Journal of Oral Science, Vol. 48, No. 3, 153-156, 2006

Original

# Association between recurrent aphthous stomatitis and salivary thiocyanate levels

Karen Cherubini[1)], Cesar Sant'Anna Lorandi[1)], Stela Maria Rigo Krapf[2)], Fabiany Rodrigues de Sousa[2)], Liliane Soares Yurgel[1)], Maria Antonia Figueiredo[1)] and Fernanda Gonçalves Salum[1)]

[1)]Division of Stomatology and Prevention of Bucomaxillofacial Cancer, Hospital São Lucas-PUCRS, Rio Grande do Sul, Brazil
[2)]Laboratory of Biochemistry, Hospital São Lucas-PUCRS, Rio Grande do Sul, Brazil

(Received 12 June and accepted 28 July 2006)

**Abstract: The aim of this work was to investigate the association between recurrent aphthous stomatitis (RAS) and salivary thiocyanate levels. The sample comprised men and women of age ranging from 15 to 55 years, who were allocated to four groups: 28 patients in RAS active phase (group 1); 28 patients in RAS remission phase (group 2); 29 smokers (group 3); 26 non-smokers without RAS (group 4). Samples of whole saliva mechanically stimulated were collected, and thiocyanate levels were measured. The results were analyzed by ANOVA and paired *t*-test. Mean salivary thiocyanate values were 0.55 mM, 0.64 mM, 2.36 mM and 0.96 mM in groups 1 (active RAS), 2 (remission RAS), 3 (smokers) and 4 (control), respectively. There was no significant difference in thiocyanate levels when groups 1 and 2 were compared with group 4. Group 3 showed a significantly higher thiocyanate concentration when compared with groups 1, 2 and 4 ($P < 0.05$). There was no significant difference in thiocyanate levels between groups 1 and 2 ($P > 0.05$). It is therefore suggested that there is no association between RAS and salivary thiocyanate levels. (J. Oral Sci. 48, 153-156, 2006)**

Correspondence to Dr. Karen Cherubini, Serviço de Estomatologia, Hospital São Lucas - PUCRS, Av. Ipiranga, 6690 Sala 231 - 2° andar, Porto Alegre, RS, CEP: 90610-000, Brazil
Tel: +55-51-3320-3254, +55-51-9981-6987
Fax: +55-51-3320-3254
E-mail: kebini.ez@terra.com.br

Keywords: aphthae; thiocyanate; pH; saliva; tobacco.

## Introduction

Recurrent aphthous stomatitis (RAS) is a chronic inflammatory disease characterized by painful recurring ulcers of the oral mucosa of unknown etiology (1,2). RAS is one of the least understood diseases of the oral cavity, and the difficulty in determining its exact nature is due in part to the non-specific histopathologic features of the ulcers and to the lack of any reproducibly identifiable endogenous or exogenous cause (3). Several theories have linked RAS etiology with local, microbiological, systemic, nutritional, immune and genetic factors, but the specific cause remains unknown (1,4).

A negative association between RAS and tobacco use has been reported (5-9), but the underlying mechanism is not clear.

Thiocyanate (SCN) is an ion found in organic and inorganic compounds. In the human body, diet (10) and tobacco (11) are the main sources. The ion represents a normal constituent of body fluids such as serum, saliva, tears and urine (10), showing significantly higher concentrations in smokers (11,12). Levels in non-smokers range from 0.5 to 2 mM with an average of 1 mM, while heavy smokers can show salivary thiocyanate concentrations as high as 6 mM (13). Salivary thiocyanate plays an important role in the peroxidase system, a non-immunoglobulin defense factor of saliva (14).

Considering the high salivary thiocyanate concentration

in smokers and interactions of this ion in the oral environment, we hypothesized that RAS patients have lower salivary thiocyanate concentrations than smokers and non-smokers without RAS.

## Materials and Methods

The sample comprised men and women of age ranging from 15 to 55 years, who were allocated to four groups.

