J Periodont Res 2007; 42: 259–266

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00942.x

The effect of age on prostaglandin-synthesizing enzymes in the development of gingivitis

B. Siegel[1], E. Weihe[2], M. Bette[2], R. M. Nüsing[3], L. Flores-de-Jacoby[1], R. Mengel[1]

[1]Department of Periodontology and [2]Institute for Anatomy and Cell Biology, Philipps University, Marburg, Germany and [3]Institute of Clinical Pharmacology, Johann Wolfgang Goethe University, Frankfurt, Germany

Siegel B, Weihe E, Bette M, Nüsing RM, Flores-de-Jacoby L, Mengel R. The effect of age on prostaglandin-synthesizing enzymes in the development of gingivitis. J Periodont Res 2007; 42: 259–266. © 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard

Background and Objective: The aim of this study was to identify the expression of cyclooxygenase-1, cyclooxygenase-2, cyclooxygenase-3, and microsomal prostaglandin E synthase-1 in young and elderly subjects.

Material and Methods: Periodontally healthy subjects were divided into young (18–30 years, $n = 7$) and elderly (46–77 years, $n = 7$). A gingival biopsy was taken at baseline. After experimental gingivitis, clinical examination was repeated and a second biopsy was taken. The expression of cyclooxygenase-1, cyclooxygenase-2, cyclooxygenase-3, and microsomal prostaglandin E synthase-1 was analyzed by means of immunohistochemistry.

Results: In both healthy age groups, cyclooxygenase-1 and microsomal prostaglandin E synthase-1 were expressed in epithelial cells, endothelial cells and fibroblast-like connective tissue cells. Cyclooxygenase-1 was found in Langerhans' cells of the epithelium. Cyclooxygenase-2 expression was observed in cells exhibiting the morphology of epithelial mitosis cells, and the expression of cyclooxygenase-2 in periodontally healthy elderly subjects was significantly lower ($p \leq 0.05$). Following experimental gingivitis, cyclooxygenase-1 and microsomal prostaglandin E synthase-1 expression did not change. However, the expression of cyclooxygenase-2 was significantly increased in both age groups ($p \leq 0.05$). Cyclooxygenase-3 was not detected in any group investigated.

Conclusion: Cyclooxygenase-1 and microsomal prostaglandin E synthase-1 were expressed constitutively in gingival tissue, and expression was unaffected by age or inflammation states. In contrast, the expression of cyclooxygenase-2 was weaker in elderly subjects. In the course of experimental gingivitis, cyclooxygenase-2 was induced in both age groups.

Prof. Dr Reiner Mengel, Department of Periodontology, School of Dentistry, Philipps University, Marburg, Georg-Voigt-Str. 3, 35033 Marburg, Germany
Tel: +49 6421 286 3279
Fax: +49 6421 286 3270
e-mail: mengel@mailer.uni-marburg.de

Key words: experimental gingivitis; prostaglandin E2; cyclooxygenase; microsomal prostaglandin E synthase-1

Accepted for publication July 24, 2006

The main cause of periodontal inflammation is microbial plaque (1). The periodontal tissue reacts to the bacteria with an immune response, which is primarily a defense mechanism. However, if inflammation persists, the immunological reaction leads to destruction of the gingiva (2). Enzymatic, cytotoxic, and phlogogenic mechanisms have been reported to be responsible for tissue damage (3,4).

In this respect, prostaglandin E2 is of great biological relevance as it promotes osteoclast bone resorption and increases vascular permeability through its vasodilatative effect. Increased prostaglandin E2 concentrations in the sulcus fluid and gingival tissue have been associated with periodontal destruction (5–9). Cyclooxygenase is a membrane-associated enzyme that is ubiquitously expressed throughout the body (10–13). It catalyses the conversion of arachidonic

acid to prostaglandin H2, which is metabolized by secondary enzymes to the various prostanoids, including prostaglandin E2. Three different isoforms of cyclooxygenase have been reported. Whereas the isoforms cyclooxygenase-1 and cyclooxygenase-2 have been clearly identified, the presence and catalytic function of a third isoform, cyclooxygenase-3, is controversial (14,15). Cyclooxygenase-1 is characterized by a more or less unregulated and constitutive expression in different cell types. In contrast, cyclooxygenase-2 is induced several fold under the influence of inflammatory cytokines, bacterial endotoxins, growth factors, and mitogens. Cyclooxygenase-2 is regarded as the main enzyme responsible for the synthesis of prostanoids, which contribute to inflammation and pain processes (16). Although previous studies have suggested its predominant role in inflammatory pathways, cyclooxygenase-2 has also been associated with protective processes, such as wound healing and bone repair (17,18), including mechanical-induced bone formation (19–21). Cyclooxygenase-2 knockout or inhibition showed impaired fracture healing and bone regeneration (22–24). Interestingly, bone morphogenic protein-2 has been shown to induce cyclooxygenase-2 in osteoblasts, and the induction of cyclooxygenase-2 contributes to the effects of bone morphogenic protein-2 on osteoblastic differentiation (25,26). Cyclooxygenase-3 is a splice variant of cyclooxygenase-1 and was described as the acetaminophen-sensitive cyclooxy genase expressed in brain tissue (14). However, the existence of a human functional cyclooxygenase-3 has been questioned (15).

