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Effect of local vitamin A administration on experimental gingivitis

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Introduction

Topical application of retinoids (synthetically-derived vitamin A analogues) has been successfully used for the treatment of diverse mucocutaneous disorders like psoriasis (Esgleyes-Ribot et al. 1994), and systemically, in lichen planus (Gorsky & Raviv 1992). However, the topical administration of vitamin A for the treatment of chronic gingivitis has been documented only in a few studies in which retinol or other vitamins were added to the active ingredients in toothpastes (Cohen et al. 1991).
Chronic gingivitis is an inflammatory lesion of the tooth-supporting soft tissues and a consequence of the local accumulation of dental plaque. The changes that occur during the development of gingivitis in humans have been previously described clinically (Löe et al. 1965) and histopathologically (Page & Schroeder, 1976). In this study, the experimental gingivitis model, as proposed by Löe et al. in 1965, was used.
Cell surface molecules and metabolic by-products of bacteria are responsible for the initiation and early development of gingivitis. However, biochemical inflammatory mediators of host origin may play a major role in the amplification and progression of local tissue destruction (Page, 1991). One important immune parameter in periodontal research is interleukin 1b (IL-1b). This is a highly inflammatory cytokine that has gained more interest in periodontology, since it is known to be the most potent osteoclast activating factor within the human organism (Alexander & Damoulis, 1994) (Dewhirst et al. 1985).
Elevated concentrations of IL-1b in GCF during experimental gingivitis in humans should be expected. Previous studies have shown that levels of this mediator in gingival crevicular fluid and tissue samples are elevated in periodontally diseased sites (Hönig et al. 1985; Masada et al. 1990). Site specific increases can be observed in untreated periodontitis and under experimental gingivitis models (Kinane et al. 1992; Preiss & Meyle 1994). Nevertheless, the role that some locally applied substances, like retinoids, play in the development, progression or regression of human gingivitis, is still unclear.

Objective

Therefore, the intent of this study was to determine the effect of local retinoid administration in experimental gingivitis as measured in the concentration of IL-1b in GCF and other clinical parameters.

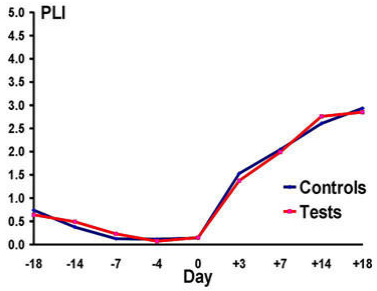

Fig.1: Longitudinal development of median plaque index

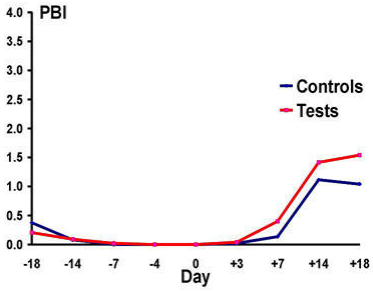

Fig.2: Longitudinal development of median papillary bleeding index

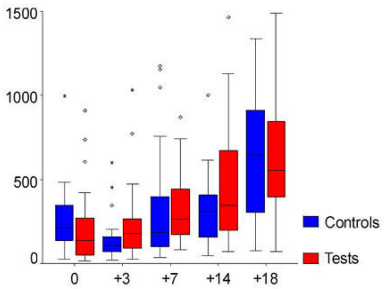

Fig.3: IL-1b in GCF (The bottom line of the boxplot coincides the first quartile, the upper line the third quartile, and the line within marks the median. Error bars include the 95% range, except extreme values which are marked with o and drop outs with

