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Effect of levamisole on treatment of recurrent aphthous stomatitis: A systematic review and meta-analysis

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Review Article

Abstract

BACKGROUND AND AIM: The aim of this study was to systematically analyze the effect of levamisole on treatment of recurrent aphthous stomatitis (RAS).

METHODS: An electronic search was executed in PubMed, Cochrane, and Scopus after determining the research question using the appropriate Medical Subject Heading (MeSH) term covering the period from 1975 to 2015. Additional publications from hand searching and the reference section of each relevant article enriched the article list. Finally, 9 articles that have assessed the effect of levamisole on the treatment of RAS and had suitable qualifications for the accomplishment of systematic review and meta-analysis were included.

RESULTS: The results showed that the chance of improvement in patients taking levamisole was 6 [odds ratio (OR) = 5.67, 95% confidence interval (CI)] times more than in patients not taking this drug.

CONCLUSION: It appears that levamisole is an effective drug for the treatment of RAS, but further appropriate studies should carryout in this context.

KEYWORDS: Levamisole; Treatment; Aphthous; Recurrent; Stomatitis

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Recurrent aphthous stomatitis (RAS) is the most common type of an inflammatory lesion of the oral cavity, affecting 5-25% of the general population.[1,2] The most characteristic symptom of the disease is the recurrent onset of single or multiple painful rounded or oval ulcers that appear mainly on non-keratinized oral mucosa of the lips, cheeks, and tongue.[1]

The etiology of RAS remains unknown.[1-3] The suggested triggering factors include genetic predisposition, infection with microorganisms, food allergies, vitamin and microelement deficiencies, increased oxidative stresses, endocrine alterations (menstrual cycle), smoking cessation, certain chemical products, mechanical injuries, and anxiety.[1,2,4] Immune changes occur in RAS, beginning with an unclear antigenic stimulation of keratinocytes, and induce the activation of T-lymphocytes, the release of cytokines [including tumor necrosis factor-alpha (TNF-α) and leukocyte chemotaxis].[2]

Since the cause of the disease is unclear, many drugs have been evaluated in an attempt to relieve the symptoms. A treatment

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is multidimensional and varies according to the predisposing factors. In all the cases, management is symptomatic with attempts to reduce inflammation and pain of the aphthae using topical or systemic treatments.[2] The choice of drug depends on the severity of the disease, the number of ulcers, their location and duration and the magnitude of pain.[4]

The use of systemic corticosteroids and immunomodulating agents has been the mainstay of the treatment for recurrent aphthous ulcers (RAU).[5] Corticosteroids are the first choice systemic treatment and immune modulators may be useful as the second line treatment.[2] One of the most effective systemic immunomodulators to be used in cases of RAU is levamisole.[3]

Steroids have been shown to provide symptomatic relief, whereas levamisole seems to provide symptomatic relief and alter the disease course.[6] It was found that in patients with RAS, the immune system's function becomes disrupted in response to some types of trigger factors. Both types of the immune response, natural and acquired (humoral and cellular), may become disturbed in patients with RAS, which is manifested with neutrophil reactivation and hyper-reactivity, elevated concentration of the complement ingredients and cytokines, increased number of natural killer (NK) cells and B-lymphocytes, and disrupted CD4/CD8 ratio.[1]

One of the agents used for systemic treatment of RAS is levamisole because it has a wide variety of immunological effects. Previously, it was used as an anti-helminthic drug. It can provide the normal phagocytic activity of macrophages and neutrophils, regulate T-cell activity, modulate the activity of human interferons (IFNs), and the serum levels of interleukin (IL-6) and IL-8. In the cases of RAU, it helps in normalization of $CD4^+/CD8^+$ cell ratio and increased level of serum immunoglobulin A (IgA) and IgM.[3]

This drug has commonly been used as monotherapy and an adjunct to treatment in a variety of diseases by gastroenterologists and dermatologists due to its wide range of immunomodulatory actions.[4] Many studies have done to evaluate the effect of levamisole in the treatment of RAS and reported varied results with different success rates.[7-12]

The present review was conducted to assess the effect of levamisole on aphthous lesions via a systematic and meta-analysis approach.