Prostaglandin E2 is formed by prostaglandin E synthase, which occurs in three isoforms (27). The first isoform, cytosolic prostaglandin E synthase, is constitutively expressed in nearly all cells. The second isoform, microsomal prostaglandin E synthase-1, is induced as a reaction towards inflammatory stimuli (28–30). For the third isoform, microsomal prostaglandin E synthase-2, constitutive and inducible expression has been reported (30,31).

Regarding the expression and function of the prostaglandin-synthesizing enzymes in gingival tissue, only limited information is available. Using human cell cultures, Noguchi *et al*. demonstrated that the stimulation of gingival fibroblasts by lipopolysaccharides derived from *Porphyromonas gingivalis* led to an increase in the expression of cyclooxygenase-2, but not cyclooxy genase-1, (32). The increase in cyclooxygenase-2 expression was paralled by an increase in prostaglandin E2 formation. A similar induction in cyclooxygenase-2 expression was observed following stimulation with interleukin-1β (33). In inflamed human gingival tissue, expression of cyclooxygenase-2 correlated with the degree of inflammation (34). No data are available regarding age-dependent expression of prostanoid-synthesizing enzymes in human gingiva under healthy and inflamed conditions.

The aim of the present study was to identify the expression pattern of cyclooxygenase-1, cyclooxygenase-2, cyclooxygenase-3 and microsomal prostaglandin E synthase-1, before and after experimental gingivitis in young and elderly subjects.

Material and methods

Subjects

A total of 14 subjects were enrolled in the present study. They were subdivided into two groups, one group of 18–30-year-old subjects (five men, two women) and one group of 46–77-year-old subjects (four men, three women). All subjects presented healthy periodontal conditions (probing depths ≤ 3 mm with no bleeding on probing) and were nonsusceptible for periodontitis. Inclusion criteria were as follows: at least 20 teeth with no large-scale prosthetic reconstructions, nonsmoker, no systemic diseases (e.g. diabetes mellitus), no pregnancy, no orthodontic therapy (e.g. brackets), no myoarthropathies, no extensive carious lesions, no medication (e.g. immunosuppressives, antibiotics, antiphlogistics), and no psychiatric disorders. After approval by the local Institutional Review Board, and after providing informed consent, the subjects were examined.

Study design

The present study is an experimental gingivitis study (Fig. 1). The subjects were instructed in oral hygiene and received professional tooth cleaning over a period of 3 wk. Thereafter, baseline examination revealed that they were periodontally healthy. Clinical data were recorded and a ginigival biopsy was taken distal from the second molar in the right side of the upper jaw of each subject. The subjects were then instructed to avoid any form of oral hygiene for the following 14 d. Clinical examination on day 15 revealed that all subjects had developed experimental gingivitis (Table 1), and a second biopsy was taken from the left side of the upper jaw. At the end of the study, all subjects received professional tooth cleaning.

Clinical examination

The clinical parameters were measured from six sites (mesial, distal, buccal, palatal/lingual) around each tooth. For each mesial and distal site, the buccal and palatal/lingual measurements were averaged to one value. Probing depth with bleeding on pro-

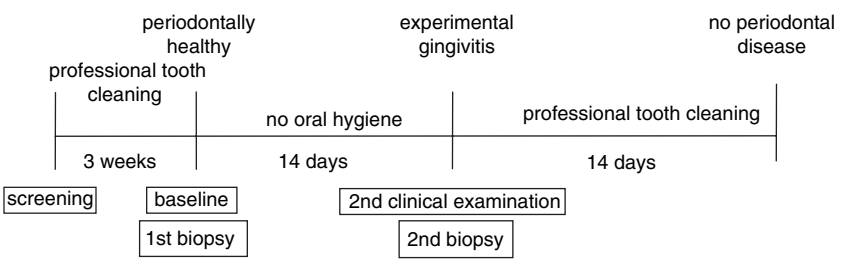

Fig. 1. Examination schedule.

Table 1. Determination of gingival inflammation

	Plaque index				Gingival index				Bleeding on probing (%)			
	18–30 years		46–77 years		18–30 years		46–77 years		18–30 years		46–77 years	
	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD
Baseline (day 0)	0.04	0.05	0.08	0.05	0.10	0.08	0.18	0.13	11	7	15	7
Second examination (day 15)	1.71*	0.30	1.82*	0.18	1.91*	0.17	2.12*	0.36	37*	21	60*	14

*$p < 0.05$.
x, experimental group; SD, standard deviation.

bing, gingival recession, clinical attachment level, gingival index (35), and plaque index (36), were recorded using a graduated periodontal probe (WHO TRS 621 probe; Hu Friedy, Chicago, IL, USA).

