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Influence of cigarette smoking on synthesis of eicosanoids, isoprostanes and lipoxygenase metabolites in apical periodontitis

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Abbreviations: AA, Arachidonic acid; AP, apical periodontitis; ASA, acetylsalicylic acid; ^{14}C-AA, ^{14}C labeled arachidonic acid; COX, cyclooxygenase; CS, current smoker; FS, former smoker; IPs, isoprostanes; LOX, lipoxygenase; NS, non-smoker; PG, prostaglandin; PPP, platelet poor plasma; PRP, platelet rich plasma; RIA, radioimmunoassay; RTLC, radio thin-layer chromatography; TX, thromboxane.

Running head: Smoking and apical periodontitis

Abstract

Arachidonic acid (AA) is metabolized to eicosanoids and isoprostanes (IPs) via different pathways. The presence of granuloma in apical periodontitis (AP) is linked with inflammation and the synthesis of metabolites of AA.

Objective: We investigated the conversion rate of ^{14}C labeled arachidonic acid (^{14}C-AA), the lipoxygenases (LOX) products and the endogenous synthesis of eicosanoids and IPs in extracted granuloma. Furthermore, we assessed if there are markers for bone destruction and the influence of cigarette smoking.

Patients and Methods: In 46 patients with symptoms and corresponding radiological signs of AP, teeth were extracted including the periapical granuloma. The endogenous synthesis of eicosanoids and IPs, the conversion rate of ^{14}C-AA and LOX products in extracted granuloma were analyzed.

Results: We found that smoking increases significantly the synthesis of IPs and LOX-metabolites in granuloma. Furthermore, smoking may have contributed to significant differences in qualitative and quantitative profile of eicosanoids, IPs and the conversion rate of ^{14}C-AA independent of the size of the granuloma.

Conclusions: Our data demonstrate that in smokers with granuloma due to AP products of lipid peroxidation as 8-iso-$PGF_{2\alpha}$ and products of the LOX-pathway are increased at the expense of cyclooxygenase products. The size of granuloma did not influence the amount of synthesized eicosanoids, IPs or LOX-metabolites out of ^{14}C-AA whereas cigarette smoking was a significantly influencing and modifiable risk factor.

Key words: Arachidonic acid; isoprostane; lipoxygenase; cigarette smoking; periodontitis

INTRODUCTION

Arachidonic acid (AA) is an essential unsaturated fatty acid and the most abundant in human cell membranes. The oxygenated metabolites are produced through biosynthetic pathways termed the "arachidonate or eicosanoid cascade". The term eicosanoid is used to embrace biologically active lipid mediators, including prostaglandins (PGs), thromboxanes (TXs), prostacyclin (PGI_2), leukotrienes and other oxygenated derivatives, which are produced primarily by cyclooxygenases (COX-1 and COX-2), lipoxygenases (LOX) and cytochrome P450 epoxygenase. Morrow et al.[1] described another pathway of AA metabolism, catalyzed by free radicals *in-vivo*, leading to compounds termed isoprostanes (IPs). When such compounds are available as free acid they initially affect the integrity and fluidity of the membranes, and consequently, the adjacent tissues, causing a state of so called in-vivo oxidative strain or oxidant stress.[2] 8-iso-$PGF_{2\alpha}$ released in conditions such as atherosclerosis, smoking and diabetes, is the most sensitive and specific biomarker of lipid peroxidation in vascular disorders[3,4] and a potent vasoconstrictor in most species and vascular beds.[5,6,7] There are several LOX that act upon different positions on the AA. LOX-metabolites like leukotrienes and lipoxins are involved in signaling or in inducing structural or metabolic changes in the cell. Furthermore, LOX can attack low-density lipoproteins directly with major implications for the onset of atherosclerosis.[8,9,10,11,12]

Kirkevang and Wenzel[13] reported for the first time in an epidemiological study on the association between tobacco smoking and apical periodontitis (AP). Recently, several reports have also studied this association with contradictory results.[8,14] Segura-Egea et al.[15] reported that smoking was significantly associated with a higher frequency of root canal treatment and with an increased prevalence of AP.

