Systemic oral isotretinoin therapy and flow rate, pH, and matrix metalloproteinase-9 activity of stimulated saliva

Kyösti Oikarinen, Tuula Salo, Meeri Kylmäniemi, Riita Palatsi, Tiina Karhunen and Aarne Oikarinen

Institute of Dentistry, University of Oulu, and Department of Dermatology and Oral and Maxillofacial Department, Oulu University Hospital, Oulu, Finland

Oikarinen K, Salo T, Kylmäniemi M, Palatsi R, Karhunen T, Oikarinen A. Systemic oral isotretinoin therapy and flow rate, pH, and matrix metalloproteinase-9 activity of stimulated saliva. Acta Odontol Scand 1995;53:369–371. Oslo. ISSN 0001–6357.

Systemic retinoids are known to cause dryness of the mouth and changes in oral and lip mucosa. The purpose of this study was to evaluate changes in salivary variables during treatment with oral isotretinoin in patients receiving the drug for 3 months for cutaneous acne. Patients were examined 1 month after initiation of medication and approximately 3.7 months after its discontinuation. Salivary flow and pH could be measured in 8 and the relative amount of matrix metalloproteinase-9 (MMP-9) of stimulated saliva in 17 patients. The mean flow rate of stimulated saliva was lower during medication than at control examination ($P = 0.0277$), but no change in the mean pH value was observed during medication. The mean activity of MMP-9 during medication was higher than at control examination ($P = 0.0442$). The enzyme activity increased in 13 of 17 and decreased in 4 of 17 cases. □ *Retinoids; salivary flow; salivary pH; xerostomia*

Kyösti Oikarinen, Institute of Dentistry, University of Oulu, Aapistie 3, FIN-90220 Oulu, Finland

Systemic retinoids (such as isotretinoin), vitamin A derivates, have been used for the treatment of acne vulgaris (1), oral lichen planus (2–4), oral leukoplakia (5), and in some other precancerous conditions (6). The influence of retinoids on the oral mucosa is mediated via retinoid-binding proteins (7), causing modulation in stratified epithelium and in epithelial differentiation (8, 9).

Patients treated with systemic oral isotretinoin complain of dry mouth, increased viscosity of the saliva, and changes in the texture of the oral mucosa (10). The mucosal changes are especially detectable in the vermillion border of the lips.

Matrix metalloproteinases (MMPs) make up a family of enzymes that play an important role in many biologic processes (11). MMP-2 and MMP-9 (also known as gelatinase A and B, respectively) are members of this family and are able to degrade, for example, basement membrane type-IV collagen (12) and to denaturate collagen and gelatin (13).

Since retinoids have an effect on mucous membranes and connective tissue (6, 10), the aim of this study was to estimate the amount of MMP-9 in stimulated saliva during the treatment with oral isotretinoin and after its discontinuation. The flow rate and pH of the stimulated saliva during and after therapy were also analyzed.

Materials and methods

The study is composed of patients receiving systemic oral isotretinoin as a treatment for acne vulgaris. All patients were healthy, without permanent medication. Patients had neither periodontal diseases nor carious teeth. Salivary flow and pH were analyzed in eight patients (six males and two females; mean age, 17.9 years). Matrix MMP-9 levels could be determined in the saliva from 9 additional patients, and the mean age of 17 patients in this part of the study was 22.7 (range, 15–45) years. Thirteen of the patients were male, and four were female.

Patients received oral isotretinoin (Roaccutane®, Roche) at a mean daily dosage of 40 mg for 3 months. The patients received the medication for acne and were informed about the side effects of the drug. Examinations were performed 1 month after the beginning of drug therapy and approximately 3.7 months after its discontinuation (range, 2–10 months).

Stimulated whole saliva was collected in the mornings, and no food intake or smoking was allowed 1 h before. The subjects were advised to chew a piece of tasteless paraffin at a stable rate, and all saliva secreted over a 5-min period was collected. Saliva secreted during the first 30 sec was discarded.

Stimulated flow rate was recorded as milliliters per minute. pH was measured immediately after collection (Orion Research SA 270, USA), and the samples were centrifuged at 1200 rpm for 10 min and frozen for MMP-9 analyses.