Tissue sampling and immunohistochemical staining

During the baseline examination (periodontally healthy) and the second examination (experimental gingivitis), a gingival biopsy ($\approx 4 \times 4$ mm) was taken from all subjects distal from the second molar in the left and right side of the upper jaw, respectively. All biopsies were taken from keratinized tissue. Wisdom teeth were not present in any subject. The soft tissue, ≈ 10 mm below the location of the intended biopsy, was anaesthetized with articaine 4% containing 1 : 200,000 adrenaline (Hoechst, Frankfurt, Germany). The gingival tissue was then fixed in Bouin Hollande solution, embedded in paraffin and cut at a nominal at 7 μm. Every fifth section was Giemsa stained and the remaining sections were used for immunohistochemistry to determine expression of cyclooxygenase-1, cyclooxygenase-2, cyclooxygenase-3, and microsomal prostaglandin E synthase-1. The following polyclonal antibodies were used.

(i) Anti-cyclooxygenase-1 (C20): sc-1752, goat IgG affinity purified; Santa Cruz (Santa Cruz, CA, USA); diluted 1 : 1000 in phosphate-buffered saline containing 1% bovine serum albumin.
(ii) Anti-human cyclooxygenase-2, rabbit IgG affinity purified; Assay Designs (Ann Arbor, MI, USA); diluted 1 : 60 in phosphate-buffered saline containing 1% bovine serum albumin.
(iii) Anti-human cyclooxygenase-3, rabbit IgG affinity purified; Alpha Diagnostic (San Antonio, TX, USA); diluted 1 : 50 in phosphate-buffered saline containing 1% bovine serum albumin.
(iv) Anti-microsomal prostaglandin E synthase-1, rabbit IgG affinity-purified; Cayman (Ann Arbor, MI, USA); diluted 1 : 50 in phosphate-buffered saline containing 1% bovine serum albumin.
(v) Biotinylated donkey antigoat and donkey antirabbit species-specific immunoglobulins, each diluted 1 : 200 in phosphate-buffered saline containing 1% bovine serum albumin (Dianova, Hamburg, Germany), were used as the second antibody.

The sections were first deparaffinized in xylene. After 10 min of incubation in 2-propanol, they were incubated for 30 min in 3% H_2O_2/methanol to block endogenous peroxidase. To increase the accessibility of the epitopes, tissue slices were incubated for 15 min in a sodium citrate buffer, pH 6.0, at 92–95°C. In order to block endogenous biotin, the avidin biotin blocking kit (Vector Laboratories, Burlingame, CA, USA) was used. After incubation with the first antibodies and subsequent incubation with biotinylated secondary antibodies, staining was visualized using streptavidin-biotin/horseradish peroxidase complex with diaminobenzidine/Ni as the substrate (Vecta-stain ABC-Kit; Vector Laboratories). The resulting oxidation product was visible as a dark brown/violet stain. Following dehydration in increasing concentrations of alcohol, the sections were coverslipped in DePeX.

As a positive control for the antibodies, human tissue known to express the relevant enzymes was used in parallel staining (data not shown). As a neagtive control, the first antibody was omitted during the immunohistochemical procedure. Staining observed under this condition was judged as nonspecific. To assess specificity of the first antibodies, pre-absorption of the antibodies with their corresponding antigens was performed (data not shown). For quantification of cyclooxygenase-1, cyclooxygenase-2, and microsomal prostaglandin E synthase-positive cells, either the number of positive cells per random area of defined tissue compartments (expressed as cells per 10,000 μm^2) or the proportional area of positive cells per random area of defined tissue compartments (expressed as percentage per 10,000 μm^2) were measured. The proportional areas were measured using an Olympus AX 70 microscope connected to an IBM PC equipped with an imaging system (MCID Elite 7.0; Imaging Research Inc., St Catherine's, ON, Canada). The immunopositive cells were displayed on the PC monitor using a defined greyscale. The software used made it possible to overlay areas of a defined size at any position on the oral epithelium or connective tissue. These areas covered an area of $\approx 10{,}000$ μm^2, of which $\approx 40{,}000$ μm^2 of each evaluation area (oral epithelium or connective tissue) was measured. Within each area, the percentage of the area whose grey rating corresponded to that of the immunopositive

cells was calculated, and so the percentage of specific signals on the entire area was determined. The amount of immunopositive cell types of the oral epithelium was determined using a DMLAM microscope (Leica, Bensheim, Germany), equipped with an imaging and analysis system (Q Win Pro; Leica). In each section, an area of $\approx 40{,}000\ \mu m^2$ from the epithelium was examined and immunopositive cells were marked.