Group 1 consisted of 28 RAS patients from the Stomatology Department of São Lucas Hospital at PUCRS, 19 women and 9 men, with a mean age of 29 years. These individuals satisfied the following inclusion criteria: (a) at least one RAS episode per month; (b) seronegativity for HIV; (c) normal values for complete blood cell count, erythrocyte sedimentation rate and fasting serum glucose level; (d) objective confirmation of RAS disease through clinical examination, according to the diagnostic criteria reported by Scully and Porter (4); and (e) current active lesion of RAS.

Group 2 included the same individuals in group 1 (n = 28) in the remission phase of the disease, i e., without active lesion. The patients who matched the selection criteria and were included in groups 1 and 2 were coincidentally all non-smokers.

Group 3 comprised 29 smokers, who had smoked a minimum of 10 cigarettes per day for at least five years prior to the study. Coincidentally, none of the individuals that matched these selection criteria had RAS. The mean age of these individuals was 35 years, 23 of them were women and 6 were men.

Group 4 (control group) was composed of 26 non-smokers who did not have RAS disease. They also met the criterion of no previous history of the disease. In this group, the mean age was 33 years, with 21 women and 5 men.

Before being enrolled, each person was interviewed and given an explanation of the aim and proceedings of the study. Only those individuals who matched the selection criteria and agreed to participate were included in the study. This study was approved by the research ethics committee of PUCRS.

### Saliva collection

Whole saliva was mechanically stimulated and collected as described elsewhere (15). The individual was asked to avoid contact of the oral cavity with any chemical agent for three hours preceding collection time. Hence, activities such as smoking, eating and toothbrushing were to be avoided in this period. Salivary flow was stimulated by chewing sterilized rubber bands (1 cm × 0.5 cm) and saliva was collected in sterilized plastic receptacles, always between 3 pm and 4 pm, under the same conditions and by the same investigator. The patient was seated, with eyes open, chewing the rubber band for ten minutes, alternating sides back and forth. Before saliva collection, RAS patients were examined to confirm the presence or absence of an active lesion at that moment. After collection, the saliva was stored in ice (no more than one hour) until time of processing. The samples were centrifuged at 3,500 rpm for ten min (16) and thiocyanate concentration was determined by colorimetric method (17). The results were analyzed by ANOVA (with Tukey's multiple comparison posthoc test) and the paired *t*-test, at a significance level of 5%.

## Results

The mean values and standard deviation observed for each group are reported in Table 1. Thiocyanate concentration did not differ significantly between RAS patients and the control group. Nevertheless, smokers showed higher levels of salivary thiocyanate than did RAS patients and controls (ANOVA, Tukey's test, $P < 0.05$). There was no statistically significant difference when the paired sample values of groups 1 and 2 were analyzed (paired *t* test, $P > 0.05$). The frequency distribution of RAS patients, smokers and controls according to the range of salivary thiocyanate values is presented in Table 2.

Table 1 Salivary thiocyanate concentration: mean values and standard deviation

| | Group 1 (Active RAS) | Group 2 (Remission RAS) | Group 3 (Smokers) | Group 4 (Control) |
|---|---|---|---|---|
| Thiocyanate (mM) | 0.55 ± 0.24 | 0.64 ± 0.25 | **2.36 ± 1.0** | 0.96 ± 0.77 |

Bold print highlights values with significant differences (ANOVA and Tukey's test, $P < 0.05$): Group 3 > Groups 1, 2, 4.

Table 2 Distribution of RAS patients, smokers and controls according to salivary thiocyanate range

| Thiocyanate (mM) | Individuals (n) | | | | |
|---|---|---|---|---|---|
| | Group 1 (Active RAS) | Group 2 (Remission RAS) | Group 3 (Smokers) | Group 4 (Control) | Total |
| 0 – 1 | 26 (92.86%) | 24 (85.71%) | 3 (10.34%) | 19 (73.08%) | 72 |
| 1.1 – 4 | 2 (7.14%) | 4 (14.29%) | 25 (86.21%) | 7 (26.92%) | 38 |
| > 4 | - | - | 1 (3.45%) | - | 1 |
| Total | 28 | 28 | 29 | 26 | 111 |

Percentage values were calculated per group.