Cysts tend to be larger than granulomas, but there was wide variation in size of both types of lesions. There was no significant correlation between the density of a lesion and its size.

The older the AP lesion, the greater is the probability of becoming a cyst but there was wide variation in size of both types of lesions. Because there are no clearly defined radiographic criteria, the distinction is usually made on the grounds of size. However, exploratory surgery and/or histopathological study of the lesion are the only tests that guarantee the diagnosis of a granuloma. We investigated the conversion rate of ^{14}C labeled arachidonic acid (^{14}C-AA) to eicosanoids, LOX products and the synthesis of eicosanoids and IPs from endogenous substrates in granuloma caused by AP. Furthermore, we assessed the suitability of markers for bone destruction and inflammation in periapical lesions and the influence of cigarette smoking on the respective AA products.

METHODS

Study design and population

Forty-five consecutive patients (16 women, 29 men) with acute or chronic AP were admitted to the Department of Conservative Dentistry, Medical University of Vienna. Patient characteristics, smoking status, and prescribed medications are depicted in **Table 1**. Current smokers (CS) were defined as those who smoked at least 1 cigarette per day during the month before enrollment, former smokers (FS) as those who had smoked at least 1 cigarette per day at any time prior to 12 months.

Oral examination and interview

Dental examinations were performed by an experienced dentist after panoramic radiography using the World Health Organization (WHO) format.[16] Pericoronitis was assessed by clinical examination and radiographic evaluation according to Armitage.[17] After oral examination a short interview was conducted to ascertain information about the patients smoking status, daily tooth brushing habits, their use of dental floss and the annual rate of

consulting a dentist.[18,19] In patients with clinical symptoms of AP and corresponding radiological signs, teeth were extracted after administration of local anesthesia including the periapical granuloma. After apicotomy periapical granuloma were frozen at <-20° C.

The study protocol was approved by our institutional Ethics Committee and has been conducted in full accordance with the Declaration of Helsinki. All participants provided written informed consent prior to any data collection.

Radiographic evaluation

Two experienced radiographers using the long-cone paralleling technique of XCP devices (Rinn Co., Elgin, IL, USA), setting of 70 kV, 8 mAs, a film-focus distance of 28cm, and Kodak Ultra Speed DF-57 film (Eastman Kodak, Rochester, NY, USA), took all periapical radiographs.[20] In teeth with severe bone resorption, radiolucent indentations on the root surface resulted in massive differences in radiographic density.[21,22,23] After visual inspection of extracted teeth, granuloma were classified into <4mm and $\geq$4mm size as determined via intraoral apical x-ray views.

Radio thin-layer chromatography (RTLC)

Frozen tissue samples were homogenized with an ultrasound homogenizer (Ultraturrax, TP 18/10 Janke & Kunkel GmbH, Staufen, Germany) at 0°C, for 10s with 50 Hz. ^{14}C-AA (Amersham Corp., Buckinghamshire, UK , 58µCi/mM/probe) and 2.5ml of 0.05 M tris-HCl-buffer (pH 7.4) were added to 1-3mg of the homogenate and incubated in a shaking water bath at 37°C for 2 h. Reaction was stopped acidifying the homogenate with 1 M HCl, thus reaching a pH of 3. After centrifugation for 10min, at 4°C, 500g, extraction of the aliquot was performed using 2ml of ethylacetate. The extract was dried under nitrogen, dissolved in 100µl ethanol (90%) and stored at -20°C. The samples were sputtered to silicagel plates (Merck, No. 5721, Darmstadt, Germany) and dissolved twice in a solvent system using the organic fraction of 110ml ethylacetate, 50ml isooctane, 20ml acetic acid glacial and 100ml H_2O. Final detection was performed using a radioactivity scanner (TLC

Linear Analyzer B283; Berthold, Wildbad, Germany). Various PGs were identified using the respective synthetic radiolabeled standards (New England Nuclear, Boston, MA, USA). The conversion rates of ^{14}C-AA were expressed in %.

