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ORIGINAL ARTICLE

Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone

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OBJECTIVE: To explore the potential of detecting the level of proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-1-alpha (IL-1-α), IL-6, and IL-8 in whole unstimulated saliva (WUS) in monitoring the therapeutic effects of topical dexamethasone on these salivary cytokines in subjects with erosive oral lichen planus (OLP).

STUDY DESIGN: Thirteen definitively diagnosed OLP subjects were enrolled in the study as were 13 age- and sex-matched controls. The OLP subjects were treated with 0.1% dexamethasone oral rinse for 6 weeks. Prior to treatment and at the end of clinical trial, the visual analog scale (VAS) for symptoms was recorded, WUS was collected and these proinflammatory cytokines were analyzed by ELISA.

RESULTS: Following the dexamethasone treatment, the levels of TNF-α, IL-1-α, IL-6, and IL-8 were decreased significantly, and IL-1-α and IL-8 were detected at a level without a statistically significant difference from controls. VAS value was decreased significantly and was found to significantly correlate with the decrease in IL-1-α and IL-8 levels.

CONCLUSIONS: These preliminary results indicate that salivary analysis of NF-κB-dependent cytokines may be applied to monitoring the therapeutic response of OLP.
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Keywords: oral lichen planus; whole unstimulated saliva; NF-κB-dependent cytokines; dexamethasone

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Oral lichen planus (OLP) is a chronic inflammatory condition involving the oral mucosal tissues which is an autoimmune disorder mediated by T-cell lymphocytes (Sugerman *et al*, 2002). Often OLP undergoes an acute phase when the superficial epithelium sloughs and leaves areas of mucosal erosion, thus erosive lichen planus (ELP). Although the etiology and mechanisms of OLP pathogenesis have not been fully disclosed, several lines of evidence have demonstrated that a complex cytokine network, especially, the activation and expression of NF-κB and its associated cytokines, plays an important role in the exacerbation and perpetuation of OLP (Sugerman *et al*, 2002; Santoro *et al*, 2003). Therefore, the detection of NF-κB and its associated cytokines in OLP is likely to have clinical potential for monitoring disease activity and therapeutic response of OLP.

As oral fluid analysis has some obvious advantages compared with blood-based analyses, such as easy access and non-invasive collection, oral fluids have been widely used in drug and disease monitoring and the detection of various oral and systemic maladies (Kaufman and Lamster, 2002; Lawrence, 2002). In our laboratory, oral fluids have been successfully applied for detection of s-IgA, apoptotic cells, and proinflammatory cytokines in patients with Sjogren's syndrome, OLP, oral leukoplakia and oral squamous cell carcinoma (Rhodus *et al*, 1998, 2005a; Cheng *et al*, 2004). Recently, our analysis has shown that NF-κB-dependent cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-1-alpha (IL-1-α), IL-6 and IL-8, can be detected at a significant higher level in three types of oral fluids from OLP patients (Rhodus *et al*, 2005b). It is evident that it is important to evaluate the practical application of these salivary analyses of NF-κB-dependent cytokines in clinical management of OLP.

The purpose of this study was to monitor therapeutic response by determining the level of several proinflammatory, proangiogenic, NF-κB-dependent cytokines in whole unstimulated saliva(WUS), in subjects with OLP

before and after treatment with an oral glucocorticod anti-inflammatory agent, dexamethasone.

Materials and methods

According to the criteria of inclusion and exclusion for participation and Eisenberg–Krutchkoff's histopathologic criteria (Krutchkoff and Eisenberg, 1985; Campisi *et al*, 2004; Rhodus *et al*, 2005b), 13 patients with oral erosive-ulcerative lichen planus (ELP) were enrolled in the study as were 13 age- and sex-matched controls (Figure 1). The average age of patients was 57.2 years (range: 28–78) and all subjects were female. All subjects were from the University of Minnesota Oral Medicine Clinic. With the approval of the Committee for the Use of Human Subjects in Research, informed consent was obtained from all subjects.

