Topical Tacrolimus in Erosive Oral Lichen Planus: An Effective Treatment Approach§

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Objective: Lichen planus is a chronic mucocutaneous disease with primary or secondary involvement of the oral mucosa. In particular in erosive lichen planus most patients experience no long-term symptom relief of conventional topical treatment such as corticosteroid ointments. Topical tacrolimus application to mucosal lesions seem to be an innovative treatment approach. We present data of a clinical phase-II study in patients with erosive lichen planus.

Patients and methods: A total of 18 patients (13 females, 5 males; mean age 64,5 years) have been recruited so far. All patients had a verified diagnosis of erosive lichen planus and had been intermittendly on local corticosteroid treatment without long-term benefit. Patients were instructed to self-administer tacrolimus 0.1% ointment on involved mucosal surfaces twice daily. Treatment period was 8 weeks with a follow-up period of 14 weeks. Blood levels of tacrolimus were assessed using an on-line SPE/HPLC/MS/MS system.Treatment response was assessed by visual analog scale (VAS range 0 – 10; 0=pain free, 10= severe pain). Treatment response was also assessed on mucosal biopsies with immunohistochemical staining for CD3, CD 4, CD 8, CD 68, CD79a and S100.

Results: Objective response rate was 100%, with a complete remission of 55%. Treatment response assessed by VAS scale revealed a significant decrease of VAS 5,96± 0.69 (week 0) to VAS 2,68±0,39 (week 8) (p=0.000). All biopsies showed a strong decrease of inflammatory cells supporting the immunomodulating effect of tacrolimus.

Conclusions: Topical tacrolimus ointment is a safe and very effective treatment approach for erosive lichen planus and deserves further investigation.

Key words: erosive oral lichen planus, tacrolimus, SPE/HPLC/MS/MS system, phase-II study, cytokines

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INTRODUCTION

Oral lichen planus is a chronic inflammatory, T-cell mediated mucosal disease of unknown origin, with an incidence of 0.2-4% within the adult population (Rodriguez-Nunez et al, 2001; Jainkittivong et al, 2002; Thornhill et al; 2003). Female patients are more commonly affected than male patients at a rate of 1.4:1 (Axéll and Rundquist, 1987).

Histologically oral lichen planus is characterised by a dense band-like subepithelial lympho-histiocytic infiltrate, increased numbers of intra-epithelial lymphocytes, and degeneration of basal keratinocytes (Sugerman et al, 2002; Dorrego et al, 2002). The subepithelial infiltrate predominantly consists of $CD4^+$ T-cells, while the intra-epithelial infiltrate consists of $CD8^+$ T-cells (Zhou et al, 2002). The chronic long-lasting clinical course of oral lichen planus may be explained by the activation of nuclear factor kappa B (NF-κB), a primary transcription factor involved in the pathogenesis of several chronic inflammatory diseases, such as asthma, psoriasis, rheumatoid arthritis (Barnes and Karin, 1997). NF-κB is released from inhibitory complexes and translocates to the nucleus, where it binds to the promotor regions of

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different genes encoding immune and pro-inflammatory mediators, such as TNF-α, IL-1β, leukocyte and vascular adhesion molecules (Barnes and Karin, 1997). Some of these products can activate NF-κB and this type of positive regulatory loop may exacerbate and perpetuate local inflammatory reactions (Barnes and Karin, 1997). Santoro et al (2003) observed that cases of erosive / atrophic oral lichen planus, which are usually associated with more severe epithelial damage, showed a lower degree of epithelial NF-κB activation than remaining oral lichen planus cases, while the number of cytotoxic activated lymphocytes was similar between the two groups. The authors speculate that in erosive/atrophic oral lichen planus, lower NF-κB activation on epithelial cells results in poor protection of keratinocytes against necrosis, whereas it does not influence the recruitment of inflammatory cells (Santoro et al, 2003).