Statistical analysis

Statistical analysis was based on a variance analysis using the *t*-test. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) software. For each group, the mean and standard deviation for each parameter were calculated. The assumption of normal distribution was checked using the Kolmogorov–Smirnov test, as modified by Lilliefors, where all the parameters tested were not rejected. As a result, the parameters can be considered as conforming to a normal distribution. Any significant differences between the examination timing within one group were determined with the *t*-test for dependent random samples. Any significant differences between the groups were determined with the *t*-test for independent random samples. The significance cut-off point was set at $\alpha = 0.05$.

Results

Twelve subjects completed the study. In the elderly group, two subjects were excluded as a result of influenza and concomitant treatment with antibiotics. The results of the clinical examination under the healthy condition (day 0) and the inflamed condition (day 15) are summarized in Table 1. Plaque index, gingival index, and bleeding on probing were significantly increased following 14 d of experimental gingivitis. Concluding from the similar increase of the different parameters, the extent of gingival inflammation did not differ between the age groups.

Immunohistochemical staining of the biopsy sections showed that cyclooxygenase-1, cyclooxygenase-2, and microsomal prostaglandin E synthase-1 are expressed in gingival tissue. In contrast, cyclooxygenase-3 protein was not detected in the tissue samples, under either the healthy or the inflamed condition (data not shown).

The gingiva sections incubated with antibodies to human cyclooxygenase-1 exhibited widespread staining. Cyclooxygenase-1 expression was observed in epithelial cells, endothelial cells, and fibroblast-like connective tissue cells (Fig. 2A). Furthermore, cyclooxy genase-1 was expressed in cells, morphologically identified as Langerhans' cells, of the gingival epithelium (Fig. 2B). Following induction of gingivitis, a slight increase in cyclooxygenase-1 expression was detectable in both study groups (Fig. 3A). However, the increase did not reach significance. Moreover, in epithelial Langerhans' cells of both age groups, cyclooxygenase-1 expression remained almost unchanged during experimental gingivitis (Fig. 3B). The number of cyclooxygenase-1-positive Langerhans' cells

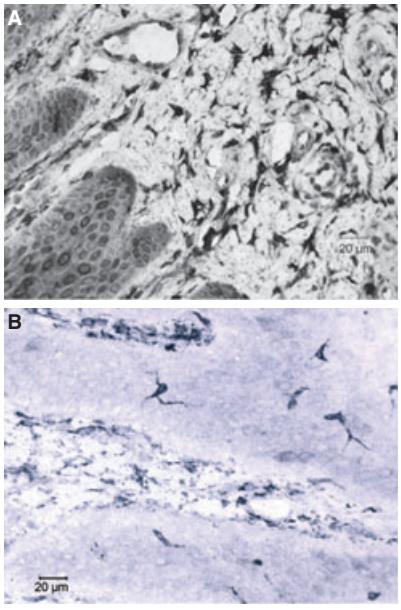

Fig. 2. Immunocytochemical staining of cyclooxygenase-1 in healthy gingival tissue. (A) Intense staining of endothelial cells, epithelial cells and connective tissue cells was observed (marked by arrow). A size bar is shown within the figures. (B) Specific staining of Langerhans cells was observed. A size bar is shown within the figures.

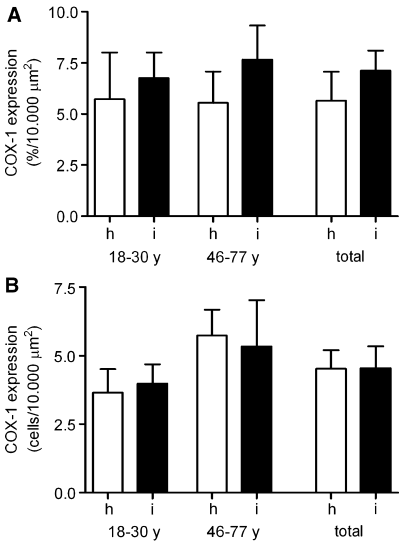

Fig. 3. Determination of cyclooxygenase-1 expression in gingival tissue. (A) Cyclooxygenase-1 expression in connective tissue. No significant differences between the groups were observed. (B) Cyclooxygenase-1 expression in Langerhans' cells. No significant differences between the groups were observed. h, healthy; i, inflamed. Results are shown + standard deviation. COX-1, cyclooxygenase-1.

was higher in the elderly subjects at both time points. The difference was, however, not statistically significant.

Cyclooxygenase-2 was found in basal epithelial cells exhibiting the morphology of cells under mitosis (Fig. 4). This was confirmed by analysis of adjacent Giemsa-stained sections (data not shown). In the periodontally healthy condition, cyclooxygenase-2 expression was significantly weaker in the older age

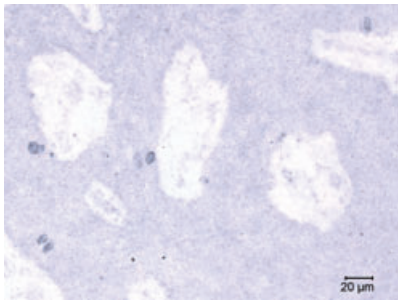

Fig. 4. Immunocytochemical staining of cyclooxygenase-2 in healthy gingival tissue. Specific staining of cells with the morphology of epithelial mitosis cells was observed. A size bar is shown within the figure.

group than in the younger age group ($p \leq 0.05$) (Fig. 5). During experimental gingivitis, quantitative evaluation showed a significant increase of cyclooxygenase-2 expression in both age groups. However, the rise in mean cyclooxygenase-2 expression was higher in the older age group ($\Delta = 390\%$) than in the younger age group ($\Delta = 180\%$).

