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Subantimicrobial dose doxycycline in the treatment of recurrent oral aphthous ulceration: a pilot study

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BACKGROUND: Recurrent oral aphthous ulceration (ROAU) is a common problem that can result in considerable pain and distress for patients. The aim of this pilot study was to evaluate the role of subantimicrobial dose doxycycline (SDD – 20 mg doxycycline twice daily) in the management of patients with ROAU.

METHODS: 50 patients with ROAU were randomly allocated to treatment with SDD or placebo for 90 days. Daily ulcer diaries were completed by the participants to record the number of new ulcers and the pain associated with the ulcers.

RESULTS: There were significantly more days with no new ulcers in the SDD group (80.4 ± 5.9) than the placebo group (69.8 ± 18.7; $P = 0.04$). Strong trends were observed towards fewer new ulcers per day, fewer new ulcers over the 90-day period, and more days with no pain in the SDD group compared with the placebo group ($P = 0.06$–0.08).

CONCLUSION: SDD shows promise as potential therapy in the management of ROAU, though this needs to be confirmed in further studies.

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Keywords: aphthous ulcer; double-blind method; doxycycline/therapeutic use; placebo-controlled study; subantimicrobial dose doxycycline

Introduction

Recurrent oral aphthous ulceration (ROAU) is a common disorder of the oral mucosa, with a reported prevalence of approximately 5–25% in the general population (1). The aetiology has yet to be defined, although a number of precipitating factors have been identified (2). Untreated lesions usually persist for 7–10 days and heal without scarring. The management of ROAU is dependent on severity but is typically directed towards symptomatic relief (1). Patients must be screened to identify any underlying cause and, where identified, treated accordingly (3, 4). For the majority of patients, however, no underlying cause can be identified and therapy is designed to reduce the incidence, duration and severity of the ulceration.

Numerous treatments have been evaluated for ROAU including topical analgesics for symptomatic relief (5), anti-inflammatory agents to suppress pathological changes (6–8) and antimicrobials to control microbial contamination and secondary infection (9). Antimicrobial agents that have been tested include antibiotics (e.g. tetracyclines) and antiseptics (e.g. chlorhexidine). Tetracyclines have been used both systemically and topically in the treatment of ROAU and it has been postulated that the beneficial effects of tetracyclines may be related to their anticollagenolytic properties (10).

It has been suggested that mast cells may play an important role in the development of ROAU lesions and mast cell counts are significantly increased at aphthous compared with traumatic ulcers (11). Numerous mast cells can be found in the subepithelial lamina propria below aphthae, and there is increased mast cell degranulation at ROAU–connective tissue–inflammatory infiltrate interfaces associated with connective tissue disruption. Mast cells activate latent matrix metalloproteinases (MMPs) and also produce and secrete MMP-9 (12). MMP-3 and MMP-9 have been co-localized to mast cells in endobronchial biopsy specimens taken from asthmatic patients (13), and elevated MMP-1 and MMP-3 expression is co-located with increased numbers of degranulated mast cells and with greater MMP activity in the shoulder regions of atherosclerotic plaques (14).

Thus, evidence exists that mast cells play a role in the development of aphthae, and that mast cells produce,

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secrete and activate MMPs. Excessive localized MMP production in the subepithelial layer may promote disruption of the epithelium–connective tissue interface, contributing to the epithelium sloughing off the underlying tissues and ulcer formation. Our hypothesis is that the inhibition of MMPs by subantimicrobial dose doxycycline (SDD) that has previously been shown to result in clinical benefits for periodontitis patients may also result in a clinical benefit for patients suffering from ROAU. Doxycycline at subantimicrobial doses (20 mg twice daily) has been shown to downregulate MMP activity in the periodontal tissues (15–17), and to result in clinically significant benefits in patients with moderate/severe chronic periodontitis when used as an adjunct to scaling and root planing (18, 19). A subantimicrobial dose of 20 mg twice daily is not associated with the development of antibiotic resistance or detrimental shifts in the normal periodontal or other microflorae (20, 21).

The primary objective of this pilot study was to evaluate the clinical effects of SDD (20 mg doxycycline twice daily) vs. placebo tablets in the relief of symptoms of ROAU.

