Clinical success of lysine in association with serumal and salivary presence of HSV-1 in patients with recurrent aphthous ulceration

Feyza Otan Ozden¹, Ahmet Yasar Turanli², Gokhan Acikgoz¹, Cafer Eroglu³

¹Department of Periodontology, Faculty of Dentistry; ²Department of Dermatology, Faculty of Medicine; ³Department of Clinical Microbiology and Infectious Diseases, Faculty of Medicine; Ondokuz Mayis University, Samsun, Turkey.

Summary
Objective: The aim of this study was to investigate the clinical effects of L-lysine monohydrochloride which is known to be a natural viral inhibitor based on a probable role of herpes simplex virus on recurrent aphthous ulcer (RAU) etiology.

Method: Thirty patients were divided into placebo and lysine treatment groups. After the end of two months' therapy, clinical changes of ulcers were recorded and the effectiveness of the study was measured by the success degrees given by the patients. Herpes simplex type-1 (HSV-1) presence was examined by ELISA in serual samples and by real-time PCR technique in salivary samples of the patients.

Results: The number and the recurrence of the ulcers decreased significantly in lysine group. Most patients in lysine group found the therapy more successful. The difference in HSV-1 IgG was statistically significant between the healthy and aphthous ulcer groups while the difference in HSV-1 DNA was not.

Conclusion: The presence of HSV in saliva suggests a possible role in RAU etiology, and it seem to be worthwhile to attempt antiviral therapies for RAU treatment.

Key words: Herpes virus; L-lysine monohydrochloride; Recurrent aphthous ulcer

Introduction
Recurrent aphthous ulceration (RAU) is one of the most common oral mucosal diseases and it affects nearly %20 of the general population. It is characterized with recurrent painful, round-shaped ulcers surrounded with an erythematous halo [1]. There is neither histopathological nor biochemical differential diagnosis for RAU and its therapy modalities are still symptomatic. Local and systemic conditions together with genetic, immunologic and microbiological factors were shown to be possible etiological factors. Various treatment modalities have been tried previously due to the proposed etiological factors. However there is still not a consensus for the successful treatment of RAU in order to prevent recurrences. Topical steroids are the most useful medicaments to shorten healing and ease the pain of ulcers [2]. Mouthwashes with chlorhexidine gluconate and tetracycline have also been used for symptomatic treatment. The side effects of tetracyclines and immunosuppressive drugs restricted their usage [3]. Some immunosupportive drugs like levamisole (Ergamisol) and LongoVital were thought to be successful [4-6]. Some alternative treatment modalities including surgical excision of the ulcers, laser, vitamine supplements, diet elimination, escape from daily stress were all tried [7]. In spite of different medicaments, none of them prevented recurrences.

There are contradictory reports about the presence of viral factors in RAU etiology. Human herpes virus (HHV) family has been the most intensively studied virus group [8-16] and herpes simplex virus (HSV) has been thought to be involved in RAU pathogenesis [17-19].

Lysine is an essential aminoacid and was used to depress the probable presence of HSV in treatment of recurrent herpes simplex infections. It inhibits arginine usage that is necessary for the growth of HSV and was indicated to decrease recurrences. Although there have been a few reports regarding it as a treatment choice for both recurrent herpes labialis infections [20-24] there is only one report discussing its effect for RAU [25].

A relationship between lysine and RAU has not been established scientifically. Lysine may suppress HSV replication in RAU as well as in herpes labialis.

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Our purpose is to evaluate the etiologic role of herpes simplex virus type 1 (HSV-1) according to HSV-1 IgG antibody and HSV-1 DNA positivity between RAU and non-RAU groups and to compare the effectiveness of the naturally viral inhibitor lysine-treated and non-treated (placebo) aphthous ulcer groups according to their HSV-1 positivity and clinical aspects.

**Materials and methods**

30 subjects (12 women and 18 men), 19 to 55 years of age (mean age, 34.0 ± 9.7) with minor recurrent aphthous ulceration (MiRAU) and 17 healthy subjects (2 women, 15 men), 22 to 45 years of age (mean age, 26.7 ± 5.5) without histories of oral mucosal disease were included in this study.