Oral lichen planus is often asymptomatic; however, the erosive and ulcerative forms are often painful, interfering with eating, swallowing and speech and subsequently affecting quality of life (Edwards and Kelsch, 2002; Dissemond, 2004). A large spectrum of treatment modalities including topical as well as systemic treatment have been proposed for symptomatic oral lichen planus (Bagan et al, 2004), however, there is still a lack of randomized controlled trials evaluating the various agents. At present topical corticosteroids remain the mainstay of therapy. Studies utilising midpotency and superpotent corticosteroids have demonstrated the efficacy of these in 30%-75% of patients (Lozada-Nur and Miranda, 1997). Topical and systemic cyclosporin A has also been used successfully (Demitsu et al, 2002). Frequently symptomatic oral lichen planus is resistant to these therapies, therefore a need for more effective topical agents exists.

The aim of our present investigation was to test the efficacy and safety of topical tacrolimus in patients suffering from erosive/ulcerative oral lichen planus refractory to previous topical application of corticosteroids.

MATERIALS AND METHODS

Our study was undertaken as an open clinical phase II trial in the Outpatient Clinic of the Department of Oral and Maxillofacial Surgery, Hannover Medical School. For all cases ethics committee approval and informed consent of participating patients was obtained.

Tacrolimus Ointment Preparation

Tacrolimus ointment (Protopic®, Fujisawa Germany, Munich) was used at a concentration of 0.1%; patients were instructed to self-administer tacrolimus ointment twice daily to the affected mucosal lesions with a cotton swap or sterile gauze.

Inclusion Criteria

Patients with biopsy-proven erosive or ulcerative oral lichen planus resistant to topical or systemic corticosteroid treatment were asked to participate in this study. Unsuccesful previous treatment was defined as persistance of erosive mucosal lesions as well as persistent painful oral symptoms. Previous medications to treat mucosal symptoms was suspended at least two weeks prior to treatment initiation. Mouthwash with 0.2% chlorhexidine for palliation of mucosal symptoms was allowed during the study. Patients had no history of renal, hepatobiliary, or malignant disease, hypertension or acute infection.

Study Design

Patients received detailed information regarding study protocol and tacrolimus application by verbal explanation and written documentation. Subsequently, informed consent was given. Each patient's data set (gender, age, medical and drug history) was recorded.

Subjective Treatment Assessment

Each participating patient reported their response to tacrolimus treatment by completing a self-administered assessment using the Visual Analogue Scale (VAS), known as a simple, reproducible instrument (Scott and Huskisson, 1976). VAS score is represented as a plain horizontal line from 0 to 10. A zero value equates to being free of pain, whereas the most severe pain is rated 10. Patients were asked to bisect the line at the point of their present oral discomfort.

Objective Assessment by the Clinician

In our study objective assessment of the involved mucosal areas was done by a clinician before treatment and at each follow-up visit. Clinical photographs from the oral mucosa were also taken at each visit using a digital camera (Fuji® Finepix S2 Pro). In addition, the involved erosive/ulcerative mucosal areas were measured and analyzed on digital photographs by using the public domain software NIH Image (http://rsb.info.nih.gov/nih-image/default.htm). The total surface area of selected lesions was estimated in square millimeters.

Mucosal Biopsies and Immunohistochemistry

In order to assess mucosal changes during tacrolimus therapy two representative mucosal biopsies of erosive / ulcerative areas were taken under local anesthesia before treatment initiation and after 8 weeks, immediately fixated in 4% buffered formalin and subsequently further processed for immunohistochemistry. Primary monoclonal antibodies specific for CD3 (DakoCytomation, Carpintera,CA, USA), CD4 (dianova, Hamburg/Germany), CD8 (DakoCytomation, Carpintera,CA, USA), CD68

(DakoCytomation, Carpintera,CA, USA), CD79a (DakoCytomation, Carpintera,CA, USA) have been used for detection of T-helper cells (CD4), T-suppressor cells (CD8), and pan T-cells (CD3). Additionally polyclonal S100 (DakoCytomation, Carpintera, CA, USA) was used for detection of Langerhans cells and CD68 marks histiocytes. Sections were viewed microscopically independently by three clinicians (A.E., B.V., O.S.) to determine expression of the forementioned antigens. Assessment of antigen expresion was made in a semi-quantitative fashion using the following scores: 0, (+), +, ++, +++. Paraffin sections were also stained with hematoxylin and eosin and PAS for diagnostic confirmation of lichen planus.