Strong expression of microsomal prostaglandin E synthase-1 protein was detected in epithelial cells, endothelial cells, and fibroblast-like connective tissue cells of the gingival tissue (Fig. 6). No significant difference in expression was observed regarding health status or age (Fig. 7A,B).

Discussion

Several studies, using cultured human gingival fibroblasts or epithelial cells, have shown that increased prostaglandin E2 synthesis provoked by various stimuli, such as lipopolysaccharide or interleukin-1β, is dependent on cyclooxygenase-2 expression (32,33,37–39). However, less information is available regarding the *in situ* expression and regulation of the cyclooxygenase system in gingival tissue. Our clinical study was designed to localize the expression of cyclooxygenase-1, cyclooxygenase-2, cyclooxygenase-3, and microsomal prostaglandin E synthase-1 under healthy and inflamed conditions, depending on age.

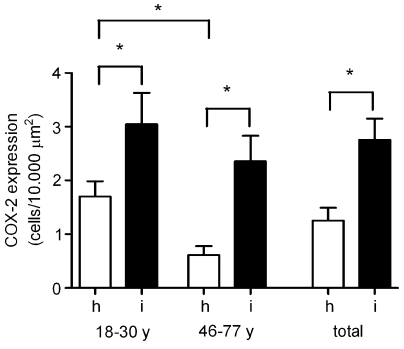

Fig. 5. Determination of cyclooxygenase-2 expression. Expression of cyclooxygenase-2 in basal epithelial cells exhibiting the morphology of cells under mitosis. In periodontally healthy elderly subjects, cyclooxygenase-2 expression was significantly weaker. During experimental gingivitis, a significant increase of cyclooxygenase-2 expression was found in both age groups. h, healthy; i, inflamed; $*p \leq 0.05$ + standard deviation.

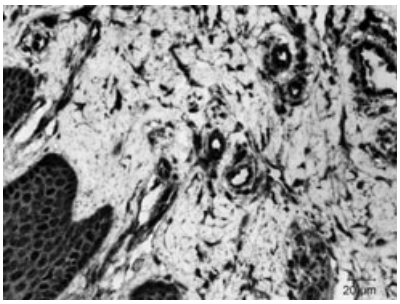

Fig. 6. Immunocytochemical staining of microsomal prostaglandin E synthase-1 in healthy gingival tissue. Staining of epithelial cells, endothelial cells and fibroblast-like connective tissue cells was observed A size bar is shown within the figure.

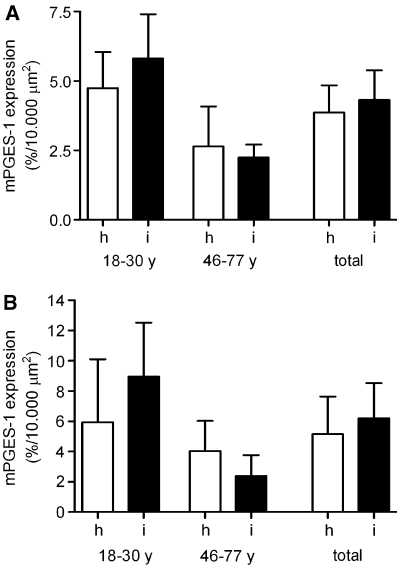

Fig. 7. Determination of microsomal prostaglandin E synthase-1 expression. Microsomal prostaglandin E synthase-1 expression in (A) fibroblast-like connective tissue cells and (B) epithelial cells. No significant differences between the groups were observed in either cell type. h, healthy; i, inflamed. Results are shown + standard deviation.

In our study, cyclooxygenase-1 was expressed in epithelial cells, endothelial cells, and fibroblast-like connective tissue cells in both periodontally healthy age groups. Additionally, cyclooxygenase-1 was identified in Langerhans' cells of human gingival epithelium. The ability of epidermal Langerhans' cells in the human skin to express cyclooxygenase-1 has been reported previously (40). Epidermal Langerhans' cells produce prostaglandin D2 as the major arachidonic acid metabolite. The prostanoid type released by gingival Langerhans' cells remains to be identified. Concluding from our data, microsomal prostaglandin E synthase-1 dependent prostaglandin E2 will be not formed, as we did not detect microsomal prostaglandin E synthase-1 in Langerhans' cells. Nevertheless, formed prostanoid/s will probably be involved in the immunological function of Langerhans' cells. During the experimental gingivitis in the present study, no significant changes in cyclooxygenase-1-positive cell density were seen in either age group. These results are in accordance with the observation of Noguchi *et al.* that the concentration of cyclooxygenase-1 remains unaffected after stimulation of human fibroblasts with lipopolysaccharide (32). However, it should be noted that protein expression of cyclooxygenase does not necessarily reflect the activity of cyclooxygenase, and increased or reduced availability of arachidonic acid may affect the tissue prostanoid level.