Radioimmunoassay (RIA)

6-oxo-$PGF_{1\alpha}$ was determined using our own non-commercially available RIA. Tissue samples were incubated at 37°C in 300µl buffer (pH 7.4) for 3min. Thereafter, the buffer was changed and the tissue samples were incubated further for 120min under identical conditions. The incubation medium was stored at -70°C until determination of 6-oxo-$PGF_{1\alpha}$. 8-iso-$PGF_{2\alpha}$ was determined after extraction and purification by chromatography. Separation of free- and antibody-bound ligand was conducted using polyethylenglycol. Values are given in pg/mg tissue wet weight.

Thromboxane B_2 (TXB_2) was determined by using 100µl aliquots for RIA (Amersham, Buckinghamshire, U.K.). RIA was performed using a specific antibody without extraction. A double antibody (OTOP 15/16, Behring, Marburg, Germany) was used for separation of free and antibody bound ligand. The values are given in pg/ml.

Bioassay to determine PGI_2

Homogenated tissue samples were incubated at 22°C in 300µl Tris-HCl buffer (pH 7.4) for 3min. 100µl of aliquots were used for the bioassay technique (platelet aggregation inhibition).

Platelet rich plasma (PRP)

For platelet aggregation testing (bioassay), blood was collected from patients who were not taking any medication during the last 14 days. Antecubital venepuncture was performed without stasis using 3.8% sodium citrate (ratio 1:9) as anticoagulant. The anticoagulated blood was sedimented at room temperature (22°C) for 5min and then centrifuged for 5min at 150g and 22°C to obtain platelet rich plasma (PRP) which was carefully removed. The remaining plasma was centrifuged again for 10min at 1500g to obtain

platelet poor plasma (PPP). PPP was adjusted with PRP to a final platelet count of 250 x 10^3/µl.

600µl of plasma with the final platelet count was warmed for 30s at 37°C. Platelet aggregation was induced with 1µM ADP 1min after addition of 100µl buffer solution (pH 7.4), containing 100µl aliquot. ADP-induced aggregation was measured in a Born-type aggregometer. 100µl aliquot of the incubation fluid was then removed to assess the PGI_2-production of the tissue samples in buffer solution or plasma, respectively, via its stable metabolite 6-oxo-$PGF_{1\alpha}$. 6-oxo-$PGF_{1\alpha}$ was measured with specific RIA. The double antibody technique was used for the separation of free and bound antigen.

Statistical analysis

Data are presented as mean and standard deviation. Dichotomous variables are described as counts and percentages. Student's *t*-test (for parametric variables) or Mann-Whitney U test (for non-parametric variables) were conducted. Chi-square test for medications was performed with Yates correction. Comparisons were made between non-smokers (NS), CS and FS using the one-way analysis of variance (ANOVA) test. If ANOVA revealed statistical significance, *post-hoc* test was performed to determine statistically significant differences between groups. *P*-values of <0.05 were considered statistically significant. All statistical analyses were conducted using SPSS version 15.0 (Chicago, Illinois, USA)

RESULTS

Synthesis of eicosanoids in our tissue samples measured by RIA and platelet-bioassay revealed 2.45$\pm$0.79pg/mg/min for 8-iso-$PGF_{2\alpha}$, 2.24$\pm$1.04pg/mg/min for 6-oxo-$PGF_{1\alpha}$, 0.16$\pm$0.14pg/mg/min for TXB_2 and 1.31$\pm$0.68pg/mg/min for PGI_2**.** There were no significant differences between men and women.