Treatment Schedule

Before treatment initiation with tacrolimus (week 0) baseline subjective and objective assessments were documented from each participating patient. Involved mucosal lesions were photographed with a digital camera. Each patient was instructed to apply 0.1% tacrolimus ointment to the painful mucosal lesions twice daily for 8 weeks using cotton swap or sterile gauze. Follow-up visits for the patients were organized and patients were reviewed by the same clinician at weeks 1, 2, 3, 4, 6, 8, 10, 14, 18 and 22. At all follow-up visits subjective and objective assessments and any adverse effects were recorded. Tacrolimus treatment was withdrawn after the 8-week-study period.

Monitoring of Tacrolimus Blood Levels

Tacrolimus absorption was monitored on weeks 1 and 2 of the study using the on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry system (SPE-HPLC-MS/MS) (Koal et al, 2004).

Statistical Aanalysis

The VAS scores, morphometric analysis of mucosal areas, and immunohistochemical expression scores were analyzed by paired t-test. A statistically significant difference was accepted if *p*-values were less than 0.05.

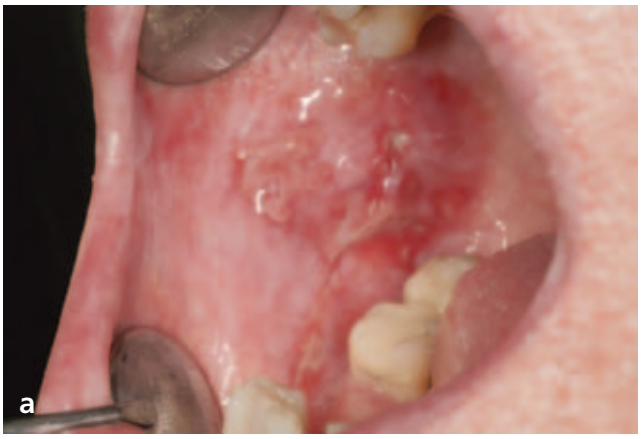
RESULTS

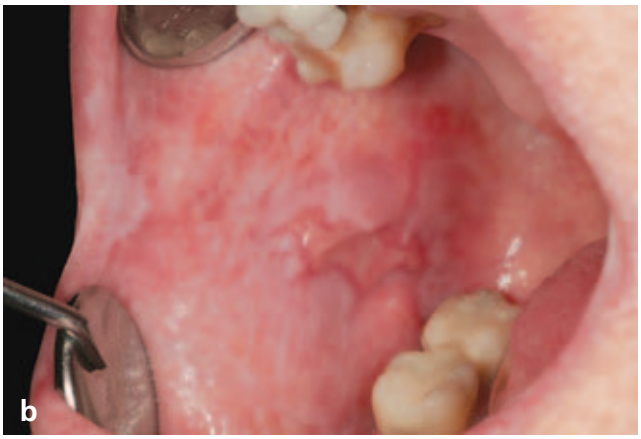
Study Group

At present a total of 18 patients (13 females, five males; mean age 64.5 years; range 47.8–81.6 years) are enrolled in this trial. There was no patient withdrawal during the eight-week study period.

Clinical Change of Mucosal Lesions

Objective assessment of response to topical tacrolimus by a clinician revealed an objective response of 100% and a 55% complete remission rate with disappearance of all erosive/ulcerative mucosal lesions after the eight-week study period. There was an 88% reduction in mean surface area of selected erosive lichen planus lesions after eight weeks of treatment. This reduction was highly statistically significant ($p<0.006$). All patients demonstrated a reduction in lesion surface area; the reduction after the eight-week study period varied from 52% to 100%. Clinical improvement of erosive/ulcerative mucosal lesions during treatment is shown in Fig 1a-c.