Methods

Search methodology and study selection

Our clinical question included four elements: population, intervention, comparison, and treatment outcomes. This research was designed to answer the question whether levamisole could be effective in improving clinical signs of recurrent aphthous patients or not. An electronic search of the PubMed, Cochrane, and Scopus databases was performed covering the period from 1975 to 2015. The following appropriate Medical Subject Heading (MeSH) terms for search were used: aphthous (aphthae, canker sore, periadenitis mucosa), recurrent (recurrence, relapse, recrudescences), stomatitis (stomatitis, oral mucositis, oromucositis), treatment (therapy, therapeutic, management), levamisole, and combination of these terms by the conjunctive operator AND and OR (Tables 1 and 2). A hand search as well as reference section of each relevant article was accomplished. Text files of the searched data from the above-mentioned databases were imported into the EndNote X7.1 for Windows & Mac, Reference management. (Thomson Reuters) software.[13] Then, after excluding duplicate records, 2365 records remained. Exclusion of the irrelevant articles was performed in the three steps of title, summary and the main text, and 29 articles remained at the end of this step. The full texts of all the related studies were evaluated by two authors separately. If there was any disagreement between these two reviewers, agreement was achieved by consulting with the third reviewer/ epidemiologist and statistical advisor.

Table 1. Description of trials

Author	Year	Sample size			Number of improvement		Index of improvement	Side effect	Dosage
		Total	Levamisole	Placebo	Levamisole	Placebo			
Lehner et al.[19]	1976	47	26	21	21	6	Number of ulcers Duration of ulcers	Nausea, Influenza	50 mg tid 2 days/week Weekly
van De Heyning[15]	1978	13	7	6	6	1	Number of ulcers Duration of ulcers Pain of ulcers Frequency of ulcers	No side effect	150 mg 3 days/week Every other week
de Cree et al.[17]	1978	18	9	9	7	2	Duration of ulcers Pain of ulcers Frequency of ulcers	Headache, Nausea	150 mg 3 days/week Interval of 2 weeks
Olson and Silverman[18]	1978	48	23	25	15	7	Duration of ulcers Pain of ulcers Frequency of ulcers	Dysgeusia, Hyperosmia, Headache	150 mg 3 days/week Weekly
Miller et al.[20]	1978	20	10	10	9	3	Number of ulcers Duration of ulcers	Nausea/diarrhea, Dysgeusia, Sleeplessness	150 mg 3 days/week Every other week
Kaplan et al.[14]	1978	65	34	31	19	5	Number of ulcers Duration of ulcers Pain of ulcers Frequency of ulcers	Dysgeusia, Hyperosmia, Headache, Nausea/vomiting	150 mg 3 days/week Every other week
Drinnan and Fischman[16]	1978	30	15	15	6	5	Number of ulcers Duration of ulcers Pain of ulcers Frequency of ulcers	Cacogeusia, Nausea	150 mg 3 days/week Every other week
Weckx et al.[21]	2009	25	15	10	7	7	Number of ulcers Duration of ulcers Size of ulcers	No side effect	150 mg 3 days/week Every other week
Sharda et al.[6]	2014	30	20	10	12	2	Number of ulcers Duration of ulcers Pain of ulcers Frequency of ulcers Size of ulcers	No side effect	150 mg 3 days/week Weekly for 3 weeks

Table 2. The Medical Subject Heading (MeSH) terms and their synonyms

Levamisole	Treatment	Aphthous	Recurrent	Stomatitis
Tetramisole	Therapy	Aphthae	Recurrence	Mucositis
Levamisole	Therapeutic	Canker sore	Relapse	Oromucositis
Decaris	Management	Sore canker	Recrudescences	Stomatitides
Dekaris		Ulcer, aphthous		
Levamisole hydrochloride		Periadenitis mucosa		

The quality evaluation of articles was performed using Critical Appraisal Skills Program (CASP) according to the Public Health Resource Unit (PHRU) (England 2006).[13]

All the articles were rated according to this checklist and the articles with desirable quality were determined. Articles rating 6 and more were included in the present study. In this step, 20 articles were excluded and 9 articles[6,14-21] were included in the study. Subsequently, the required data were extracted and imported into an Excel (version 2007) sheet. The main author's name, publication date of the article, quality assessment rating of each study, type of study, sampling method, sample size, study groups' assignment, treatment period duration, dose of the drug used, age (range, average), male and female ratio, the patients' response to the treatment, clinical outcomes, and side effects were systematically recorded.