These ELP patients were then placed on therapy with 0.1% dexamethasone mouthwash three times a day over a period of 6 weeks. Patients were instructed to rinse with 5 ml for 3 min and then to expectorate, and also instructed not to use any other medications for the treatment of ELP during this study period.

Prior to treatment and at the end of the 6-week clinical trial, the evaluation of visual analog scale (VAS) to evaluate subjective symptomatology was performed in all subjects. Briefly, the participants indicated the severity of their pain and/or discomfort on a 100 mm VAS. The scale (a 100 mm horizontal line) was designed with zero level of discomfort (no pain) on the far left of the horizontal line and 10 (worst pain imaginable) on the far right. The subject made a vertical line across the point where they felt symptoms.

Similarly, the WUS were collected from all participants prior to treatment and at the end of therapeutic intervention. The WUS were then analyzed by ELISA technique. The detail procedures of the WUS collection and analysis were described in our previous report (Rhodus *et al*, 2005b).

Statistical Analysis System Software (Version 8.1; SAS Institute Inc., Cary, NC, USA) was used to analyze the data. Results were expressed as mean ± standard deviation (s.d.). First, Wilcoxon signed rank test was used for comparison of the change of each cytokine level and VAS value between before and after treatment in OLP group, and then, Wilcoxon rank sum test was performed to demonstrate the difference of each cytokine level between OLP and control group. Furthermore, Spearman's rank test was used to evaluate correlations between each cytokine and VAS. $P \leq 0.05$ was considered to be statistically significant.

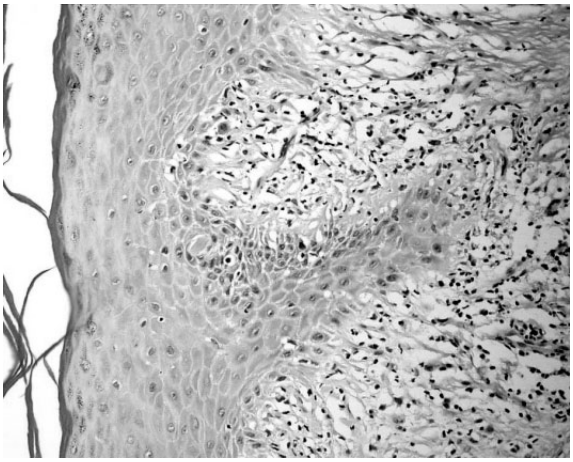

Figure 1 Histopathologic speciman of OLP, note basalar 'retepeys' and bank-like lymphocytic infiltrate

Results

Following treatment with the topical dexamethasone mouthwash for 6 weeks, the levels of TNF-α, IL-1-α, IL-6 and IL-8 decreased significantly, moreover, it was noted that IL-1-α and IL-8 were detected at a level not significantly different from controls. Also, subject symptoms as indicated by VAS value was decreased significantly (Table 1 and Figure 2). Additionally, the results from Spearman's rank test showed that there was a significant correlation between VAS improvement and decreases in IL-1-α and IL-8 (Table 2 and Figure 3).

Discussion

As the activation and enhanced expression of NF-κB and its associated cytokines have been demonstrated to contribute to the pathogenesis of OLP from our previous study and other reports (Sugerman *et al*, 2002; Santoro *et al*, 2003; Rhodus *et al*, 2005b), it is reasonable for clinicians to focus on the clinical application of detection of NF-κB and its associated cytokines in tissues and different body fluids. In this preliminary study, with a small sample size, we found a statistically significant reduction in the salivary levels of proinflammatory cytokines, TNF-α, IL-1-α, IL-6 and IL-8, in patients with erosive OLP following treatment for 6 weeks with a dexamethasone mouthwash. Additionally, the subjects experienced significant subjective improvement in discomfort symptoms following the dexamethasone treatment which was positively correlated with the decrease of IL-1-α and IL-8. Our results, to the best of our knowledge, present for the first time evidence that the detection of these cytokines in saliva may have some utility in monitoring the clinical management for OLP.