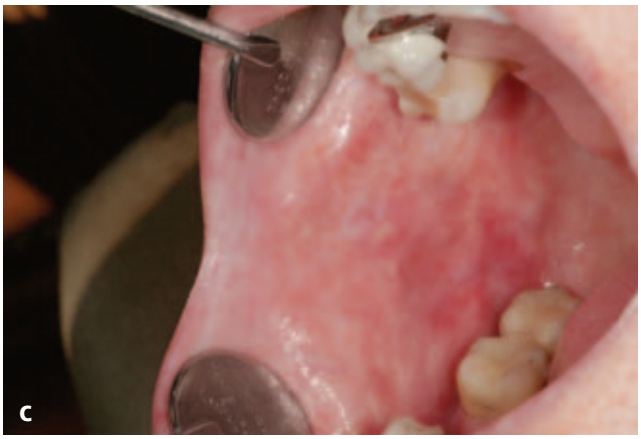


Fig 1a-c Erosive oral lichen planus with typical involvement of buccal mucosa before treatment (1a). During treatment in week 4 marked shrinkage of involved mucosal area (1b) and furthermore also stable complete remission in week 14 (1c).

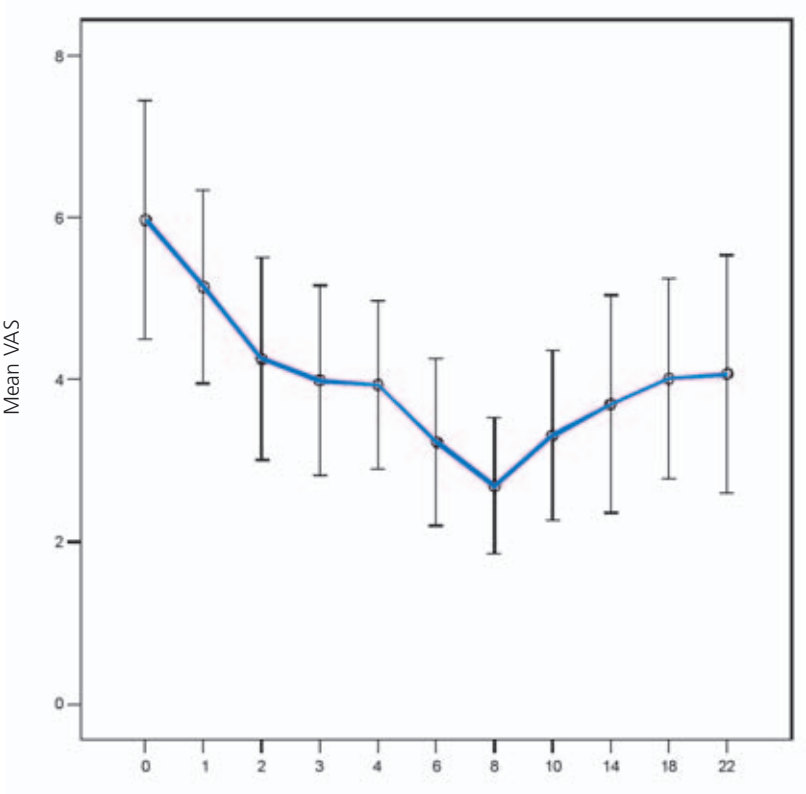


Fig 2 Mean VAS in 18 patients with erosive oral lichen planus over a period of 22 weeks with an eight-week treatment period of topical tacrolimus. Error bars represent 95% confidence interval.

Symptom Relief

Topical tacrolimus therapy significantly reduced painful mucosal symptoms as recorded on the VAS (Fig 2). 94% of the study population reported major symptom relief, whereas only 6% of the patients reported no change of symptoms. Mean VAS in week 0 (5,69 ± 0,69; 95% C.I. 4,50 – 7,43) significantly differs from mean VAS in week 8 (2,68 ±0,39; 95% C.I. 1,84 – 3,53) (p=0.000).

Adverse Effects

Initial burning at the site of application was the only side effect reported by the patients. These temporary symptoms does not lead to study withdrawal.

Systemic Absorption of Tacrolimus

Twelve of 18 patients had systemic absorption of tacrolimus within the therapeutic range (0-2,90 ng/ml). Mean systemic tacrolimus level on day 0 was 0.05 ng/ml and increased to 1,16 ng/ml on day 8. No relation could be demonstrated between systemic absorption of tacrolimus and ulcerated mucosal areas.

