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Effects of experimental gingivitis on crevicular PGE_2 in a split mouth trial

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Abstract

Objective: The study aimed to analyse the effects of experimental gingivitis on crevicular prostaglandin E_2 (PGE_2). An increase of PGE_2 was expected.

Methods: Fourteen medical students refrained for 28 days from any oral hygiene procedures in two antagonistic quadrants while they maintained close to perfect oral hygiene in the remaining quadrants. Crevicular fluid samples were taken at baseline and at days 7, 14, 21 and 28 of experimental gingivitis both from quadrants with and without oral hygiene. PGE_2-concentrations (ng/ml) and absolute levels (pg/sample) were analysed for quadrants with and without oral hygiene.

Results: Comparison of quadrants with and without oral hygiene by repeated measures ANOVA revealed no effects of experimental gingivitis both on crevicular PGE_2-concentrations and absolute levels.

Conclusion: The study does not support the notion that experimental gingivitis induces an increase of crevicular PGE_2. The data are discussed in the context of other studies on PGE_2 concentrations in gingivitis. Close inspection of these studies reveals no clear evidence for an increase of local PGE_2 in gingivitis.

Key words: experimental gingivitis; gingival crevicular fluid; prostaglandin E_2

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Prostaglandin E_2 (PGE_2) is an immunoactive host-produced agent the release of which is dependent on availability of arachidonic acid from which it is a metabolite. PGE_2 acts as a proinflammatory agent; it stimulates bone resorption and acts as an inflammatory adjuvant to the effects of other inflammatory agents, namely interleukin-1β. Numerous studies indicate PGE_2 to be an important immune factor in periodontal disease (for review see Offenbacher et al. 1993, Offenbacher 1996, Gemmell et al. 1997). Several studies found increased levels of PGE_2 in gingival crevicular fluid (GCF) and tissue samples of periodontitis sites as compared with healthy sites or gingivitis (e.g. El Attar 1976, Ohm et al. 1984, Offenbacher et al. 1993, Offenbacher 1996, Cavanaugh et al. 1998, Leibur et al. 1999, Needleman et al. 2000, Rassmussen et al. 2000). Furthermore, in vivo and in vitro studies indicate an increased preparedness of periodontitis patients to secrete PGE_2 upon bacterial challenge (Shapira et al. 1994, 1996, Johnson et al. 1997, Offenbacher & Salvi, 1999, Fokkema et al. 2002). Thus, clear evidence exists to suggest periodontitis to go ahead with elevated PGE_2 concentrations and release. Data on gingivitis are yet less conclusive. While some authors describe significantly increased PGE_2 concentrations in spontaneous gingivitis (Ohm et al. 1984), others find no or only tentative elevations (Nakashima et al. 1994). Correspondingly, data on experimentally induced gingivitis are equivocal. Heasman et al. (1993) observed a sudden and profound increase of PGE_2 in the fourth week of experimental gingivitis while Johnson et al. (1997) found no significant alterations and Preshaw et al. (1998) described a reduction of PGE_2 at experimental gingivitis sites.

None of these studies on experimental gingivitis effects included a control condition assessing spontaneous (non-systematic) *intra*individual variations of PGE_2. Such variations have been described repeatedly (Jeffcoat et al. 1995, Needleman et al. 2000, Preshaw & Heasman 2002) and may lead to misinterpretations if not controlled. This control can be provided by maintaining oral hygiene in some quadrants while plaque accumulation is allowed in the other. The present study was thus designed to analyse the effects of experimental gingivitis on PGE_2 alterations in a split mouth design. As PGE_2 is an inflammatory agent and release of PGE_2 can be induced by bacterial endotoxin we expected an increase of PGE_2 at experimental gingivitis sites as compared with sites with oral hygiene.

Material and Methods

GCF-samples analysed for this study are aliquots of samples from the control group of a study on effects of plaque, stress and gender on crevicular II-1β and II-1ra already published in this journal (Waschul et al. 2003).

Participants

Fourteen students of medicine (eight male, six female) built the final sample of this study. Exclusion criteria – applied either due to potential risks for the subjects during the experimental gingivitis period or due to potential impacts on gingival health – were diseases of the immune system as well as known infections of any kind; diabetes; psychiatric diseases; drug abuse; nicotine consumption of more than five cigarettes/day; pregnancy; current orthodontic or dental treatments; untreated caries; defect fillings; inadequate dental restoration; probing depths above 3 mm; major periodontal recessions; regular use of calcium-antagonists, anticonvulsives, immunostimulants or immunosuppressives prior to the study and any use of these substances from one week prior to the study until the end of the study; use of antibiotics or antiphlogistics within a period of six weeks prior to the study until the end of the study; bleeding on probing at more than one site of intended measurement at the beginning of the study; papillary bleeding index (PBI) of more than one at any site at the beginning of the study. While these exclusion criteria were applied at the time of recruitment further criteria were applied at the end of the study: to make sure that we were indeed analysing the effects of plaque accumulation on crevicular PGE_2, only those participant whose plaque indices (PI) were one and higher on at least 1/4 of experimental gingivitis sites at day 7 and on at least 1/2 of sites at day 28 of the study were included into analyses.