Cyclooxygenase-2 was expressed in healthy gingiva in cells exhibiting the morphology of epithelial mitosis cells. We also observed that a significantly lower number of cyclooxygenase-2-positive cells were present in the healthy gingival tissue of elderly subjects compared with younger subjects. This may be a result of the fact that older persons may have a reduced regeneration and mitosis capability, and a lower level of immune defence followed by a stronger inflammatory reaction, once inflammation is manifested. During the experimental gingivitis there was a statistically significant increase in cyclooxygenase-2 cell density in both age groups. In accordance, in a rat study using topical application of lipopolysaccharide on periodontal tissue, an induction of cyclooxygenase-2 was observed in a small number of junctional epithelium cells, whereas cyclooxygenase-1 expression was

unaffected (41). Our data also support the previous observation, by Morton & Dongari-Bagtzoglou, that increased cyclooxygenase-2 expression is detectable in inflamed gingival tissue (34). However, the total number of cyclooxygenase-2-positive cells was lower in the group of elderly subjects, and the induction of cyclooxygenase-2 during the course of experimental gingivitis was more pronounced. It has to be elucidated whether this reflects a stronger inflammatory reaction, although we observed no difference in the plaque index or gingival index, or in bleeding on probing, between the two age groups. However, the elderly group showed slightly greater bleeding on probing at the time of experimental gingivitis. While these differences in this pilot study did not approach statistical significance, a larger number of samples might have given the appropriate power to these observations to make a definitive judgment of significance or nonsignificance. Furthermore, it also remains speculative whether older patients may benefit more, or may be more vulnerable to, treatment with cyclooxygenase-2-specific inhibitors.

The fact that cyclooxygenase-2 was strongly detected in epithelial mitosis cells may indicate an increased 'turnover' of gingival epithelium cells. As the experimental gingivitis caused an increase in the number of cyclooxygenase-2-positive cells, it would be interesting for future clinical studies to clarify whether local application of cyclooxygenase-2 inhibitors causes a reduction in the number of these cells. The prostanoid pattern formed by these mitotic cells has to be identified. From our study we conclude that microsomal prostaglandin E synthase-1 is not involved. It also remains speculative whether the rise in number of cyclooxygenase-2-positive mitotic cells may be indicative for a higher carcinogenic risk of older patients. Increased expression of cyclooxygenase-2 in neoplastic tissue, and decreased incidence of carcinoma following long-term consumption of cyclooxygenase inhibitors, has prompted the hypothesis that cyclooxygenase-2 is involved in carcinogenesis (42). As chronically inflamed cells can degenerate and become malignant, further studies are required to identify such a link between periodontal inflammation and malignant degeneration of the oral mucosa.

Cyclooxygenase-3 represents a cyclooxygenase-1 splice variant, which is expressed in the cerebral cortex and, to a lesser extent, in the heart and aorta (14). It is believed that cyclooxygenase-3 is of significance in the case of chronic inflammation and that it catalyses biosynthesis of the prostaglandins responsible for pain and fevers (43). However, the existence of cyclooxygenase-3 in humans is controversial (15). Our clinical study did not provide any evidence for the expression of cyclooxygenase-3 in human gingival tissue.

In the present study, microsomal prostaglandin E synthase-1 demonstrated a cellular distribution similar to that seen for cyclooxygenase-1. During experimental gingivitis, no statistically significant alteration in microsomal prostaglandin E synthase-1 expression was observed in either age group. Taking into account that higher levels of prostaglandin E2 are detectable in the sulcus fluid of patients with gingivitis and chronic periodontitis (5,8,44), we speculate that *in situ* this increase is caused by cyclooxygenase-1/microsomal prostaglandin E synthase-1 activation combined with increased cyclooxygenase-2 expression. The contribution of each cyclooxygenase isoform to the amount of prostaglandin E2 formed has to be determined. Although microsomal prostaglandin E synthase-1 is considered to be an inducible enzyme, we speculate that, at least in gingiva, a strong, constitutive expression of microsomal prostaglandin E synthase-1, together with cyclooxygenase-1, is present. Possibly this reflects the continous exposure of the gingiva towards bacteria or other agents. This assumption is supported by the observation that Triclosan (2,4,4′-trichlor-2′hydroxy-diphenylether), which inhibits prostaglandin E2 synthesis, reduces microsomal prostaglandin E synthase-1 expression in human gingival fibroblasts (45). On the other hand, we cannot exclude that other isoforms of prostaglandin E synthase – cytosolic prostaglandin E synthase or microsomal prostaglandin E synthase-2 – are involved, or even induced, during gingival inflammation. Additional studies are required to clarify this issue.