MMP-9 in frozen salivary samples was assayed using the zymography method described by Heussen & Dowdle (14). Zymography was performed in 1.5-mm

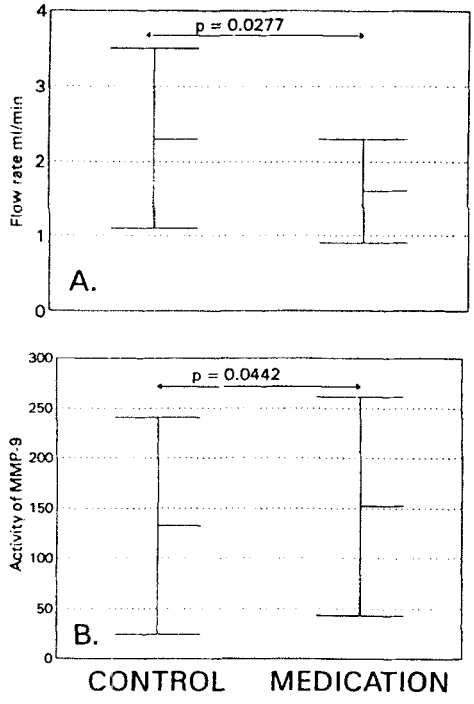

Fig. 1A. Mean flow rate of stimulated saliva during systemic oral isotretinoin treatment and several months after its withdrawal. 1B. Mean activity of matrix metalloproteinase (MMP-9) during medication with systemic oral isotretinoin treatment and several months after its withdrawal (densitometer units).

10% polyacrylamide slab gels containing 1 mg/ml gelatin. The gels were photographed under long-wave ultraviolet illumination; cleavage rates were estimated by determining the rates of disappearance of the gelatin by densitometric scanning of the negatives of the photographed gels, using a Computing Densitometer model 300A (Molecular Dynamics, USA). Twenty microliters of the saliva were usually used for the assay.

Statistical analyses of the paired measurement during medication and after it were performed with non-parametric Wilcoxon's signed-rank test.

Results

Flow rate

The flow rate of stimulated saliva decreased during medication in six patients and remained the same in two.

The flow rate of stimulated saliva during medication varied between 0.7 and 3 ml/min (mean, 1.6 ml/min; SD, 0.7 ml/min) and at control examination between 1.0 and 5 ml/min (mean, 2.3 ml/min; SD, 1.2 ml/min), and there was a statistically significant difference in the flow rate with and without medication ($P = 0.0277$) (Fig. 1A).

MMP-9 activity

The relative MMP-9 activity in the measurement during medication was higher in 13 and lower in 4 subjects. The mean relative MMP-9 activity was 152.6 (SD, 109.2) at medication and 132.6 (SD, 108.4) at control. The difference was statistically significant ($P = 0.0448$) (Fig. 1B).

pH

Oral treatment with isotretinoin decreased the pH of stimulated saliva in six (mean decrease, 0.17; SD, 0.14) and increased it in two subjects (mean increase, 0.19; SD, 0.14) as compared with the samples collected several months after discontinuation of the therapy. The mean pH of eight patients tested was 7.26 (SD, 0.31) during treatment and 7.34 at control (SD, 0.24), but the difference was not statistically significant ($P = 0.2076$).

Discussion

The results of this controlled trial showed that withdrawal of systemic oral isotretinoin increases the secretion of saliva and has some decreasing effect on the activity of MMP-9 in stimulated saliva.

In these patients the MMP-9 activity of saliva was often higher during medication than at control. Earlier studies on the effect of retinoids on the expression of MMP-9 in cell cultures have shown that retinoids increase their expression in cultured epithelial cells (15). Still, some in vitro studies have shown an inhibition of the activity of collagenases by retinoic acid in fibroblastic cells (16). In this study the increase in enzyme activity during medication could be anticipated to be partly dependent on the decrease in salivary flow, as the flow was lower at medication than at control examination.

The most significant change in these series was the decrease in salivary secretion. This is not an unexpected result, as oral side effects are usual in combination with oral retinoid medication (17, 18).

In a study by Reynolds et al. (18) it was shown that in addition to decreased salivary volume during isotretinoin medication some changes in salivary glycoproteins also occur. Changes in the salivary secretion might be due to the effect of retinoids on epidermal cell turnover rate (18).