Pre-treatments

To achieve perfect gingival health and thus to standardize gingival baseline conditions all participants received a professional tooth cleaning at least 2 weeks prior to the study. At that time participants were also instructed in maintaining perfect oral hygiene. Either they were taught the modified bass technique or – if their hygiene was already close to perfect – only few individual instructions were given in further improving their cleansing. All participants were instructed in using dental floss at all sites. The compliance in these instructions was controlled at least weekly by assessing plaque levels and bleeding on probing. If necessary, instructions were repeated until gingival health and oral hygiene were perfect. Compliance in oral hygiene instructions was excellent; in fact, only one subject had to be excluded due to bleeding on probing on more than one site at the beginning of the study.

Experimental gingivitis

All participants were randomly assigned either to refrain from oral hygiene at teeth 3–8 of the first and fourth or of the second and third quadrants for 28 days. Perfect hygiene had to be maintained at the contralateral sites. Participants were not allowed to provide oral hygiene at sites of perfect hygiene by other means than a standardized medium-soft toothbrush (blend-a-dent), a standardized sodium flouride toothpaste (blend-a-med) and dental floss.

Collection of gingival crevicular fluid

GCF was collected at mesiobuccal, distobuccal, mesiopalatal and distopalatal sites of teeth 14–17 and 24–27, respectively (if one of these teeth was missing, the respective canine was included into measurements). Prior to sampling the respective region was dried by isolation with cotton rolls and a gentle air stream of 5 s duration with a 90° angle to the tooth axis. In a previous study, we had demonstrated that the presence or absence of dental plaque had no effect on the crevicular volume sampled (Deinzer et al. 2000). A periopaper (Harco, New York, USA) marked with pencil 1 mm from the lower edge was inserted 1 mm into the gingival crevice and remained there for exactly 30 s. Periopapers with visible blood contaminations were discarded. The absorbed fluid volume was determined using a Periotron 8000 (Harco, New York, USA), which had been calibrated on each study day as described previously (Deinzer et al. 1999). The precision (reliability) of this method is very good; duplicate measurement within 5 min reveals retest correlations of $r_{tt} > 0.80$ (Deinzer et al. 2000). Immediately after volume assessment the white tip of the periopaper was cut off and pooled together with the other tips of the same quadrant into a microtest tube containing 800 μl PBS-buffer solution. The two tubes of one subject were then centrifuged at 3000 rpm for 5 min and aliquots of 100 μl of the sample solution were shock frozen at CO_2 within 5 min after centrifugation and then stored at -80°C until analyses.

Immunoassays

Diluted GCF samples were analysed for PGE_2 by a commercially available EIA Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the instructions of the manufacturer. Reliability of this method was good with a correlation of $r = 0.93$ of duplicate measurements. Samples of one person were always run at a time and with the same assay. Both, absolute GCF–PGE_2-values and concentrations were computed. For determination of absolute GCF values (pg) concentrations of the diluted samples (pg/ml) were multiplied by diluent volume (i.e. 0.8 ml); PGE_2 concentrations in crevicular fluid were computed by multiplying concentrations measured in the diluted samples with the respective dilution factor.

Clinical parameters

To determine the clinical effects of experimental gingivitis, PI (Sillness & Loe as modified by Rateitschak et al. (1989)) and papillary bleeding indices (PBI; Saxer & Mühlemann as modified by Rateitschak et al. (1989)) were assessed at all sites of GCF sampling (i.e. 16 sites per hygiene condition) by two trained and calibrated examiners.

Design

Crevicular fluid samples and clinical parameters were taken at days 0, 7, 14, 21 and 28 of the experimental gingivitis period. All assessments were done between 14:00 and 22:00 hours. Within one subject time of the day of consecutive measurements did not vary by more than $\pm$ 30 min.

Statistical analyses

Effects of experimental gingivitis on PGE_2 are reported as differences to baseline (day 0) values. Repeated mea-

sures ANOVA (hygiene × time) were computed after univariate normal distribution was confirmed by Kolmogorov–Smirnov goodness-of-fit tests with exact significance computations (all $p > 0.09$). Baseline PGE_2 values were included as covariates. To control for gender effects, ANOVAS were first computed with gender included as separate factor. Gender remained in analyses if there was any substantial gender main-effect or interaction ($p < 0.10$), otherwise analyses were rerun without a gender factor.