Methods

A single centre, 3-month, placebo-controlled, double-blind, parallel group study of SDD (20 mg doxycycline twice daily) in patients with ROAU was undertaken. Ethical approval was obtained from the relevant Research Ethics Committee prior to study commencement. Written informed consent was obtained from all subjects. Patients presenting with long-standing histories of ROAU at the specialist Oral Medicine Clinic at Newcastle Dental Hospital were recruited to the study. Prior to entry, venous blood samples were obtained as part of routine clinical screening for full blood count, serum ferritin, serum vitamin B_{12} and red cell folate levels.

Subjects were male or female, aged 18–65 years, and had a history of ROAU necessitating appointments on a specialist oral medicine clinic ≥2 times per year. Exclusion criteria included any patient with deficiencies in serum ferritin, vitamin B_{12} and/or folate; pregnant or nursing women; patients with a known hypersensitivity to tetracyclines; women of child-bearing potential not taking adequate contraceptive precautions; patients on clinically significant concomitant drug therapy (e.g. steroids, immunosuppressants, anti-inflammatory drugs); patients with diabetes mellitus, systemic infection, kidney or liver disease; patients requiring prophylactic antibiotic coverage for routine dental therapy.

At the baseline (day 0) examination, subject demographics and history of ROAU were recorded. An oral soft tissue examination was conducted to confirm the presence and numbers of any ulcers. A predetermined, computer-generated randomization schedule (based on a permuted blocks of four design) was used to determine the group assignment (SDD or placebo) and study medication was dispensed. Subjects were requested to take their assigned medication twice daily (one tablet in the morning, one in the evening) for the next 90 days, whether or not ulcers were present in the mouth. Placebo and SDD tablets were identical in appearance. Subjects were given study diaries to complete over the next 3 months (days 0–90). The study diaries assessed on a daily basis (i) number of new ulcers present in the mouth, (ii) the pain associated with the ulcer(s) by means of visual analogue scales (VAS) and (iii) whether the subject needed to use any additional ulcer treatments on that day. The boundaries of the VAS were labelled 'no pain at all' (0 mm) and 'the worst possible pain imaginable' (100 mm). All subjects were permitted to use any additional ulcer management therapies at any time during the study.

Subjects were contacted by telephone once per month to check on clinical status with a view to improving compliance with the study protocol. At the day 90 visit, the subject returned for further evaluation including a full oral soft tissue examination, and the number of ulcers and discomfort experienced by the subject at the time of exit from the study were recorded. Completed diaries were collected from the subjects, together with any unused study medication.

Statistical considerations

Descriptive statistics were calculated and comparisons made between treatment groups with respect to demographics and efficacy parameters. Data recorded from ulcer diaries were summarized and mean values and standard deviations calculated. Outcome variables included the incidence of ulcers (derived from the numbers of new ulcers recorded on a daily basis), days with no new ulcers, pain scores (VAS scores) and the need (or not) of subjects to use additional means of ulcer treatment on a daily basis.

Due to the lack of previous research in this area, commonly observed standard deviations from VAS were used for power calculations. Assuming $\sigma = 15$ mm, a mean difference between groups of 15 mm and using $\alpha = 0.05$, approximately 20 subjects per group were required to complete the study to provide an 85% power for detecting a significant difference between treatments. Thus, to allow for drop-outs, 50 subjects were recruited.

Results

Fifty subjects were recruited to the study, 25 in each group. The demographics of the study population are presented in Table 1. There were no significant differences between the SDD and placebo groups with respect to gender distribution, age, race/ethnicity or smoking status ($P > 0.05$). When considering the smokers, there were no significant differences in the pack-years of smoking between the SDD group (8.3 ± 10.3 pack-years) and the placebo group (13.7 ± 12.4 pack-years; $P > 0.05$).

At the baseline appointment, the subjects were asked to estimate the number of years for which they had suffered from ulcers, and the number of ulcers they felt they experienced per month. There were no significant differences between the SDD and placebo groups in respect of these parameters ($P > 0.05$; Table 2). The

Table 1 Baseline demographic characteristics of study population

Characteristic	*SDD group (n = 25)*	*Placebo group (n = 25)*	*P-value*
Age (years)			
Mean (SD)	36.8 (13.9)	43.0 (13.3)	0.11*
Range	19–64	18–62	
Gender, *n* (%)			
Male	11 (44)	7 (28)	0.38†
Female	14 (56)	18 (72)	
Race/ethnicity, *n* (%)[a]			
White	25 (100)	24 (96)	1.00†
Asian or Asian British	–	1 (4)	
Tobacco use, *n* (%)			
Non-smoker	14 (56)	16 (64)	0.81‡
Ex-smoker	8 (32)	6 (24)	
Smoker	3 (12)	3 (12)	

P-values determined: *using independent samples *t*-test, †using the Cochran-Mantel-Haenszel test, ‡using chi-squared statistics.
[a]Level 1 classification of ethnic groups, National Statistics 2001.