Although the activation of NF-κB and enhanced expression of its dependent cytokines were found in serum, oral keratinocytes and tissue-infiltrated mononuclear cells of OLP patients (Fayyazi *et al*, 1999; Simark-Mattsson *et al*, 1999; Sklavounou *et al*, 2000; Khan *et al*, 2003; Santoro *et al*, 2003), their clinical significance remains unclear. In previous study, serum IL-6 was found as a useful marker in evaluating therapeutic effects and in monitoring the disease status of OLP (Sun *et al*, 2002), and IL-6 level of serum and oral exfoliated mucosal cells from OLP lesions was significantly higher in patients with ulcerative OLP (Gu *et al*, 2004). Recently, the NF-κB-dependent cytokines, TNF-α, IL-1-α, IL-6, and IL-8 were successfully detec-

Table 1 The change of NF-κB-dependent cytokine levels and VAS in OLP patients treated with dexamethasone

	TNF-α	*IL-1-α*	*IL-6*	*IL-8*	*VAS*
Before	10.97 ± 7.56	260.37 ± 65.97	74.01 ± 9.67	1912.00 ± 297.92	6.72 ± 1.38
After	7.72 ± 5.40	202.71 ± 48.60	28.51 ± 10.29	1531.70 ± 258.11	2.26 ± 0.62
Control	2.95 ± 1.86	117.05 ± 39.09	1.35 ± 1.33	1579.00 ± 205.78	
Before *vs* after	S	S	S	S	S
Before *vs* control	S	S	S	S	
After *vs* control	S	NS	S	NS	

S, significant; NS, non-significant.

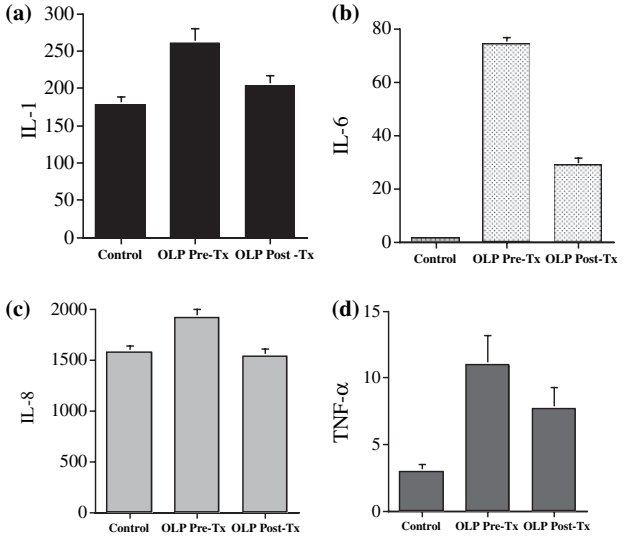

Figure 2 Mean cytokine levels of IL-1 (**a**), IL-6 (**b**), IL-8 (**c**), and TNF-α (**d**) measured in pg ml^{-1} from control patients compared with levels in patients with OLP before and after topical glucocorticoid treatment. Error bars = s.e.m. $n = 13$ per group

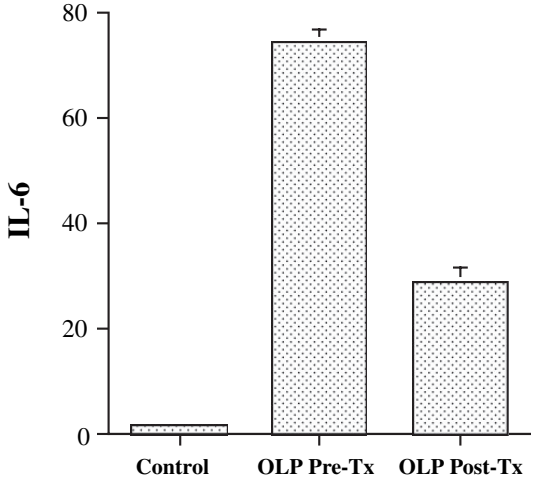

Figure 3 Visual analog scale (VAS) (100 mm) of symptomatic improvement in OLP before and after treatment with dexamethasone