Material and Methods

Twenty four healthy young men were voluntary included in the study (mean age: 28 years). All had at least 28 teeth, a high standard of oral hygiene and healthy gingiva. For 2 weeks before the experiments the subjects were regularly examined and their oral hygiene monitored to achieve maximum gingival health. Plaque accumulation was assessed by the Quigley-Hein plaque index (1962). The Saxer & Mühleman papillary bleeding index (1975) was used for evaluation of the degree of gingival inflammation. After baseline values had been measured the volunteers were instructed to stop all oral hygiene measures, with the exception of flossing, that was ceased the day before. Subjects were randomly assigned either to the test or the control group (n = 12 each). Each of them received a tar with a paste that had to be applied carefully with a finger until all the gingiva around the teeth was covered, three times a day. Subjects received these instructions individually. They were not allowed to provide any other oral hygiene procedure for 18 days and had to fill out a nutritional status during the complete experiment. For GCF collection, the following test teeth and sites were chosen in all participants: upper right premolar (mb) and upper left canine (mb). None of the test teeth had a crown or extensive class II restorations with defective margins. Clinical measurements and GCF samples were taken at baseline and then at days 3, 7, 14 and 18. The clinical measurements were recorded by 3 trained investigators, which were blinded for the ingredient in the paste. GCF sampling was performed with periopaper. The collection of the samples have been described in detail previously. The absorbed fluid volume was determined using a Periotron 8000 (Vetter Laborbedarf, Ammerbuch, Germany) and immediately stored at -80 °C until laboratory analysis. IL-1b concentration in GCF was assayed by a commercial ELISA kit (Cistron Biotechnology, Pine Brook, New Jersey). The procedure will be shortly described: first, 10 µl of PBS were added to the samples. Samples were spun at 3000 rpm for 15 min at 4 °C as described by Wilton et al. (1992). The procedure was repeated until 200 µl were reached. Microtiter plates were supplied with 100 µl of polyclonal IL-1b mature antiserum (rabbit). 100 µl of standard solutions, or test samples were added to the wells. Double testing of the samples was performed. All wells were washed three times, and 100 µl of diluted horseradish peroxidase-conjugated anti-rabbit-IgG was added to each well. The microtiter plates were covered and incubated at room temperature for 20 min. After decanting and washing, 100 µl of peroxidase substrate solution (TMB) was added to each well and incubated uncovered at room temperature. The enzymatic reaction was stopped with 4N sulfuric acid and adsorption read at 480 nm. The amount of IL-1b in each sample was calculated based on the dilutions and referenced to the standard curve.

Results

The changes in clinical signs of inflammation and plaque accum ulation are summarized in Figs. 1 and 2 (days 18 to + 18). The plaque control program undertaken in the pre-study period ensured that plaque and gingival indices of "0" or near "0" were achieved at all teeth at baseline in both groups. Gingival inflammation, expressed simply as a median score, decreased almost linearly from day 18 to baseline (from 0.4-0.2 to 0), remaining stable until day 3 and increasing considerably from day 7 to 18 in both groups (from 0 to 1.5 in tests and 1.0 in controls) (Fig. 1). After abstaining from oral hygiene, dental plaque accumulated rapidly. This observation correlates with Fig. 2, that shows an almost identical development of plaque median scores in both groups. The IL-1b levels for the subjects during the 18-day experimental gingivitis are shown in Fig. 3. The IL-1b concentration increased with plaque accumulation and in advance of the subsequent gingival inflammation. Hence, although this inflammatory mediator was produced in increasing quantities over time, the increases were similar for both groups. Overall, measured concentrations ranged from approximately 137-211 ng/ml at baseline, to 555-644 ng/ml at day 18.

Discussion and Conclusions

In the present study, the development of gingival inflammation and dental plaque following the cessation of oral hygiene procedures was consistent with that which was classically reported by Löe and coworkers in 1965, and diversely observed by other investigators. However, no significant differences in PBI and PLI were observed in both groups.
IL-1b expressed in the GCF by experimental gingivitis was detected in the study. Previous studies reported an increase of IL-1 (Kinane et al. 1992) and IL-1b (Deinzer et al. 1999) under an experimental gingivitis model. Like in these studies, IL-1 concentration increased throughout the experimental gingivitis period although no group differences were observable. However, as far as we know, determination of IL-1b concentration in GCF under the effect of a specific substance, locally applied, has not been investigated in the past.
Based on the clinical and laboratory results obtained in this study, we conclude that a daily local application of vitamin A during a period of 18 days, doesn't markedly influence clinical signs of experimental gingivitis and the concentration of IL-1b in GCF. Consistency and retention characteristics of the toothpaste used, as well as individual instructions for ointment are factors that probably have an important effect on these results.