The review of literature was confined to English papers with randomized clinical trial studies. The meta-analysis was carried out on the clinical outcomes.

An estimation of each treatment effect was reported as odds ratio (OR) index. In fact, OR was measured for every study and then pooled using a fixed-effect model. The investigation of total variation between findings of studies (the estimations of treatment/intervention effects from final studies) was carried out using Cochran's test for heterogeneity and I^2 index. This index shows what percentage of differences observed between the indexes of the study are due to the heterogeneity between the studies.

The Cochrane guidelines for classification of this index are as follows:

Cochrane Handbook 2008 categories:

- 0-40%: might not be important
- 30-60%: moderate heterogeneity
- 50-90%: substantial heterogeneity
- 75-100%: considerable heterogeneity.

Results

Initially, 3837 articles were found using the electronic search and hand search. Repetitive 2365 articles were omitted. Based on the title, abstract and full text 1443 irrelevant articles were discarded, leaving 29 studies (Figure 1).

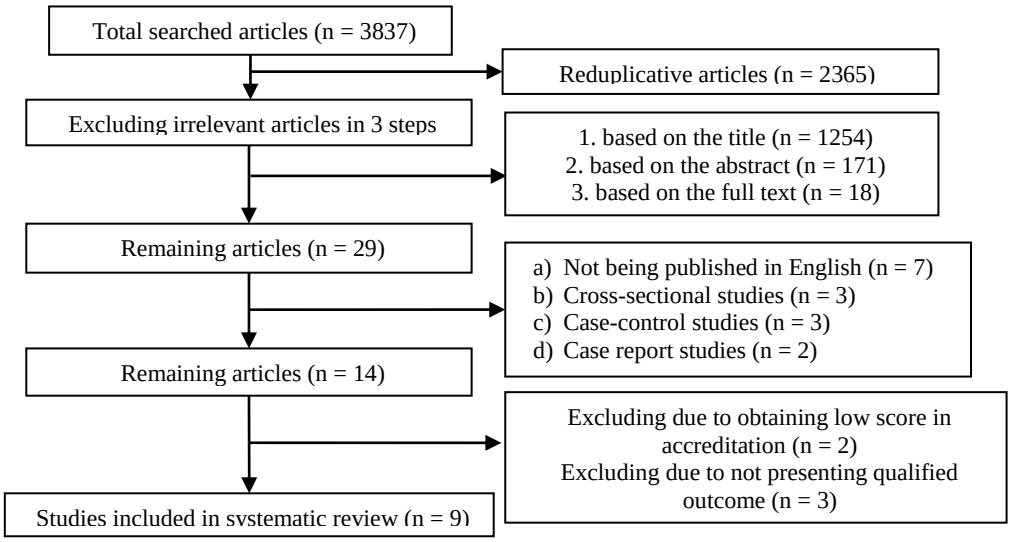

Figure 1. Flow diagram of studies

Table 3. The combination of keywords

Levamisole and treatment	Treatment and recurrent and aphthous
Levamisole and aphthous	Treatment and recurrent and stomatitis
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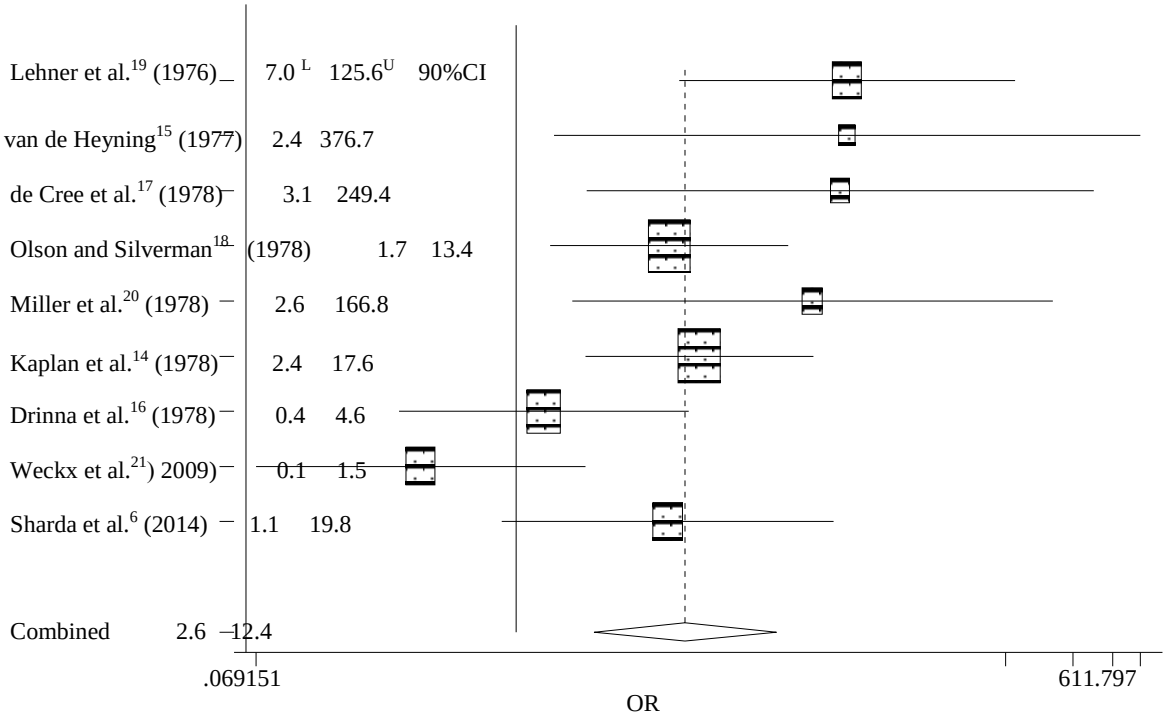

Figure 2. The overall odds ratio (OR) in a fixed model

Two case reports, 3 case-control, 3 cross-sectional, and 7 non-English articles were separated. Quality assessment of the 14 studies was executed according to the PHRU and 9 articles had suitable qualifications (Score 6 or more) for the accomplishment of systematic review and meta-analysis. Table 3 shows the basic information of these 9 studies.

Meta-analysis results of levamisole are presented in figure 2. The chance of improvement in patients taking levamisole was 6 [OR = 5.67, 95% confidence interval (CI)] times more than in patients not taking this drug, and the difference was significant (P = 0.001).