In summary, our study demonstrates that cyclooxygenase-1 and microsomal prostaglandin E synthase-1 are constitutively expressed in human gingival tissue, independent of age and health status of the subjects. The similarity in level and extent of expression leads us to suggest that both enzymes are involved in gingival prostaglandin E2 synthesis, probably under inflammatory conditions. The fact that cyclooxygenase-1 is expressed in gingival Langerhans' cells indicates that cyclooxygenase-1 is involved in the primary immune defence system of gingival tissue. In contrast, cyclooxygenase-2 appeared to be age-dependently expressed, with a weaker expression level in periodontally healthy elderly subjects. During experimental gingivitis, cyclooxygenase-2 expression increased in both age groups. Therefore, we assume that the prostaglandin E2 levels observed in inflamed gingival tissues are only, in part, cyclooxygenase-2-dependently formed.

In conclusion, the present study provides new understanding of the possible roles of prostaglandin-synthesizing enzymes in the healthy and diseased gingiva at different ages. The relationship between cyclooxygenase-2 expression and age-dependent gingivitis, and the therapeutic application of cyclooxygenase-2 inhibitors, merits further investigation.

Acknowledgements

This study was supported, in part, by Gaba International (Basel, Switzerland).

References

1. Löe H, Theilade E, Jensen SB. Experimental gingivitis in man. *J Periodontol* 1965;**36:**177–187.
2. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 1976;**33:**325–249.

