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ORIGINAL ARTICLE

Clinical assessment of the effect of a matrix metalloproteinase inhibitor on aphthous ulcers

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Abstract

Objective. Aphthous ulceration is a common form of recurrent ulceration of the oral mucosa. Numerous treatments have been tried as a means of relieving pain, disinfecting the ulcer base, and reducing inflammation, but with limited success. Tetracycline and its derivatives have been shown to be inhibitors of matrix metalloproteinases (MMPs), which are part of the inflammatory response and contribute to the breakdown of tissue in the ulcer. Of the commercially available tetracyclines, doxycycline has shown the best inhibition of the MMPs. The aim of this study was to test clinically whether the inhibitory effect of a low-dose doxycycline in a hydrogel on MMPs would speed the recovery of oral ulceration. ***Material and Methods.*** Forty-nine patients participated in a randomized, double-blind, placebo-controlled trial. ***Results.*** Sixty-eight percent of ulcers had healed by the third day of treatment with the doxycycline gel, whereas only 25% of the patients receiving the placebo reported healing of their ulcers within 3 days. Patients treated with the docycline gel recounted faster reduction in pain during the treatment period than the placebo group did. ***Conclusions.*** Incorporation of low-dose doxycycline in a muco-adhesive gel has been demonstrated to have potential in the treatment of recurrent oral ulceration. It is concluded that MMP enzymes can be inhibited by low doses of doxycycline below levels likely to disrupt the oral flora.

Key Words: *Hydrogel, low-dose doxycycline, mucosa, ulceration*

Introduction

Aphthous ulceration is a common form of recurrent ulceration of the oral mucosa, affecting between 5% and 60% of certain populations or groups within populations [1]. The ulcers are shallow and painful, usually characterized by a serous exudate in the ulcer floor and surrounded by a halo of inflammation. There is likely to be an overgrowth of bacteria in the serum-rich floor of the ulcer that may then exacerbate the inflammation and the pain felt. The flora of the base of aphthous ulcers has been shown to be diverse, although there is no agreement on a possible microbiological cause of aphthae [2]. Aphthous ulceration is more common in women than in men [1,3] and can occur throughout life, but it is particularly common in childhood and adolescence [1,3]. The etiology is unknown, but mild trauma, deficiencies in serum iron, folate, or vitamin B12 [4], and sensitivity to certain foods and sodium lauryl sulphate in toothpaste have all been linked to aphthous ulceration [5,6]. Cross-reactivity between oral streptococci heat shock proteins (HSPs) and oral mucosa has also been suggested as a possible etiological factor in aphthous ulceration, as antibodies to HSPs have been demonstrated in patients with this type of ulceration [7]. Numerous treatments have been tried in attempts to relieve pain, disinfect the ulcer base and reduce inflammation. No treatments have been particularly successful in reducing the recurrence of aphthous ulceration, other than detecting and correcting any underlying deficiency that may be present. Treatments that can relieve the symptoms and/or reduce inflammation include topical, or even systemic, corticosteroids, benzydamine HCl, chlorhexidine and other antiseptics [1]. Intra-lesional steroids have also been used for aphthous ulceration in HIV-positive patients [8]. Although the cause of aphthous ulceration is not

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always clear, increased activity of matrix metalloproteinases (MMPs) has been detected in ulcers [9]. This is not particularly surprising, as MMPs are a component of the normal process of mucosal renewal but also contribute to the inflammatory process by promoting breakdown of tissues. Thus excessive MMP activity is seen in diseases such as aphthous ulceration [10], periodontitis [11], rheumatoid arthritis and bullous diseases of the skin. These enzymes are found in tumor metastases [12]. MMPs are generally structured in five parts: the signal peptide, propeptide, catalytic site, hinge region, and pexin-like domain. When activated, the enzyme first loses the signal peptide and then the internal bonds of the propeptide are disrupted. Disruption of the Cystein-Zn^{++} bond is a prerequisite to activation of the enzymes, which allows Zn^{++} to catalyze one or more cleavages [13].