Patients in this study also complained of dry mouth and especially of changes in the mucosa of the lips, which in all cases was cured after withdrawal of the drug. It is known that 30% of patients receiving systemic oral isotretinoin complain of dry mouth, and every tenth patient describes a subjective change in the texture of the oral mucosa (10).

A double-blind trial on the effect of acitretin on lichen planus showed that 88% of the patients had some adverse effect during medication and 52% during pla-

cebo treatment (17). The most usual complaints were dryness of the lips, mouth, nose, eyes, and skin, which all were reversible.

The duration of medication here was, on the average, 3 months. The patients were all healthy young individuals with no dental or oral problems before treatment. This is why no special attention was paid to the prophylaxis of the possibly increased risk of caries or periodontal diseases, and the decrease in salivary secretion was so small that no irreversible decay could have taken place. The patients should, however, be informed of these adverse effects before treatment.

Acknowledgement.—This study was supported by a grant from the Medical Research Council of the Academy of Finland.

References

1. Peck GL. Retinoids in clinical dermatology. Prog Dis Skin 1982; 1:227–309.
2. Regezi JA, Ellis CN, Stewart JCB, Giustina TA. Histologic changes associated with the topical use of isotretinoin on oral lichen planus. Oral Surg Oral Med Oral Pathol 1986;61:479–84.
3. Sloberg K, Hersle K, Mobacken H, Thilander H. Topical tretinoin therapy and oral lichen planus. Arch Dermatol 1979;115:716–8.
4. Giustina TA, Stewart JC, Ellis CN, Regezi JA, Annesley T, Woo TY, et al. Topical application of isotretinoin gel improves oral lichen planus. A double-blind study. Arch Dermatol 1986;122: 534–46.
5. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, et al. 13-cis-retinoic acid in the treatment of oral leucoplakia. N Engl J Med 1986;315:1501–5.
6. Oikarinen A, Peltonen J, Kallioinen M. Ultraviolet radiation in skin ageing and carcinogenesis. The role of retinoids for treatment and prevention [review]. Ann Med 1991;23:497–505.
7. Siegenthaler G, Samson J, Bernard J-P, Fioro-Donno G, Saurat J-H. Retinoid-binding proteins in human oral mucosa. J Oral Pathol 1987;17:106–12.
8. Sporn MB, Roberts AD. Role of retinoids in differentiation and carcinogensis. Cancer Res 1983;43:3034–40.
9. Vahlqvist A, Törmä H. Retinoids and keratinization. Current opinions. Int J Dermatol 1988;27:81–95.
10. Windhorst DB, Nigra T. General clinical toxicology of oral retinoids. J Am Acad Dermatol 1982;6:675–82.
11. Emonard H, Grimaud J-A. Matrix metalloproteinases. A review. Cell Mol Biol 1990;36:131–53.
12. Salo T, Turpeenniemi-Hujanen T, Tryggvason K. Tumor-promoting phorpol esters and cell proliferation stimulate secretion of basement membrane (type IV) collagen-degrading metalloproteinase by human fibroblasts. J Biol Chem 1985;260:8526–31.
13. Birkendal-Hansen H, Moore WGI, Bodden MK, Windsor LJ, Birkendal-Hansen B, Le Carlo A, et al. Matrix metalloproteinases. A review. Crit Rev Oral Biol Med 1993;4:197–250.
14. Heussen C, Dowdle EB. Electrophoretic analysis of plasminogen activators in polyacrylamide gels containing sodium dodecyl sulfate and copolymerized substrates. Anal Biochem 1980;102:196–202.
15. Oikarinen A, Salo T. Effects of retinoids on type IV collagenolytic activity in melanoma cells. Short reports. Acta Derm Venerol (Stockh) 1986;66:346–8.
16. Bauer EA, Selter JL, Eisen AZ. Inhibition of collagen degradative enzymes by retinoid acid in vitro. J Am Acad Dermatol 1982;6: 603–7.
17. Laurberg G, Geiger J-M, Hjorth N, Holm P, Hou-Jensen K, Jacobsen KU, et al. Treatment of lichen planus with acitretin. A double-blind, placebo-controlled study in 65 patients. J Am Acad Dermatol 1991;24:434–7.
18. Reynolds NJ, Gough M, Clamp JR, Burton JL. Effect of oral isotretinoin therapy on saliva volume and composition [letter]. Br J Dermatol 1991;125:189–90.

Received for publication 8 August 1994
Accepted 27 February 1995