## Discussion

Although many theories have been proposed to explain the cause of RAS, it still remains obscure (1,4). On the other hand, the negative association between RAS and tobacco use (5-9) has been proved, but the mechanism responsible for that is not known. A chemical agent liberated by tobacco, acting in a topical or systemic way, has been suggested (5,6), but no specific agent has been identified.

Promotion of specific and non-specific mechanisms of resistance, protection against toxic and mutagenic agents, and promotion of regenerative processes in wound healing and hair growth are some of the effects of thiocyanate (18). Salivary thiocyanate plays an important role in the peroxidase system, a non-immunoglobulin defense factor of saliva (14). Oral bacteria excrete $H_2O_2$, which is toxic to host tissues. Thiocyanate reacts with hydrogen peroxide ($H_2O_2$) under the catalytic action of peroxidase enzyme producing hypothiocyanite, which has antibacterial properties and is less harmful to human cells than hydrogen peroxide (19). Salivary peroxidase may also play a role in the destruction of $H_2O_2$ along the mucosa. Salivary peroxidase may play an important role in preventing damage to host tissues by eliminating this reactant as well as preventing bacteria from producing this metabolite (20). It has also been reported that aphthous ulcerations may be responsive to enhancement of the salivary peroxidase system. (21). Based on these properties as well as the fact that smoking can inhibit RAS and also increase thiocyanate levels, we hypothesized that thiocyanate could be the smoke agent responsible for RAS inhibition.

However, the results of this study rejected the proposed hypothesis. There was no statistically significant difference between RAS patients (groups 1 and 2) and control group in the salivary thiocyanate concentration, suggesting that there is no association between RAS and salivary thiocyanate. As expected, smokers showed significantly higher levels of thiocyanate when compared with RAS patients and control group, with a mean concentration of 2.36 mM. Glatz et al. (22) reported a mean thiocyanate concentration of 2.05 mM in smokers, while in non-smokers it was 1.05 mM. Studies have demonstrated that levels of salivary thiocyanate are elevated in smokers (11,12,23,24). However, the clinical implications of this finding are poorly understood, ranging from thiocyanate being considered a protective substance to being viewed as an aggressive agent according to sources consulted. Although values of thiocyanate did not differ significantly between RAS patients and controls, they were higher in the control group. Controls (group 4) showed thiocyanate values closer to those of smokers (group 3) than RAS patients with (group 1) or without (group 2) lesions. This finding suggests the possibility that a larger sample would change the results of this work, showing significant difference between controls and RAS patients.

When the paired samples of groups 1 (active RAS) and 2 (remission RAS) were compared, thiocyanate levels did not differ significantly, suggesting that there is no association between thiocyanate concentration and RAS status (active or in remission).

It was speculated that the negative association between RAS and tobacco could result from the thickness of the keratin layer and other epithelial alterations that this drug induces in the oral mucous membrane (5). However, these epithelial alterations are morphologic changes that probably depend on the action of a chemical, and this agent may also constitute the key for understanding the RAS/tobacco relationship. It seems reasonable that identification of a

chemical mediator responsible for the tobacco-mediated reversal of RAS would be a logical way to better understand this disease.

## Conclusion

The results of the present study could not establish any association between RAS and salivary thiocyanate levels.