This Poster was submitted on 08.02.01 by Jens Martin Herrmann.

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Poster Faksimile:

Effect of local vitamin A administration on experimental gingivitis

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Biochemical Institute, Universitaet Hohenheim, Stuttgart **, Germany

Abstract

Several studies have focused on the potential role of interleukin 1 (IL-1) in periodontitis and gingivitis. To determine the influence of a toothpaste on the concentration of IL-1β in experimental gingivitis, we performed a double blind longitudinal study using a sample with vitamin A on a test group and placebo on a control group. Twenty four young healthy volunteers participated and were randomly divided into 2 groups. The following clinical parameters were assessed: Quigley-Hein plaque index (PLI) and Saxer & Mühlemann gingival index (GI). Examinations were performed at days 18, -14, -7, -4 before baseline, and days 3, 7, 14 and 18. All oral hygiene procedures were ceased at baseline. GCF was collected from the mesiobucal site of the upper right canine using periopapers. After measuring the volume at the Periotron 8000®, the samples were analysed at our lab for the detection of IL-1β concentration by ELISA with a commercial kit (Cistron technology, USA). Our results showed a reduction in PLI and GI prior to baseline in the two groups (almost 0), both incrementing after baseline (PLI= from 0.1 to 3.0, GI = 0 to 2.0). IL-1β concentration increased from baseline to day 18 in both groups (mean: from 200 ng/ml to 510 ng/ml and from 120 ng/ml to 445 ng/ml). The local vitamin A application did not influence the inflammatory reaction. We conclude that the use of vitamin A in this form of application, doesn't markedly influence clinical signs of experimental gingivitis as measured in the concentration of IL-1β in GCF.

Material and Methods

Twenty four healthy young men were voluntary included in the study (mean age: 28 years). All had at least 28 teeth, a high standard of oral hygiene and healthy gingiva. For 2 weeks before the experiments the subjects were regularly examined and their oral hygiene monitored to achieve maximum gingival health. Plaque accumulation was assessed by the Quigley-Hein plaque index (1962). The Saxer & Mühleman papillary bleeding index (1975) was used for evaluation of the degree of gingival inflammation. After baseline values had been measured the volunteers were instructed to stop all oral hygiene measures, with the exception of flossing, that was ceased the day before. Subjects were randomly assigned either to the test or the control group (n = 12 each). Each of them received a tar with a paste that had to be applied carefully with a finger until all the gingiva around the teeth was covered, three times a day. Subjects received these instructions individually. They were not allowed to provide any other oral hygiene procedure for 18 days and had to fill out a nutritional status during the complete experiment. For GCF collection, the following test teeth and sites were chosen in all participants: upper right premolar (mb) and upper left canine (mb). None of the test teeth had a crown or extensive class II restorations with defective margins. Clinical measurements and GCF samples were taken at baseline and then at days 3, 7, 14 and 18. The clinical measurements were recorded by 3 trained investigators, which were blinded for the ingredient in the paste. GCF sampling was performed with periopaper. The collection of the samples have been described in detail previously. The absorbed fluid volume was determined using a Periotron 8000 (Veltler Laborbedarf, Ammerbuch, Germany) and immediately stored at -80 °C until laboratory analysis.

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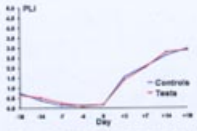
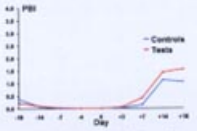


Fig.1: Longitudinal development of median plaque index



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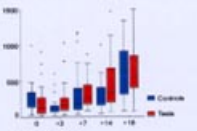

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