3. Socransky SS. Relationship of bacteria to the etiology of periodontal disease. *J Dent Res* 1970;**49:**203–222.
4. Nisengard RJ. The role of immunology in periodontal disease. *J Periodontol* 1977; **48:**505–516.
5. Offenbacher S, Farr DH, Goodson JM. Measurment of prostaglandin E in cervicular fluid. *J Clin Periodontol* 1981; **8:**359–367.
6. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. *J Periodontol* 1993;**64:**432–444.
7. Offenbacher S, Odle BM, Braswell LD *et al*. Changes in cyclooxygenase metabolites in experimental periodontitis in Macaca mulatta. *J Periodont Res* 1989;**24:** 63–74.
8. Offenbacher S, Odle BM, Gray RC, Van Dyke TE. Cervicular fluid prostaglandin E_2 levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. *J Periodont Res* 1984; **19:**1–13.
9. Heasman PA, Collins JG, Offenbacher S. Changes in cervicular fluid levels of interleukin-1®, leukotriene B_4, prostglandin E_2 and tumor necrosis factor α in experimental gingivitis in humans. *J Periodont Res* 1993;**28:**241–247.
10. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* 1998;**38:**97–120.
11. Garavito RM, DeWitt DL. The cyclooxygenase isoforms: structural insights into the conversion of arachidonic acid to prostaglandins. *Biochim Biophys Acta* 1999;**1441:**278–287.
12. Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. *Inflamm Res* 2000;**49:**367–392.
13. FitzGerald GA. COX-2 and beyond: approaches to prostaglandin inhibition in human disease. *Nat Rev Drug Discov* 2003;**2:**879–890.
14. Chandrasekharan NV, Dai H, Roos KL *et al*. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA;* 2002;**99:**13926–13931.
15. Kis B, Snipes JA, Busija DW. Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties. *J Pharmacol Exp Ther* 2005;**315:** 1–7.
16. Xie WL, Robertson DL, Simmons DL. Mitogen-inducible prostaglandin G/H synthase: a new target for nonsteroidal anti-inflammatory drugs. *Drug Dev Res* 1992;**25:**249–265.
17. Schmassmann A, Peskar BM, Stettler C *et al*. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. *Br J Pharmacol* 1998;**123:**795–804.
18. Rys-Sikora KE, Konger RL, Schoggins JW, Malaviya R, Pentland AP. Coordinate expression of secretory phospholipase A2 and cyclooxygenase-2 in activated human keratinocytes. *Am J Cell Physiol* 2000;**278:**822–833.
19. Forwood MR. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading *in vivo*. *J Bone Miner Res* 1996;**11:**1688–1693.
20. Kawata A, Mikuni-Takagaki Y. Mechanotransduction in stretched osteocytes – temporal expression of immediate early and other genes. *Biochem Biophys Res Commun* 1998;**246:**404–408.
21. Kunnel JG, Igarashi K, Gilbert JL, Stern PH. Bone anabolic responses to mechanical load *in vitro* involve COX-2 and constitutive NOS. *Connect Tissue Res* 2004;**45:**40–49.
22. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. *J Bone Miner Res* 2002;**17:**963–976.
23. Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. *J Clin Invest* 2002;**109:**1405–1415.
24. Damrongsri D, Geva S, Salvi GE, Williams RC, Limwongse V, Offenbacher S. Cyclooxygenase-2 inhibition selectively attenuates bone morphogenetic protein-6 synthesis and bone formation during guided tissue regeneration in a rat model. *Clin Oral Implants Res* 2006;**17:**38–47.
25. Koide M, Murase Y, Yamato K, Noguchi T, Okahashi N, Nishihara T. Bone morphogenetic protein-2 enhances osteoclast formation mediated by interleukin-1alpha through upregulation of osteoclast differentiation factor and cyclooxygenase-2. *Biochem Biophys Res Commun* 1999; **259:**97–102.
26. Chikazu D, Li X, Kawaguchi H, Sakuma Y *et al*. Bone morphogenetic protein-2 induces cyclo-oxygenase-2 in osteoblasts via a Cbfal binding site: role in effects of bone morphogenetic protein-2 *in vitro* and *in vivo*. *J Bone Miner Res* 2002;**17:**1430–1440.
27. Tanikawa N, Ohmiya Y, Ohkubo H *et al*. Identification and characterization of novel type of membrane-associated prostaglandin E synthase. *Biochem Biophys Res Commun* 2002;**291:**884–889.
28. Jakobsson P-J, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc Natl Acad Sci USA* 1999;**96:**7220–7225.
29. Murakami M, Naraba H, Tanioko T *et al*. Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E_2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem* 2000;**275:**32783–32792.
30. Stichtenoth DO, Thoren S, Bian H *et al*. Microsomal prostaglandin E synthase is regulated by pro-inflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J Immunol* 2001; **167:**469–474.
31. Murakami M, Nakashima K, Kamei D *et al*. Cellular prostaglandin E_2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. *J Biol Chem* 2003;**278:**37937–37947.
32. Noguchi K, Shitashige M, Yanai M *et al*. Prostaglandin production via induction of cyclooxygenase-2 by human gingival fibroblast stimulated with lipopolysaccharides. *Inflammation* 1996;**20:**555–568.
33. Noguchi K, Iwasaki K, Shitashige M, Murota S, Ishikawa I. Prostaglandin E2 downregulates interferon-gamma-induced intercellular adhesion molecule-1 expression via EP2 receptors in human gingival fibroblasts. *Inflammation* 1999;**23:**481–493.
34. Morton RS, Dongari-Bagtzoglou AI. Cyclooxygenase-2 is upregulated in inflamed gingival tissues. *J Periodontol* 2001;**72:**461–469.
35. Löe H, Silness J. Periodontal disease in pregnancy. 1. Prevalence and severity. *Acta Odontol Scand* 1963;**21:**533–551.
36. Silness J, Löe H. Periodontal disease in pregnancy. 2. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;**22:**121–135.
37. Noguchi T, Shiba H, Komatsuzawa H *et al*. Syntheses of prostaglandin E2 and E-cadherin and gene expression of beta-defensin-2 by human gingival epithelial cells in response to *Actinobacillus actinomycetemcomitans*. *Inflammation* 2003;**27:** 341–349.
38. Nakao S, Ogata Y, Yamamoto Y *et al*. Platelet-derived growth factor-induced arachidonic acid release for enhancement of prostaglandin E(2) synthesis in human gingival fibroblasts pretreated with interleukin-1beta. *J Cell Biochem* 2004;**92:**579–590.
39. Rausch-Fan X, Ulm C, Jensen-Jarolim E *et al*. Interleukin-1beta-induced prostaglandin E2 production by human gingival fibroblasts is upregulated by glycine. *J Periodontol* 2005;**76:**1182–1188.
40. Rosenbach T, Czernielewsky J, Hecker M, Czarnetzki B. Comparison of eicosanoid generation by highly purified human Langerhans cells and keratinocytes. *J Invest Dermatol* 1990;**95:**104–107.
41. Miyauchi M, Hiraoka M, Oka H *et al*. Immuno-localization of COX-1 and COX-2 in the rat molar periodontal tissue after

topical application of lipopolysaccharide. *Arch Oral Biol* 2004;**49:**739–746.

42. Dannenberg AJ, Zakim D. Chemoprevention of colorectal cancer through inhibition of cyclooxygenase-2. *Semin Oncol* 1999;**26:**499–504.
43. Schwab JM, Schluesener HJ, Meyermann R, Serhan CN. COX-3 the enzyme and the concept: steps towards highly specialized pathways and precision therapeutics? *Prostaglandins Leukot Essent Fatty Acids* 2003;**69:**339–343.
44. Nakashima K, Roechrich N, Cimasoni G. Osteocalcin, prostaglandin E_2 and alkaline phosphatase in gingival cervicular fluids: their relations to periodontal status. *J Clin Periodontol* 1994;**21:**327–333.
45. Mustafa M, Wondimu B, Yucel-Lindberg T *et al*. Triclosan reduces microsomal prostaglandin E synthase-1 expression in human gingival fibroblasts. *J Clin Periodontol* 2005;**32:**6–11.