## References


1. Ship JA (1996) Recurrent aphthous stomatitis. An update. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 81, 141-147
2. Sun A, Chang JG, Kao CL, Liu BY, Wang JT, Chu CT, Yuan JH, Chiang CP (1996) Human cytomegalovirus as a potential etiologic agent in recurrent aphthous ulcers and Behçet's disease. J Oral Pathol Med 25, 212-218
3. Vincent SD, Lilly GE (1992) Clinical, historic, and therapeutic features of aphthous stomatitis. Literature review and open clinical trial employing steroids. Oral Surg Oral Med Oral Pathol 74, 79-86
4. Scully C, Porter S (1989) Recurrent aphthous stomatitis: current concepts of etiology, pathogenesis and management. J Oral Pathol Med 18, 21-27
5. Axéll T, Henricsson V (1985) Association between recurrent aphthous ulcers and tobacco habits. Scan J Dent Res 93, 239-242
6. Grady D, Ernster VL, Stillman L, Greenspan J (1992) Smokeless tobacco use prevents aphthous stomatitis. Oral Surg Oral Med Oral Pathol 74, 463-465
7. Tüzün B, Wolf R, Tüzün Y, Serdaroglu S (2000) Recurrent aphthous stomatitis and smoking. Int J Dermatol 39, 358-360
8. Atkin PA, Xu X, Thornhill MH (2002) Minor recurrent aphthous stomatitis and smoking: an epidemiological study measuring plasma cotinine. Oral Dis 8, 173-176
9. Rivera-Hidalgo F, Shulman JD, Beach MM (2004) The association of tobacco and other factors with recurrent aphthous stomatitis in an US adult population. Oral Dis 10, 335-345
10. Wood JL (1975) Biochemistry. In Chemistry and Biochemistry of Thiocyanic Acid and its Derivatives. Newman AA ed, Academic Press, New York, 156-221
11. Luepker RV, Pechacek TF, Murray DM, Johnson CA, Hund F, Jacobs DR (1981) Saliva thiocyanate: a chemical indicator of cigarette smoking in adolescents. Am J Public Health 71, 1320-1324
12. Morabia A, Bernstein MS, Curtin F, Berode M (2001) Validation of self-reported smoking status by simultaneous measurement of carbon monoxide and salivary thiocyanate. Prev Med 32, 82-88
13. Tenovuo J (1989) Nonimmunoglobulin defense factors in human saliva. In Human Saliva: clinical chemistry and microbiology, Vol II, Tenovuo JO ed, CRC Press, Boca Raton, 55-91
14. Tenovuo J, Pruitt KM (1984) Relationship of the human salivary peroxidase system to oral health. J Oral Pathol 13, 573-584
15. Atkinson JC, Dawes C, Ericson T, Fox PC, Gandara BK, Malamud D, Mandel ID, Navazesh M, Tabak LA (1993) Guidelines for saliva nomenclature and collection. Ann NY Acad Sci 694, xi-xii
16. Tenovuo J, Valtakoski J, Knuttila MLE (1977) Antibacterial activity of lactoperoxidase adsorbed by human salivary sediment and hydroxyapatite. Caries Res 11, 257-262
17. Pruitt KM, Adamson M, Arnold R (1979) Lactoperoxidase binding to Streptococci. Infect Immun 25, 304-309
18. Rosin M, Kocher T, Kramer A (2001) Effects of $SCN^-/H_2O_2$ combinations in dentifrices on plaque and gingivitis. J Clin Periodontol 28, 270-276
19. Hänström L, Johansson A, Carlsson J (1983) Lactoperoxidase and thiocyanate protect cultured mammalian cells against hydrogen peroxide toxicity. Med Biol 61, 268-274
20. Carlsson J (1987) Salivary peroxidase: an important part of our defense against oxygen toxicity. J Oral Pathol 16, 412-416
21. Hoogendoorn H, Piessens JP (1987) Treatment of aphthous patients by enhancement of the salivary peroxidase system. J Oral Pathol 16, 425-427
22. Glatz Z, Nováková S, Sterbová H (2001) Analysis of thiocyanate in biological fluids by capillary zone electrophoresis. J Chromatogr A 916, 273-277
23. Galanti LM (1997) Specificity of salivary thiocyanate as marker of cigarette smoking is not affected by alimentary sources. Clin Chem 43, 184-185
24. Tenovuo J, Mäkinen KK (1976) Concentration of thiocyanate and ionizable iodine in saliva of smokers and nonsmokers. J Dent Res 55, 661-663