Table 2 Ulcer data recorded at day 0 and day 90

	SDD group, mean (SD)	*Placebo group, mean (SD)*	*P-value**
Years suffered from ulcers	14.6 (13.7)	15.0 (17.0)	0.94
Number of ulcers per month	5.8 (6.2)	8.0 (8.6)	0.32
Number of ulcers present at day 0	1.7 (2.1)	1.4 (1.8)	0.61
Number of ulcers present at day 90	0.7 (0.8)	1.2 (1.3)	0.17

*Determined using independent samples *t*-test.

actual number of ulcers present in the mouth at baseline was recorded by the clinician (Table 2), and again, there were no significant differences between the groups at baseline. Table 2 also presents the number of ulcers present in the mouth at the day 90 appointment, and there were less ulcers in the SDD group (0.7 ± 0.8) than in the placebo group (1.2 ± 1.3) at day 90, although this difference failed to achieve statistical significance ($P = 0.17$).

Table 3 presents data derived from the subject-completed daily ulcer diaries. From these diaries, we were able to identify the pain experienced arising from the ulcers each day (VAS scores), the number of new ulcers forming per day, the number of days for which the subject needed to use additional forms of treatment for their ulcers, the number of days in which no new ulcers formed and the number of days with no pain. Of all of the parameters presented in Table 3, a statistically significant difference between the treatment groups was only identified in respect of the number of days with no new ulcers, with significantly more days with no new ulcers in the SDD group (80.4) compared with the placebo group (69.8; $P = 0.04$). However, there were also strong trends in favour of a beneficial effect of SDD in this cohort of subjects when considering the number of new ulcers occurring per day (fewer in the SDD group, $P = 0.07$) and the total number of new ulcers over 90 days (fewer in the SDD group, $P = 0.08$). Furthermore, there were more days without pain in the SDD group (61 days) compared with the placebo group (45 days), though this just failed to achieve statistical significance ($P = 0.06$).

Visual analogue scale pain scores tended to be highly variable throughout the study, supporting the fact that pain responses are very different between individuals. Both total pain scores and mean daily pain scores were less in the SDD group than the placebo group, though due to the variability of the data, these differences were not statistically significant.

Discussion

Severe ROAU results in considerable pain and distress for patients, and presents a difficult management challenge for the dental practitioner, as evidenced by the wide range of treatments that have been proposed for these lesions. The aetiology is unknown, but likely involves immune mechanisms and a genetic predisposition (22). Contemporaneous treatment strategies include primary, secondary and tertiary approaches. Primary treatment options include topical gels, creams, ointments and rinses, for example, topical glucocorticoids (8). Other first-line approaches that have been reported include converting the ulcer into a wound by cautery (23) or lasers (24). Stress management, relaxation and imagery training may also have additional therapeutic benefits (25). For patients whose symptoms are not alleviated by the primary lines of treatment, a secondary, more aggressive, treatment modality may be

Table 3 Data from subject daily ulcer diaries

	SDD group [n = 25; mean (SD)]	*Placebo group [n = 25; mean (SD)]*	*P-value**
Number of new ulcers per day	0.17 (0.15)	0.36 (0.37)	0.07
Total number of new ulcers over 90 days	16.0 (12.9)	32.4 (33.4)	0.08
Number of days with no new ulcers	80.4 (5.9)	69.8 (18.7)	0.04
Number of days with no pain	60.5 (20.8)	45.3 (26.5)	0.06
Mean VAS pain scores per day	10.4 (9.2)	15.4 (15.1)	0.25
Total summed VAS scores over 90 days	932.8 (824.9)	1386.5 (1361.5)	0.25
Number of days on which subject needed to use additional treatment	11.5 (14.9)	13.4 (12.9)	0.68

*Determined using independent samples *t*-test.

indicated, for example, systemic steroid therapy. Prednisone can be used in conjunction with topical gels and rinses (26), or with another immunosuppressive agent, azothiaprine (27). Tertiary lines of treatment include thalidomide, which has been shown to be an effective treatment for severe oral ulceration, despite the potential for significant side effects (28).

