topical and systemic steroids in an attempt to manage RAS is based on the presumption that aphthae are the result of a non-infectious inflammatory process. The anti-inflammatory actions of corticosteroids include: (1) a reduction in exudation of leukocytes and plasma constituents; (2) maintenance of cellular membrane integrity with prevention of cellular swelling; (3) inhibition of lysozyme release from granulocytes and inhibition of phagocytosis; (4) stabilization of membranes of intracellular lysozymes containing hydrolytic enzymes; (5) decreased scar formation by inhibiting proliferation of fibroblast; and (6) possible effect on antibody formation when administered in large doses\(^2,3\).

Pain reduction is a recognized feature of steroid treatment for aphthous ulceration. Rhodus and Bereuter\(^4\) found that 20% of patients treated with triamcinolone acetonid reported pain reduction in the first 3 days of treatment. Al-Na’ma\(h\) et al\(^5\) compared a paste containing dexamethasone and triamcinolone acetonid in Orabase in aphthous ulceration treatment. They reported faster healing of the ulcer after the dexamethasone treatment compared to triamcinolone acetonid, although statistically not significant.

In this study, no significant difference was found in pain reduction between the 2 groups at day 3 and 7. Since MIR and IR were also similar in the groups, and no significant difference was found between the groups at day 3 and 7. According to the results of VAS scores in both groups, we found noticeable reduction in pain with both ABS and Kenacort, and concluded that both agents improved the pain symptoms significantly.

Reduction in ulcer size is another important issue for RAS. Al-Na’mah et al\(^6\) reported reduction in ulceration size 2 days after application of the dexamucobase preparation. They found that size reduction of ulcers was significantly faster in the dexamucobase group (66.0%) compared to the Kenalog in Orabase group (18.9%).

Rodriguez et al\(^7\) designed a study to compare a corticosteroid (0.05% clobetasol propionate) prepared as an oral paste versus 5% amlexanox oral paste, which in large-scale studies carried out in the USA proved effective in the treatment of RAS. Reduction of pain score of the index ulcer on a VAS and reduction in the surface area of the same lesion following treatment were considered the main outcomes of interest. Both medications significantly reduced pain magnitude and the index ulcer’s size on days 2 and 5 compared with day 0 without adverse reactions, and no statistical differences between groups of the study medications were found.

In this study, the mean ulcer size was similar in both groups at day 0. No statistically significant difference was found between the groups at days 3 and 7. However, the mean ulcer size was statistically reduced, both in ABS and Kenacort group, at days 3 and 7 and these results show that both agents were effective in ulcer healing. MIR and IR were also similar in both groups at days 3 and 7.

Although ABS significantly reduced ulcer size and alleviated ulcer pain, the mechanism is still unclear. Given the broad biological composition of the substance, different theories can be probable. If the infectious agents are considered a cause of RAS than the antibacterial effects and bacteriostatic activity of ABS might be the therapeutic mechanism. If the immune or inflammatory factors are important in RAS etiology, than the anti-inflammatory properties of some extracts included in ABS might be the effective mechanism.

Combination of these plants in ABS appears to provide a unique composition for tissue oxygenation and physiological haemostatic process. The effects of ABS on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cellular mediators should be further investigated in order to determine its potential role in many pathological states and oral mucosa healing.

In conclusion, the effects of ABS spray in RAS treatment was found similar to that of Kenacort-A orabase cream. 2 medications decreased the ulcer size and pain suffering in same manner. ABS spray provides an alternative management of RAS as a natural product when compared with Kenacort and may be used effectively and safely in RAS treatment. Since our study is a preliminary one, additional studies with larger number of subjects, investigating its antibacterial and immunologic effects, will help to understand the treatment mechanism of ABS and evaluate clinical benefits and any possible adverse effects of the ABS spray application.

References


**Correspondence and request for offprints to:**
Assoc.Prof. Meltem KORAY
İstanbul Üniversitesi, Dis Hekimliği Fakültesi
Agz, Dis, Cene Cerrahisi Anabilim Dalı 6. Kat
Fatih, İstanbul
E-mail: mkoray@istanbul.edu.tr