In this study, heterogeneity of chi-squared results was Q = 20.732 on 8 degrees of freedom (P = 0.008), which was significant at 5% level of significance. Therefore, the study results were heterogenic.

Discussion

This study was performed based on Cochrane systematic review and meta-analysis. The research question was designed to answer whether levamisole is effective in improving clinical signs and symptoms of RAS or not. According to the results of meta-analysis, levamisole improves the clinical signs of RAS. RAS is a common disease and despite many research studies in this field, the etiology of this condition is unknown and there is no definitive treatment for it.[2]

As the mentioned earlier, the use of

systemic corticosteroids and immunomodulating agents has been the mainstay of treatment for RAU.[5] One of the most effective systemic immunomodulators to be used in cases of RAU is levamisole.[3]

The exact mode of action of levamisole remains unclear. Levamisole reportedly decreases the frequency, duration and number of oral ulcers. Levamisole has been found to immunomodulate T-cell-mediated immunity. Normalization of the decreased $CD4^+/CD8^+$ cell ratio and increased serum levels of IgA and IgM has been found in RAU patients after levamisole treatment.[3] The serum TNF-α level may be associated with the severity of RAS. It has been concluded that levamisole can modulate serum TNF-α levels in RAS patients.[22] IL-6 and IL-8 are pro-inflammatory cytokines that affect cellular and humoral immunities and levamisole can modulate the serum level of these cytokines.[3,23]

This drug has proven to increase hemoglobin concentration of the patient along with regulating immune system of RAS patients.[4] Based on evidence available, it seems that levamisole could be effective in the treatment of RAS.[2-5,22,23] Many studies have evaluated this subject. Outcomes of some of these studies confirm the effect of levamisole on improving clinical signs of aphthous lesions including reduction of frequency, number, duration, size, and pain of ulcers.[6,14-21]

We assess the results of 9 selected articles in different dimensions.