In this pilot study, the efficacy of SDD (20 mg doxycycline) taken twice daily for 90 days in alleviating the symptoms associated with ROAU was assessed. SDD has become established as an adjunctive treatment for the management of patients with both chronic and aggressive periodontitis (19, 29). SDD reduces the local production of excessive levels of MMPs in diseased periodontal pockets (15–17) and thus downregulates the destructive processes associated with progressing periodontal disease. The long-term administration of SDD for periods of 9–18 months has not been associated with significant adverse events, or the development of antibiotic resistance in the periodontal microflora or the microflorae of the gastrointestinal or genitourinary tracts (20, 21). SDD has also been evaluated in the dermatological setting, and a 6-month study revealed that SDD (20 mg doxycycline twice daily) resulted in a significant improvement in inflammatory lesions in facial acne with no evidence of changes in antibiotic susceptibility or colonization by potential pathogens. The drug was well tolerated and had no detectable antimicrobial effects on the skin flora, and did not result in an increase in doxycycline-resistant organisms (30).

Subantimicrobial dose doxycycline was chosen as a possible treatment for ROAU in this study because of its known anticollagenolytic properties. Tetracyclines have previously been reported as efficacious in the treatment of ROAU though it is not clear whether this reflects an antibiotic effect in reducing secondary infection of ulcers, or whether it is related to non-antimicrobial effects such as MMP inhibition, or a combination of both. It has previously been reported that doxycycline *in vitro* inhibits salivary collagenase levels (10) and the same authors also identified that salivary MMP-8 levels were elevated in patients with aphthae. In a later study, it was identified that mast cells express high levels of MMP-8 (and also MMP-1; 31). The finding that mast cells can be found localized in the subepithelial connective tissue layer beneath aphthae (11) supports the concept that mast cells are actively involved in ROAU pathogenesis. Thus, a drug that inhibits MMP-8 activity (together with other MMPs) may theoretically be beneficial in the treatment of aphthous ulceration.

In this small pilot study, a clinical benefit of SDD therapy was seen, although statistically significant differences between treatment groups were not apparent for all parameters studied. Thus, there were significantly more days on which no new ulcers occurred in the SDD group compared with the placebo group ($P < 0.05$; Table 3), and strong trends towards fewer new ulcers per day, fewer new ulcers over the 90-day period, and more days with no pain in the SDD group compared with the placebo group ($P = 0.06$–0.08). These findings represent tangible clinical benefits for the subjects, who were patients with long-standing, somewhat intractable problems with ROAU over many years, necessitating regular follow up and review on a specialist Oral Medicine clinic at a regional referral centre. Although less pain was recorded in the SDD group compared with the placebo group during the study, the data were very variable, and there were no significant differences between the groups. We attribute this to the great variability that is inherent in pain responses between different individuals. Indeed, it is well recognized among clinicians that some patients with large ulcers seems to complain very little about pain, whereas others with small ulcers experience seemingly much more pain (22).

Our interpretation of the data is that SDD shows promise as a possible therapeutic in the management with ROAU, and this effect may be mediated through a non-antimicrobial mechanism, i.e. inhibition of MMPs, possibly in mast cells. In support of this, recent data have reported that chemically modified tetracyclines (CMTs), which possess only anti-MMP activity and no antibiotic properties whatsoever, limit the viability and proliferation of mast cells and inhibit their production of inflammatory cytokines such as TNF-α (32, 33). Further long-term studies to test the efficacy of SDD in the treatment of ROAU are required, involving larger numbers of subjects and extended time periods. SDD has a demonstrated safety profile with minimal adverse effects, and has been shown to be effective in the management of periodontitis and acne. The data in this report support the concept that SDD may be considered as an additional drug therapy for the management of patients with ROAU, although it is presently not licensed for this indication. If this is confirmed in subsequent studies, SDD may prove to be a useful treatment, given its low incidence of unwanted effects in comparison with many of the other drug treatments that are routinely prescribed for patients with long-standing and persistent ROAU.

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