It has been shown that tetracycline and its derivatives inhibit MMPs and this has been used, for example, to reduce periodontal inflammation [14,15]. The British National Formulary no. 42 [16] recommends a strong concentration of tetracycline ("250 mg in a small amount of water") as an unlicensed indication as a mouth wash for the treatment of severe oral ulceration, and the British National Formulary no. 52 states that doxycycline rinsed in the mouth may be of value for recurrent aphthous ulceration [17].

Of the commercially available tetracyclines, doxycycline has proved to be the best inhibition of the MMPs [18,19]. The mechanism of this inhibition was first proposed to be the ability of the tetracyclines to inhibit already active MMPs, which was shown by Golub et al. [20]. Later it was found that the tetracyclines also inhibited pro-MMP activation and downregulated the expression of MMPs [21]. It has also been shown that in low doses the tetracyclines do not induce the emergence of tetracycline resistance bacterial strains [22–24]. Chemically modified tetracyclines have been made to rid the tetracyclines of their anti-bacterial properties and to identify the site of the anti-collagenase property [14].

The aim of this study was to test clinically whether the inhibitory effect of low-dose doxycycline in a hydrogel on MMPs would speed the recovery of oral ulceration.

Material and methods

Formulation

Doxy-Gel, a gel containing a low dose of doxycycline (1.5 mg/g) (LDDg), was developed and produced by Bio-Gels Pharmaceuticals. Tubes of gel for the study were labeled with a code number, placed in a box, and stored at 4°C until dispensing to patients.

Clinical trial

Permission for the clinical trial was obtained from the Icelandic Medicines Control Agency (K-11-01) and the National Bioethics Committee (VSN 01-076-S1) in Iceland. A randomized, double-blind clinical trial was to be performed on 50 volunteers suffering from recurrent aphthous ulceration.

Sample size determination

A priori calculations of sample size, using an alpha level of 0.05, a beta level of 20.0 and a subjectively determined clinically relevant difference between treatment and placebo group of 40%, yielded a minimum sample size of 46. To account for possible attrition and dropout, the initial sample size was set slightly higher.

Participants

Male and female adult outpatients (aged between 18 and 65 years) with aphthous ulcers were eligible for the trial. Patients attended a specialist oral medicine clinic or the Faculty of Odontology, University of Iceland for treatment of their oral ulceration. All were examined by a trained clinician (W.P.H.) prior to being invited to participate in the study. Subjects had to have at least a 1-year history of minor recurrent oral ulceration and have had at least one occurrence in the previous 12 months. Subjects were not enrolled in the study until an ulcer resembling an aphthous ulcer was observed by the clinician. Excluded from the clinical trial were those with major aphthae, herpetiform ulceration and those who had used any antibacterial products in the 2 weeks prior to the experiment, including antiseptic mouthwash. Similarly, participants in other concurrent investigations were excluded from study enrolment or continued participation. Prospective participants were informed of the objectives of the study, and the design of the study protocol was discussed with them. Questions were allowed and were answered. After the meeting, volunteers who still wished to participate in the study gave their written and informed consent.

Study design

Randomization was done by the sponsor of the trial, i.e. tubes containing test and placebo gel were assigned random study numbers. Patients meeting the entry criteria selected their tube of medicament at random. Randomization was irrespective of age, gender, or perceived severity of the ulcer present. The clinician (W.P.H.) and the patients were blinded to the contents of the tube of medication handed to the patient on enrolment in the clinical trial. The laboratory and pharmaceutical staff knew the patient only by a study number.

Subjects were instructed, orally and in writing, to apply the gel four times a day and to record the time of day of application in the diary as well as acceptability by commenting on taste of the formulation and recording pain in the ulcer using a scale from 0 to 10, 0 being no pain and 10 being severe pain. The patients also had the possibility to record any other observations they might have during the treatment. Participants were requested to refrain from all other treatments for aphthous ulceration while entered in the trial.

At the end of the study, diaries were returned to the clinical examiner, who also examined the oral mucosa. Age-eligible participants were recruited from February 2002 to February 2003. If a subject was suspected of being deficient in serum iron, folate, and vitamin B12, then a hematological investigation was arranged. The subject participated in the trial while awaiting the results of the hematological investigation, but was excluded if the hematological investigation revealed an iron or B-vitamin deficiency.