Greenhouse–Geisser corrections (which result in a more conservative hypothesis testing) were applied to all ANOVA computations including time-effects to correct for potential violations of the sphericity assumption. This was done irrespective of the result of the sphericity test (Mauchly test of sphericity), since this might have had a low power due to small sample sizes; original degrees of freedom and Greenhouse–Geisser ε are reported. Effect sizes are reported as partial η^2 (η^2) and d corrected for correlated measures according to the suggestions of Dunlap et al. (1996), respectively. The intended significance level was $p = 0.05$.

Ethics

Written informed consent was provided by all participants. The study design was approved by the local ethics committee and was found to conform to the guidelines issued in the Declaration of Helsinki.

Results

Effects of experimental gingivitis on plaque and bleeding of male and female participants of this study have been published in detail elsewhere (Waschul et al. 2003). Briefly, we observed significant main effects of hygiene on number of sites with plaque ($\eta^2 = 0.978$, $F(1/13) = 572.4$; $p < 0.0001$) and bleeding on probing ($\eta^2 = 0.550$, $F(1/12) = 14.670$; $p = 0.002$) and significant time × gender ($\eta^2 = 0.294$, $F(2/24) = 4.997$; $p = 0.029$; $\varepsilon = 0.699$) and hygiene × time × gender ($\eta^2 = 0.249$, $F(2/24) = 3.973$; $p = 0.032$; $\varepsilon = 0.999$) interactions on number of sites showing bleeding on probing. After 28 days in study an average of 12.1 ($\pm$ 2.4(SD)) out of 16 sites without hygiene showed PI above zero but only 2.5 ($\pm$ 2.28) of 16 sites with hygiene. At the same time, bleeding on probing was observed at an average of 2.6 ($\pm$ 1.7) and 3.2 ($\pm$ 2.7) sites in experimental gingivitis quadrants of males and females, respectively. In quadrants with oral hygiene the corresponding figures were 0.5 ($\pm$ 0.76) and 1.2 ($\pm$ 1.6) sites for male and female participants, respectively.

Effects of experimental gingivitis on PGE_2 are shown in Fig. 1. No significant gender effects were observed (all $p > 0.48$; all $\eta^2 < 0.07$). Gender was thereby excluded from repeated measures ANOVAS. Repeated measures hygiene × time ANOVAS exerted no significant effects on PGE_2 concentrations and absolute levels (Table 1). No baseline (day 0) differences of PGE_2 between quadrants were found (Fig. 1) both with respect to PGE_2 concentrations (means $\pm$ SD: with hygiene: 3.42 ± 2.07 ng/ml; without hygiene 3.46 ± 2.26 ng/ml; $d = 0.01$; $t(13) = 0.085$, $p = 0.933$) and absolute levels (with hygiene: 6.83 ± 1.58 pg; without hygiene 6.66 ± 1.35 pg; $d = 0.11$; $t(13) = 0.655$, $p = 0.524$). Correlations of PGE_2 baseline values between quadrants with and without hygiene were $r = 0.81$ and $\rho = 0.81$ for PGE_2 concentrations and $r = 0.79$ and $\rho = 0.65$ for PGE_2 absolute levels, respectively.

Discussion

In the present study, experimental gingivitis did not induce any significant effect on crevicular PGE_2 concentrations or absolute levels. This unexpected result cannot be readily explained by weaknesses of experimental design or biochemical measurements. The experimental design controlled for spontaneous variations of PGE_2 by including a within-subjects control condition (split mouth design). Experimental gingivitis procedures were clinically effective in as far as they induced highly significant increases of plaque and bleeding. They were immunologically effective, proven by profound increases of II-1β observed in aliquots of the samples analysed here (Waschul et al. 2003). PGE_2 measurements were highly reliable which can be seen from correlations of parallel samples taken from the two quadrants at baseline. The results are even difficult to attribute to small sample size (and thereby low test power) or high variability of PGE_2 as the direction of effects was unexpected at least as far as PGE_2 concentrations are concerned, which seems to be a more reliable measure than PGE_2 absolute levels (see parallel test correlations reported above). As there is no trend of PGE_2 at all to increase during the experimental gingivitis period, one can also not argue that differences between quadrants were not visible because levels at control sites were influenced by experimental gingivitis occurring at test sites. Therefore, our results strongly indicate that 4 weeks of plaque accumulation in a split mouth design have no meaningful effect on crevicular PGE_2. This result is in line with the results of Johnson et al. (1997) and Preshaw et al. (1998) who did not observe significant increases of PGE_2-absolute levels and concentrations, respectively, during experimental gingivitis in otherwise healthy subjects. Our result contradicts Heasman et al. (1993) who observed a sudden increase of crevicular PGE_2-concentrations in the fourth week of experimental gingivitis in $n = 7$ subjects. Unfortunately, like the others, these authors did not include a