Levamisole and frequency of ulcers

Six articles showed that levamisole could reduce the frequency of aphthous periods and increase the interval of episodes.[6,14-18]

Levamisole and duration of ulcers

All the 9 articles supported the effect of levamisole on decreasing duration of ulcers in the mouth and accelerating recovery of ulcers.[6,14-21]

Levamisole and the number of ulcers

Seven studies demonstrated a decrease in the number of lesions in different sites by levamisole.[6,14-16,19-21]

Levamisole and size of ulcers

In two investigations, the diameter of ulcers were measured and a reduction in ulcer sizes was noted after taking levamisole.[6,21]

Levamisole and pain of ulcers

The results of six trials confirmed the effect of levamisole on decreasing pain of aphthous ulcers.[6,14-18]

Levamisole and types of aphthous lesions

Two studies described their results based on the classification of aphthous lesions including minor, major, and herpetic form. Olson and Silverman[18] reported that levamisole had more effects on improvement of minor aphthous than major aphthous ulcers, whereas Lehner et al.'s study[19] showed that the efficacy of levamisole in recovery of major aphthous ulcers was more significant than minor aphthous ulcers.

Method of administration

Five methods had been used in the articles reviewed.

A. 150 mg daily for 3 consecutive days/weeks[18]

B. 150 mg for 3 consecutive days every other week[14-16,20,21]

C. 50 mg 3 times daily for 2 consecutive days every week[19]

D. 150 mg three times daily for 3 consecutive days/weeks[6]

E. 150 mg daily for 3 consecutive days/weeks with an interval of 2 weeks[17]

The duration of trials and follow-up periods of patients were different in different studies, from 2 to 6 months but all of them reported that no clinical changes were seen 1 month after initiation of treatment. Differences in methods of administration and trial protocols in a wide range of duration resulted in differences between the results of studies.

Adverse effects of levamisole are mild and infrequent and include rash, nausea, abdominal cramps, alopecia, arthralgia, hyperosmia, dysgeusia and a flu-like

syndrome and rarely agranulocytosis.[3,4] The most common adverse effects of levamisole in the mentioned studies were headache, nausea, dysgeusia, and hyperosmia.[14,16-20]

Overall, 6 articles confirmed the efficacy of levamisole in improving clinical signs of RAS,[6,14,15,17-19] while 3 articles did not support the influence of levamisole for recovery of clinical signs of aphthous stomatitis.[16,20,21]

In general, the study results show that studies were heterogenic. The heterogeneity was attributed to differences in methods of administration of levamisole (differences in doses and duration), carrying out the trials in different years and lack of a standard index for improvement between different studies. Moreover, this review showed that there is a time lag between the studies. Most studies conducted in the years 1976-1978 and only two studies recently conducted (2009 and 2014).[6,21] However, despite the effectiveness of levamisole in the improving of clinical signs of RAS that in most older studies referenced,[14,15,17-19] the reason of this time lag is not specified. Therefore, further studies are necessary on this topic.

Limitations

The most important factor was the number of appropriate studies carried out in this context; therefore, further studies are necessary on this topic. Since levamisole was administered at different doses using different protocols in different studies, it is difficult to evaluate discrepancies between studies with differences in their data. It is suggested that future studies use standardized variables and similar conditions including evaluation of size, number, duration, frequency and pain of ulcers, to facilitate comparisons between the results of different studies. The types of aphthous ulcers (minor, major and herpetic form) should be considered for more accurate assessment of the influence of levamisole. Administration of an equal dose of levamisole with the same prescription order and similar period of follow-up make it possible to compare the results of different studies.

Conclusion

Many studies have been undertaken to find an appropriate treatment for RAS and numerous topical and systemic interventions have been used.[24,25] Administration of levamisole is one of the systemic interventions for the treatment of RAS because of its immunomodulatory action. The results of this study showed that the chance of improvement in patients taking levamisole was 6 times more than that in patients not taking it. Although several studies supported its efficacy, further studies are necessary in this field.

Conflict of Interests

Authors have no conflict of interest.

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