The primary objective was to investigate and evaluate the efficacy of low-dose doxycycline (LDD) hydrogel on ulcerated and inflamed buccal mucosa. Assessment of efficacy was made from the diary record of the patients' assessments of the conditions on the ulcer; no change or healed. The secondary objective was to investigate acceptability of the preparation and to evaluate whether the formulation had any irritating effects on the oral mucosa.

Statistical methods

The collected data were analyzed using Fisher's exact probability test to compare the groups. The test was one tailed and $p<0.05$ was considered statistically significant.

Results

Clinical trial

Demographics. – Fifty-six patients met the eligibility criteria and were assigned randomly to the two treatment arms. Four patients were excluded from the trial because of hematinic deficiencies (Figure 1).

More women than men participated; the placebo arm had 14 women (58%) and 10 men (42%), while the LDDg arm had 18 women (72%) and 7 men (28%). The initial pain scores of the placebo and LDDg groups were 5.3 and 5.1, respectively, i.e. not significantly different.

Efficacy evaluations. – The primary objective was to evaluate the effect of the doxycycline formulation on the ulcers. Data from the patients' diaries were transferred into the computer, analyzed, the code broken and subjects assigned to the test or placebo group.

From the information contained in the diaries, it was noted that 68% (17 of 25) of ulcers had healed by the third day of treatment with the doxycycline gel, whereas only 25% (6 of 24) of the patients receiving the placebo reported healing of their ulcers within 3 days. Healing was determined by the subjects as the time when they were no longer aware of the presence of the ulcer, either visually or by perceiving pain or discomfort at the site of the ulcer. The duration of ulcers treated with LDDg was statistically significantly shorter than for the ulcers treated with placebo ($p<0.005$). No patient perceived delays in the healing of their ulcer compared with their normal experience. It was also noted that the LDDg group showed faster reduction in pain during the treatment period (Table I) than the placebo group did.

The diary reports gave an opportunity for reporting recurrence of the ulceration, but recurrent ulceration had not happened by the time most of the diaries were returned and, consequently, no conclusion can be reached concerning the reduction of recurrence of ulcers.

Acceptability. – Both groups found the formulations acceptable and only reports of mild adverse reactions were made. One subject reported a short burning sensation from the ulcer after application of the gel. Furthermore, subjects from both groups, 13 in all (26.5%), noted that after applying the gel to the ulcers there was a short-term relief of pain (30–60 min); this was equal between groups. When interviewed, they reported that a protective film formed over the ulcers, which was probably responsible for the short-term pain relief. Three participants (6%) reported increased pain for a short time after gel application.

Discussion

Aphthous ulceration is a common, painful condition that can often interfere with the patient's lifestyle. Consequently, many remedies have been proposed and tried in order to reduce pain, promote healing, and reduce the frequency of recurrence [1]. There seems to be no reliable curative treatment, although correcting of iron, folate, and vitamin B12 deficiencies may lead to long-term relief from recurrence. The aim of treatment has thus been to reduce the pain and discomfort of the ulcer and to reduce the frequency of recurrence [1]. Most commonly used treatment regimens are either obtainable over-the-counter or on prescription from a general medical or dental practitioner. Systemic treatments are not usually indicated but, where necessary, corticosteroids are the most commonly prescribed medication for systemic use. Topical agents used include disinfectants such as chlorhexidine,