The participants were selected for a history of at least two or more MiRAU attacks in a month of the previous year and diagnosis had been made by the clinical characteristic of the lesions followed by a careful anamnesis by the same dermatologist. Selection criteria for this form of RAU depended on its high prevalence in the population and elimination of other possible oral mucosal disorders. They were all systemically healthy otherwise. Approval of the local ethic committee was obtained and patients were asked if they would try a supplement to reduce their RAU complaints and written permissions were obtained.

Saliva samples were evaluated for the detection of HSV-1 DNA by real-time polymerase chain reaction (RT-PCR), serum samples were evaluated for the detection of HSV-1 IgG by enzyme-linked immunosorbent assay (ELISA).

At the beginning of the study serum and saliva samples were collected from the control group and from subjects with MiRAU and all samples were analyzed for HSV-1 IgG and HSV-1 DNA at the Microbiology Department of Ondokuz Mayis University. ELISA technique was performed in microtitration plates (Enzygnost Anti-HSV/IgG test plate, Dade Behring Marburg GmbH, Marburg, Germany) according to the instructions of the manufacturer and analyzed at 450 nm waveband by photometry (Genesis RMP 150 immunoassay analyzer, Tecan Group Ltd., Mannedorf, Switzerland). Unstimulated saliva was collected from the subjects for 10 minutes (once-twice in a minute) preferably before meals and smoking in order to prevent contamination. For the RT-PCR detection of HSV-1 DNA, fortysseven saliva specimens 2 ml of each were stored at -70°C until assay. Positive results were evaluated under the light of the manufacturer’s given instructions for cut-off values. (Roche Diagnostics GmbH, Mannheim, Germany).

Thirty patients who had a complaint of RAU were randomly divided into two groups; fourteen patients began with a placebo, and sixteen patients started with lysine tablets. Volunteers in the placebo group took tablets containing starch powder twice a day. Volunteers of the lysine group were instructed to take two tablets of Herpetrol® (Alva-Amco Pharmacal Cos., Inc., Niles, IL, USA) daily with meals and to take four when prodrome developed. Herpetrol® dietary supplement is an enhanced lysine anti-oxidant complex containing 630 mg of L-lysine as monohydrochloride (LMH) per two tablets.

Patients were followed for two months of trial according to the criterias of ulcer recurrence (number of episodes), ulcer duration, number of ulcers per attack and severity of symptoms (redness, itching, burning). Patients were also asked for the effectiveness of the therapy and directed to give a success degree (from 1 to 10) to the treatment in order to measure the degree of success. At the end of the study a questionnaire was completed for each patient and recorded by the same investigator.

Post treatment values for RT-PCR detection of HSV-1 DNA and HSV-1 IgG detection by ELISA were repeated with the same protocol as described for pre-treatment. Statistical differences between the two groups were evaluated by chi-square test while the success points were compared statistically by Student’s t test.

**Results**

In the pre-treatment phase of the study, HSV-1 IgG antibody was detected in 13 of 17 healthy subjects (76.5%) while all 30 patients with RAU (100%) tested positive; this difference was found to be statistically significant (χ²=7.71, P<0.05). HSV-1 DNA was present in 1 of 17 healthy controls (5.9%) and in 4 of 30 RAU patients (13.3%); the difference was estimated insignificant (χ²=2.44, P>0.05) (Fig.1).

**Figure 1.** HSV-1 IgG and HSV-1 DNA positivity between the control and the patient group.
After two months’ therapy, serumal HSV-1 IgG and salivary HSV-1 DNA positivities were re-evaluated. 100% positive results for HSV-1 IgG did not change and there was also no significant change in the HSV-1 DNA values. The pre- and post-treatment results are shown in Table 1.