Table 1. Results of repeated measures ANOVAS of PGE_2 measures*

measure	effect	statistics
PGE_2 concentration (ng/ml)	hygiene	$\eta^2 = 0.082$, $F(1/10) = 0.977$; $p = 0.344$; $\varepsilon = 1$
	time	$\eta^2 = 0.026$, $F(3/33) = 0.296$; $p = 0.784$; $\varepsilon = 0.798$
	hygiene × time	$\eta^2 = 0.078$, $F(3/33) = 0.935$; $p = 0.407$; $\varepsilon = 0.660$
PGE_2 absolute level (pg)	hygiene	$\eta^2 = 0.001$, $F(1/10) = 0.010$; $p = 0.921$; $\varepsilon = 1$
	time	$\eta^2 = 0.094$, $F(3/33) = 1.139$; $p = 0.334$; $\varepsilon = 0.589$
	hygiene × time	$\eta^2 = 0.187$, $F(3/33) = 2.533$; $p = 0.077$; $\varepsilon = 0.962$

*Analyses on weekly measures of differences to baseline during 28 days of a split mouth trial of experimental gingivitis vs. good oral hygiene; the respective baseline values are included as covariates; partial η^2 and Greenhouse–Geisser ε are reported together with original degrees of freedom.

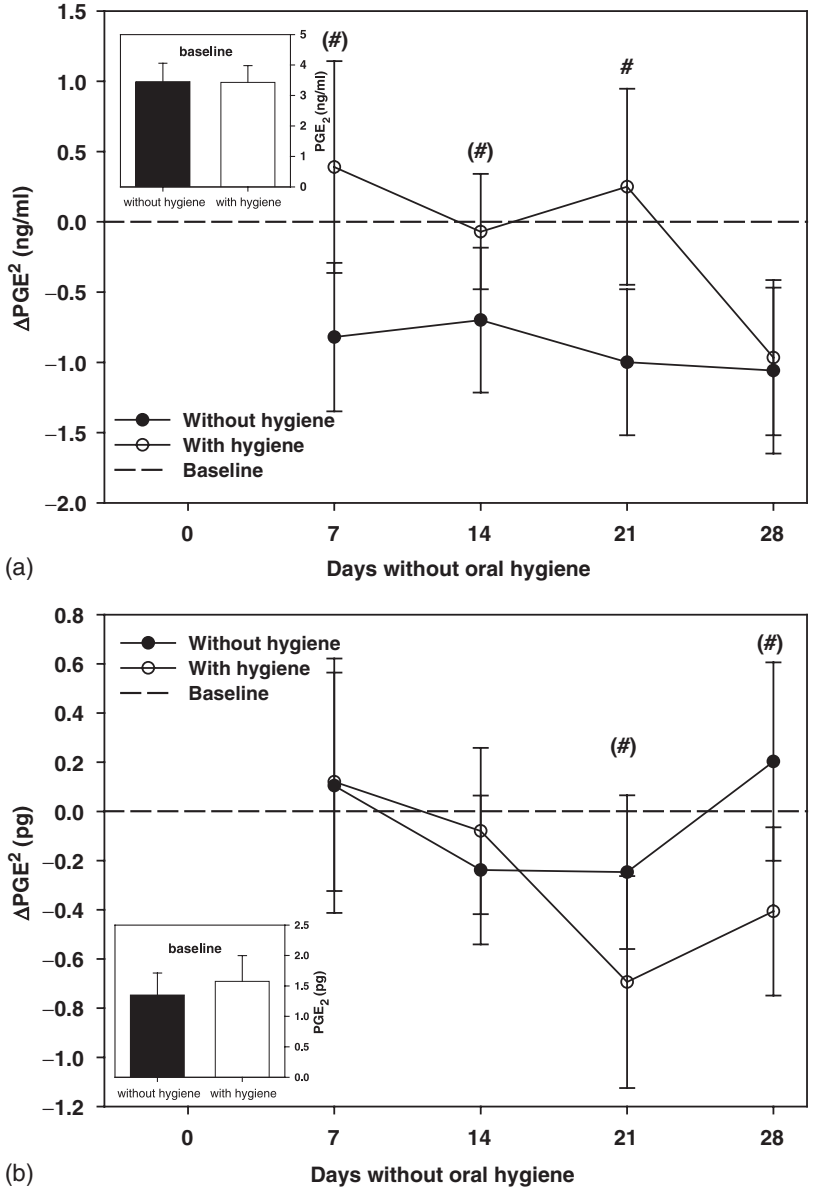

Fig. 1. PGE concentrations (A) and absolute levels (B) in quadrants with and without oral hygiene. Data are shown as mean differences from the respective baseline ± SEMs. Baseline data are given in nested graphs. Repeated measures hygiene × time ANOVA revealed no significant hygiene main effects or interactions. Effects of sizes for comparisons between sites with and without hygiene are given with the following symbols: #$d \geqslant 0.2$; #$d \geqslant 0.5$; ##$d \geqslant 0.8$.

control condition. Whether the effect they observed was due to experimental gingivitis or due to other factors unknown is thus not clear from their study.

Our results, however, not only contradict those of Heasman et al. (1993), but also challenge results of studies repeatedly cited to add evidence to the hypothesis that gingivitis increases PGE_2 (Offenbacher et al. 1993, Offenbacher 1996). Yet, closer inspection of these studies questions the strength of evidence. Albers et al. (1979) compared PGE concentrations of 12 healthy tissue samples from patients subjected to extraction of third molars with 20 gingivitis tissue samples obtained from periodontitis patients during periodontal surgery. The authors do not provide any information on how they differentiate between gingivitis and periodontitis tissue samples nor did their analysis differentiate between PGE_1 and PGE_2. Furthermore, several studies indicate the PGE_2 response of periodontitis patients to bacterial challenge to be increased in general and not only at periodontitis sites (Shapira et al. 1994, 1996, Johnson et al. 1997, Offenbacher & Salvi, 1999, Fokkema et al. 2002). Thus, if gingivitis samples are taken from periodontitis patients we do not know whether the alterations observed are due to periodontal disease and concomitantly altered immune responsiveness or to gingivitis as such. El Attar (1976) analyse tissue samples from patients with ''deep periodontal pockets and chronic inflammation''; they thus rather study periodontitis samples than gingivitis samples. Holmes & El Attar (1977), also repeatedly cited in this context, measured PGE_2 concentration in no more than two inflamed and three healthy tissue samples; inflammation was further defined by ''moderately dense accumulation of inflammatory cells in isolated areas with sparse distribution in other areas''—a definition which does not allow for a clear differentiation between gingivitis and periodontitis. Goodson et al. (1974) just differentiate between healthy and inflamed tissue without further specifying what is meant by ''inflamed'' indicating that this could mean periodontitis as well. Nakashima et al. (1994), who find no difference between healthy and gingivitis sites considering crevicular PGE_2, include six healthy patients defined as patients with ''clinically healthy gingiva'' and three gingivitis patients into their study. Samples of gingivitis sites, however, are taken both from gingivitis patients and from healthy patients, which obscures the authors' diagnostic criteria. Mean age of patient groups analysed by Ohm et al. (1984) differs by more than 12 years indicating that the authors did not thoroughly control for potential disturbing factors and thereby questioning the internal validity of any conclusion drawn from this data.

Taken together studies on spontaneous gingivitis reveal no clear evidence that gingivitis goes ahead with increased local levels of PGE_2 in otherwise healthy patients. If gingivitis is induced experimentally – as has been done in the present study and in three studies before – no effect on PGE_2 is observed when plaque accumulation occurs at a limited number of sites (present study, Johnson et al. 1997, Preshaw et al. 1998). Interestingly, the only study observing an increase of PGE_2 after 4 weeks of plaque accumulation induced experimental gingivitis in the full mouth (Heasman et al. 1993). Unfortunately, however, this study is

missing a control group and refers to data of seven subjects, only. Nevertheless, the contradiction between this and our study might give an important hint for future studies. PGE_2 might increase only if a certain threshold of antigen accumulation is crossed. If this would be the case restricting plaque accumulation to two quadrants might be a below threshold stimulus. Future studies should further elucidate this notion by running full mouth experimental gingivitis in a control group design with one group maintaining oral hygiene and one group allowing for continuous plaque accumulation. To increase the validity of interpretation from these studies participants must be assigned randomly to plaque vs. hygiene conditions. A crossover design would further be helpful to control for interindividual differences. As long as such study is missing, the best we know currently on the effects of plaque accumulation and gingivitis on PGE_2 remains that there is no clear evidence for an increase of PGE_2 under theses conditions in otherwise healthy patients.

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