Immunohistochemistry

Expression of cytokines on mucosal biopsies assessed in a semi-quantitative fashion demonstrated a decrease over the 8-week study period. The mean expression scores assessed by three clinicians (A.E., B.V., O.S.) showed significant decrease in most inflammatory cells: CD4 positive T-helper cells ($p<0.001$), CD8 positive T-suppressor cells ($p<0.001$), CD68 positive histiocytes($p=0.000$), CD79a positive plasma cells ($p=0.153$), and S100 positive Langerhans cells ($p=0.095$), supporting an immunomodulating effect of topical tacrolimus.

Mucosal Relapse after Cessation of Tacrolimus Therapy

After the eight-week period of topical tacrolimus treatment, 10 of the 18 patients (62.5%) suffered a relapse of erosive oral lichen planus within three to 10 weeks.

DISCUSSION

Erosive oral lichen planus, a chronic inflammatory T-cell-mediated disase, continues to be a therapeutic challenge, since most of the present topical or systemic treatment concepts using corticosteroids only yield short-term remissions and limited or inconsistent symptom relief in the majority of patients. Furthermore prolonged treatment with systemic steroids or retinoids bears the risk of substantial adverse effects. This may explain the intensive search for newer more effective treatment modalities with fewer side effects. A promising new treatment for oral lichen planus is the topical use of the immunomodulator tacrolimus (FK506), a

Table 1 Clinical trials of topical tacrolimus in erosive lichen planus

Author	Patients (n)	Tacrolimus concentration	Treatment duration	Symptom relief
Vente et al, 1999	6	0.1%	4-12 weeks	yes
Olivier et al, 2002	10	0.1%	6 months	yes
Morrison et al, 2002	6	0.1%	3 months	yes
Kaliakatsou et al, 2002	19	0.1%	8 weeks	yes
Rozycki et al, 2002	13	0.03%-0.1%	4-8 weeks	yes
Hodgson et al, 2003	50	0.1%	2-39 months	yes
Thomson et al, 2004	23	0.1%	4-29 months	yes

hydrophobic, polycyclic lactone, which is involved in interleukin 2 (IL-2) production mediated by T cells (Schreiber and Crabtree, 1993). Tacrolimus is currently used for the prevention of allograft rejection following kidney, liver, and heart transplantation (Ruzicka et al, 1997). Furthermore, tacrolimus has been used systemically for the treatment of chronic immunedermatoses. In recent years several groups reported on promising results by using topical tacrolimus in chronic inflammatory skin disease as well as chronic inflammatory mucosal disease like oral lichen planus (Vente et al, 1999; Lener et al, 2001; Kaliakatsou et al, 2002; Olivier et al, 2002; Rozycki et al, 2002; Morrison et al, 2002; Hodgson et al, 2003; Thomson et al, 2004). All authors came to the conclusion that topical tacrolimus is highly effective in erosive lichen planus with rapid symptom relief and disappearance of inflammatory mucosal symptoms (Table 1).

Interstingly, and this was also reported in other studies (Vente et al, 1999; Kaliakatsou et al, 2002), the majority of our patients (10/18) experienced mucosal relapse after treatment discontinuation. It appears therefore recommendable to extend topical application of tacrolimus. For atopic dermatitis as well as for oral lichen planus long-term treatment with topical tacrolimus seem to be feasible without alteration of the safety profile (Reitamo et al, 2000; Hodgson et al, 2003).

Our present study clearly shows that erosive oral lichen planus can be treated very effectively by topically applied tacrolimus and results in rapid symptom relief.

The efficacy in lichen planus further supports the concept that T lymphocytes play an essential role in the pathogenesis of this disease. Despite our promising results which have also been reported by other groups (Vente et al, 1999; Lener et al, 2001; Kaliakatsou et al, 2002; Olivier et al, 2002; Rozycki et al, 2002; Morrison et al, 2002; Hodgson et al, 2003; Thomson et al, 2004), further recommendations and interpretation must be made with caution. Although topical immunomodulators seem to offer a new treatment option for symptomatic treatment of erosive oral lichen planus, a recent meta-analysis and Cochrane review does not support the superiority of conventional interventions over placebo for the treatment of symptomatic oral lichen planus (Cribier et al, 1998; Chan et al, 2004). We would therefore emphasise that randomised clinical trials are necessary to better define in particular the role of new immunomodulators for symptomatic treatment of oral lichen planus.

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