Three of sixteen (18.7%) patients in the lysine group never experienced aphthous ulcers during the treatment period. Ulcers never developed in 4 of 14 placebo patients (28.5%). Ten patients of the lysine group found that ulcer recurrence was decreased (62.5%) while 3 of them told the difference was not significant (18.7%). In placebo group, only two patients (14.2%) found a decrease in ulcer recurrence although one patient (7.1%) experienced an increase in ulcer recurrence. Seven placebo patients recorded significant decrease in ulcer recurrence (50%). Seven patients of each group told that the duration didn’t change (43.7% and 50% for lysine and placebo groups, respectively). Six patients of lysine group (37.5%) and three patients of placebo group (21.4%) reported decreased duration of the lesions. There was a statistical significance in the recurrence of ulcers between both of the groups ($\chi^2=7.97$, P<0.05), but the duration of the lesions did not change significantly ($\chi^2=1.01$, P>0.05).

Severity of the symptoms (itching, burning, redness) didn’t change significantly in both of the groups (4 and 8 in lysine and placebo groups, respectively) ($\chi^2=5.96$, P>0.05). One patient in lysine group experienced severe lesion once. Compliances were weaker in 8 patients (50%) of lysine and in 2 patients (14.2%) in placebo group (Fig.2).

In lysine group, 9 of 16 patients (56.2%) experienced a decrease in number of ulcers per attack during the therapy period according to their previous experiences. In 4 patients of lysine (25%) and 9 patients of placebo group (64.2%) the number of ulcers didn’t change. Only one patient in placebo group told that the number of ulcer per attack decreased (7.1%). Number of ulcers decreased significantly in lysine group ($\chi^2=8.37$, P<0.05). The duration, recurrence, severity and number of ulcers in the groups are compared in Fig.2.

Therapy was found to be very effective by 5 (31.2%) and effective by 10 patients (62.5%), but ineffective by only one patient of lysine group. The average success degree was 7.5 ± 1.4 in lysine group. In placebo group, none of the patients found the therapy very effective, 6 patients found it effective (42.8%) and 8 patient found the therapy unsuccessful (57.1%). The average success degree was 3.8 ± 2.0 in for the placebo group. There was a significant difference for the effectiveness and success degree between lysine and placebo groups ($t=5.85$, P<0.001) (Fig.2, Table 2).

Most patients in lysine group found the therapy more successful when compared with the patients using placebo. No side effects were recorded in both of the groups.

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**Table 1.** Pre- and post-treatment results of HSV-1 IgG and HSV-1 DNA in lysine and placebo groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSV-1 IgG</td>
<td>HSV-1 DNA</td>
</tr>
<tr>
<td>Lysine (N=16)</td>
<td>100% (16)</td>
<td>6.3% (1)</td>
</tr>
<tr>
<td>Placebo (N=14)</td>
<td>100% (14)</td>
<td>2.1% (3)</td>
</tr>
<tr>
<td>Total (N=30)</td>
<td>100% (30)</td>
<td>13.3% (4)</td>
</tr>
</tbody>
</table>

Figure 2. The comparison of the groups according to the duration, recurrence, severity and number of ulcers between lysine (L) and placebo (P) groups, post-treatment.
Table 2. Average degrees given for the success of the therapies between lysine and placebo groups (r=5.85, P<0.001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Success degree</th>
</tr>
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<tbody>
<tr>
<td>Lysine</td>
<td>7.5 ± 1.4</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.8 ± 2.0</td>
</tr>
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Discussion
RAU is a real problem in the developing world. Approximately 10-25% of the general population suffers from these painful and untolerable ulcers [26] and this occurrence varies and may reach to 66% in some cultures [27]. Genetic factors, stress, trauma, hormonal disturbances, hematonic deficiencies, gastrointestinal problems, immunologic imbalances, viral and infectious agents were thought to play an important role in RAU etiology [28]. Despite much research, the cause remains idiopathic or a result of predisposing factor. HSV-1 has been one of the suspected viral agents in RAU etiology [17-19].

Some studies that performed to detect antibody levels of HSV-1 showed higher seral antibody levels against this virus [17, 18]. Tantivanich et al [18] stated higher ELISA antibody levels of HSV in 38 of 52 patients with RAU. Anti-HSV-1 IgG antibodies were found to be higher in some of RAU patients when compared with the non-RAU control groups [17]. Consistent with these results, we found that the number of individuals having antibodies to HSV-1 were significantly higher for the RAU group than for the control group (100% vs. 76.5%, respectively) and this led us to think that patients with MiRAU met with this virus more common in their daily life.