Table 2 The relationship between NF-κB-dependent cytokines and VAS (1.0 cm)

	Correlation coefficient			
	TNF-α	*IL-1-α*	*IL-6*	*IL-8*
VAS	0.5468	0.6052	0.3741	0.6244
P	0.5431	0.0284	0.2079	0.0225

ted at significant elevated levels in three types of oral fluids from OLP patients (Rhodus *et al*, 2005b), and the change of NF-κB-dependent cytokines in saliva reflected in part the trend of malignant transformation of OLP (Rhodus 2005c). These results, together with the data presented here, strongly indicate that the detection of NF-κB-dependent cytokines in different biological specimens indeed has some clinical potential in monitoring therapeutic response and the disease activity status of OLP.

VAS is one method to evaluate pain and other subjective symptoms of disease. In this study, the reduction of subject's VAS was noted to parallel to the decrease of these proinflammatory cytokines, especially, IL-1-α and IL-8. This finding may implicate that IL-1-α and IL-8 play more important role in the development of symptoms of erosive OLP. Although more research advances have disclosed the peripheral mechanisms of inflammatory and neuropathic hyperalgesia mediated by proinflammatory cytokines (Lee *et al*, 2004; Sommer and Kress, 2004), the precise role of proinflammatory cytokines in pain is still unknown and the relationship between proinflammatory cytokines and VAS or other symptoms/signs is controversial. For example, levels of serum IL-1-β and TNF-α was reported to correlate with self-rated health in women but not in men (Lekander *et al*, 2004), and the concentrations of IL-1-beta, IL-6 and IL-8 in the synovial fluid of temporomandibular joint disorders were found to positively associate with pain/hyperalgesia (Alstergren *et al*, 1998), and the joint effusion grade (Segami *et al*, 2002). In contrast, no correlation was found between the measurements of IL-8 and other inflammatory mediators and the symptoms assessed with the VAS in chronic venous disease (Howlader and Smith, 2003) or patients with disc herniation and sciatica (Brisby *et al*, 2002). Therefore, further studies are necessary to elucidate the role of proinflammatory cytokines in the symptoms of erosive OLP.

A series of topical medications, including steroids, cyclosporine, retinoid and tacrolimus, have been applied to the treatment of OLP, especially erosive/ulcerative OLP (Chainani-Wu *et al*, 2001; Byrd *et al*, 2004). Dexamethasone, a glucocorticoid drug has been widely used as an anti-inflammatory agent for OLP and other mucocutaneous inflammatory conditions (Rosenberg *et al*, 1997; Chainani-Wu *et al*, 2001). For example, dexamethasone has been shown to decrease NF-κB transcription of IL-1-β and MMP-9 in animals and humans (Eberhardt *et al*, 2002), to inhibit the expression of IL-6 in human airway smooth muscle cells in patients with COPD (Huang *et al*, 2003) and suppress IL-8 and macrophage inhibitory protein in PMNs of newborns with bronchopulmonary dysplasia (Irakam *et al*, 2002). In the present study, dexamethasone mouthwash was observed to result in a significant decrease of these proinflammatory cytokines and satisfactory clinical outcome in erosive OLP. As analysis of evidence-based medicine has shown that there was some weak evidence for the superiority of the assessed interventions over placebo for palliation of symptomatic OLP (Chan *et al*, 2000), our results may suggest that dexamethasone mouthrinse may be an efficacious choice of therapy.

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