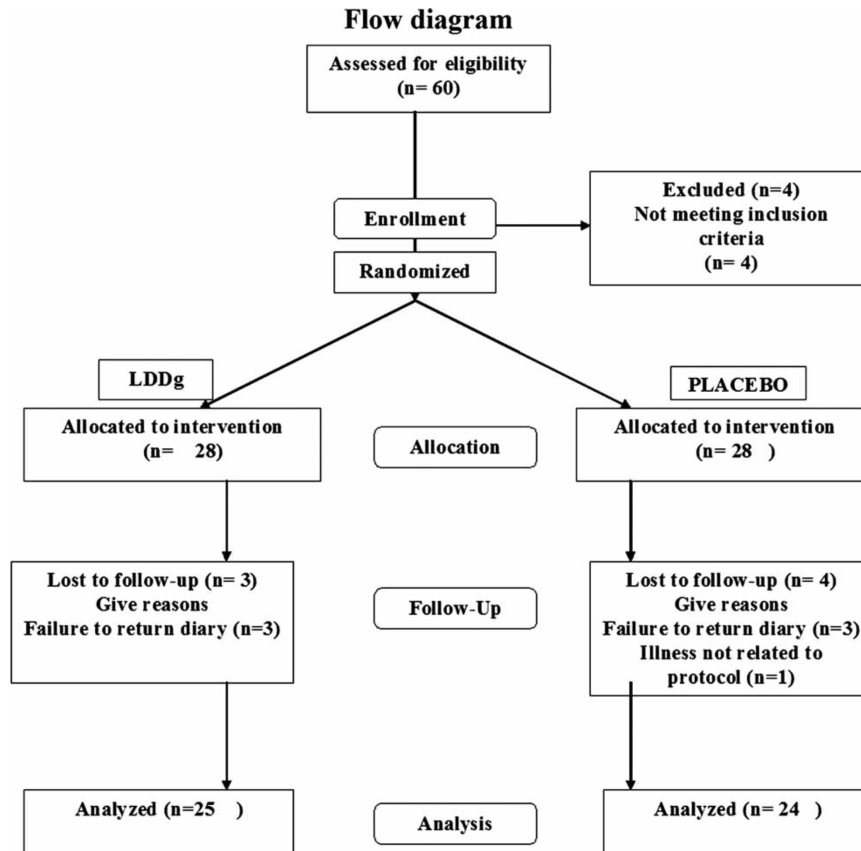

Figure 1. Flow diagram for the clinical trial.

anti-inflammatory agents such as amlexanox, benzydamine HCl, and topical corticosteroids [1]. Tetracyclines in the form of chlortetracycline mouthwashes have been used as a form of topical treatment of aphthae for many years [15]. The mode of action of tetracycline is suggested to be antimicrobial, disinfecting the base of the ulcer thereby promoting healing. Golub et al. [14] have shown the topical action of tetracyclines on mucosa to be inhibitory to MMP activity. As a consequence of that inhibition, the normal growth of the mucosal cells allows the ulcer to heal rapidly.

The text accompanying the tetracycline mouthwash described in the British National Formulary [16] for the treatment of oral ulceration implies that the mode of action is that of disinfection [17]. The use of topical tetracyclines in sub-antimicrobial concentrations in the treatment of chronic periodontitis, usually as an adjunct to conventional therapy [24], indicated the MMP-inhibiting potential of tetracycline without disruption of the normal oral flora. Some reviews still suggest, however, that the action of tetracyclines in management of periodontal disease is based on their antimicrobial action [25]. Chlortetracycline and doxycycline have been tried and compared as a treatment of RAS, the results indicating that doxycycline is a more active treatment [10,26]. Previous investigations show that the MMP inhibiting activity of doxycycline on MMP-2 and MMP-9 [27] may be an additional mode of action.

The doxycycline hydrogel formulation proved acceptable to patients. It was easy to apply and there were no complaints about irritation. Film-forming properties of the gel were noted to bring relief of pain in both the placebo group and the active group, as the gel seemed to protect the ulcers for a reasonable period of time after application. The results of this randomized, double-blind, placebo-controlled trial are sufficiently promising to support the concept of activity of low-dose doxycycline in the treatment of aphthous ulceration. This treatment modality for the treatment of recurrent aphthous ulceration warrants further refinement followed by a subsequent larger-scale clinical trial.

MMP enzymes can be inhibited by doses of doxycycline below levels likely to disrupt the oral

Table I. Scores of pain during treatment

	Average score		
Score of pain	day 1(s.d.)	day 2(s.d.)	day 3(s.d.)
Placebo	5.3(2.5)	4.8(2.6)	4.0(2.9)
LDDg	5.1(2.5)	3.9(2.4)	2.9(2.9)*

*Significant reduction from day 1 to day 3($p<0.05$).

flora. Incorporation of low-dose doxycycline in a muco-adhesive gel has been demonstrated to have potential as a means of treatment of recurrent oral ulceration.

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