Eglin et al showed HSV-1 DNA hybridization in the peripheral blood mononuclear cells in a significant number of patients with RAU of minor type and of patients with Behçet’s syndrome. HSV-1 was thought to play a role in MiRAU [19]. In another study, HSV-1 DNA was found in 2 of 11 lesional tissues of RAU patients [17]. The results of this study are consistent with our finding that HSV-1 DNA can be detected by RT-PCR in the saliva of 4/30 RAU patients. It is difficult to compare our results with other studies because although others have investigated salivary HSV DNA in Behçet’s disease patients [29]; to our knowledge, there are no other reported studies evaluating HSV DNA in saliva samples from RAU patients.

Though, HSV-1 shedding in saliva which is a rather common event might complicate the results of this study and thus making hard to compare with other studies. This study is limited only by HSV-1 virus. A better understanding of the pathogenesis of oral ulceration may only be possible after elimination of each other viruses. HSV can cross-reactivate by other herpes viruses and therefore a possible effect of these virus family members cannot be excluded.

There are multiple treatment modalities for RAU in accordance with the complex etiological background for RAU. Due to the viral etiological basis, drugs that have antiviral activities, such as acyclovir, had been inspected. Wormster et al [30] disagreed the effectiveness of acyclovir while Pedersen [31] reported ulcer remission and decrease of symptoms in 6 of 8 patients with RAU with a dosage of 1600 mg of daily acyclovir.

In our placebo-controlled study, we examined the effectiveness of lysine treatment based on viral etiological background, especially on HSV-1, in a group of patients with RAU. Lysine acts as a viral inhibitor while competing with arginine, a natural viral activator of HSV-1, in the small intestine [20, 23, 32]. Lysine blocks arginine levels for HSV-1 and was reported to prevent from herpes labialis lesions [20, 23, 33, 34]. HSV replication was inhibited by higher lysine and lower arginine concentrations [35].

Although lysine successfully treated herpes simplex labialis in the literature, there have been almost no clinical trials using lysine as a remedy for canker sores. In a retrospective study of Wright, 27 of patients with RAU, 14 patients with herpes labialis and 1 patient with both of the lesions; patients were advised to take 500 mg lysine tablets daily for prophylaxis and directed to take two tablets every 6 hours when the prodrome developed. A telephone survey was conducted 6 months later. Lysine prophylaxis was found to be 100% effective in both of the the patient groups. Lysine shortened lesion duration in 25-50% of herpes labialis patients and 50% of RAU patients. RAU patients found lysine therapy more successful as compared with the herpes labialis group [25].

Different from Wright, we followed up our patient group for 2 months and recorded their complaints. Beside this, the patient group was compared with a placebo group and patients were exposed to a 630 mg of daily L-lysine as monohydrochloride (LMH). In accordance with Wright’s study [25], lesion number and recurrence was decreased significantly in lysine group when
compared with the placebo group. But the duration and the severity of the lesions didn’t change. Successful degrees were given to measure the difference between the pre- and post-treatment periods. Patients who used lysine for 2 months noted higher successful degrees as compared with the placebo group.

RAU etiology is still unclear and whether herpes viruses have a primary effect on RAU or lead to some causative immune mechanism still needs to be elucidated. It is also possible that viruses do not play a direct role; rather, factors that reactivate herpes viruses might trigger RAU. However, because a viral etiology has not been excluded, antiviral therapies for RAU should not be ruled out. It is worth to try different treatment modalities in patients suffering from RAU which is still an unsolved phenomenon and there is a need for long-term, placebo-controlled studies investigating the role of the HHV family in the pathogenesis and etiology of RAU.

In conclusion, lysine is a natural viral inhibitor and seems to be effective clinically for patients with RAU. The clinical success of lysine does not seem to reflect the same success for HSV presence, but lysine is cheap and easy to perform; so it may be tried on wider population groups and in patients who are resistant to other treatment modalities.

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References


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35. Marcason W. Will taking the amino acid supplement lysine prevent or treat the herpes simplex virus? J Am Diet Assoc 2003; 103:351.