The conversion rates of ^{14}C-AA were 2.84$\pm$0.72% for the LOX-metabolites and for $PGF_{2\alpha}$ 0.13$\pm$0.1%, 6-oxo-$PGF_{1\alpha}$ 0.53$\pm$0.37%, PGE_2 0.35$\pm$0.21% TXB_2 0.05$\pm$0.05%. RTLC

revealed a significantly higher rate of metabolized ^{14}C-AA via the LOX- than the COX-pathway. The highest conversion rate was measured for 6-oxo-$PGF_{1\alpha}$. There were no significant gender differences.

Granuloma

The diameter of extracted granuloma was 1.5-9mm, mean 3.2±1.5mm, (≥4mm, mean 4.9±1.4mm, <4mm, mean 2.4±0.6mm, p<0,001). There was no significant difference in synthesis of eicosanoids and isoprostanes comparing granuloma ≥4mm vs. <4mm in diameter. The size of the granuloma showed no significant differences between CS, vs. NS and FS.

41.3% of the patient study group had undergone previous endodontic treatment. Eicosanoid- and IPs-synthesis and the conversion rate of ^{14}C-AA were independent of previous root canal treatment, except TXB_2 which was significantly increased after endodontic treatment, 0.21±0.15pg/mg/min vs. 0.12±0.12pg/mg/min (p=0.048).

Pain

AP with painful teeth was reported in 54.3%. All painful teeth were sensitive to pressure, 91.7% were sensitive to heat and 83.3% to ice. 8-iso-$PGF_{2\alpha}$ was significantly increased in patients with pain, 2.7±0.67pg/mg/min vs. 2.14±0.84pg/mg/min (p=0.001). PGE_2 revealed no significant increase in painful teeth in CS. In NS with pain PGE_2 was significantly elevated vs. NS without pain, 0.45±0.24% vs. 0.24±0.1% (p=0.017). In patients with painful teeth the conversion rate of ^{14}C-AA to eicosanoids was not significantly different to painless teeth, whereas LOX-metabolites were significantly increased, 3.09±0.69% vs. 2.55±0.65% (p=0.009).

Location

Granuloma were found in 65% at the apex, in 25% periapically, lateral in the distal third of the root, in 6% at the bifurcation, and in 4% at the trifurcation. Tooth 4-6 (Federation Dentaire Internationale) (19 in the universal tooth numbering chart) was the most involved (in 15.2%) at the mesial root. 8-iso-$PGF_{2\alpha}$ was highest in granuloma at the bifurcation and lowest

at the trifurcation, 3.28±1.26pg/mg/min vs. 1.88±0.43pg/mg/min ($p<0.001$). 6-oxo-$PGF_{1\alpha}$ and PGI_2 were significantly decreased at the bifurcation, 1.62±1.38pg/mg/min and 0.8±0.73pg/mg/min, respectively, in comparison to lateral granuloma 2.63±1.2pg/mg/min and 1.57±0.84pg/mg/min, respectively ($p=0.006$). Metabolites of ^{14}C-AA showed no significant differences in this respect.

Dental care

Tooth-brushing was performed 1.6±0.8 times/day. There was no significant difference in eicosanoid- and IPs synthesis or LOX metabolites whether brushing was done <1 or >1 time/day. Use or no use of dental floss (28% of patients) and the annual rate of consulting a dentist (43.5% annually) or consulting a dentist because of clinical symptoms only (56.5%) revealed no significant difference in eicosanoids, IPs and LOX-metabolites. Periodontitis and self-reported gingival bleeding during teeth brushing was reported in 74%. In gingival bleeding no significant increase in eicosanoids, IPs or LOX-metabolites compared to patients without gingival bleeding could be documented.

Smoking status and eicosanoids

In relation to smoking status 8-iso-$PGF_{2\alpha}$ and products of the LOX-pathway were significantly increased ($p<0.0001$), whereas 6-oxo-$PGF_{1\alpha}$, PGI_2 and $PGF_{2\alpha}$ were significantly decreased in CS vs. FS and NS ($p<0.0001$) **(Figure 1)**. In contrast, PGE_2, and TXB_2 revealed no significant difference.

The size of the granuloma in conjunction with the smoking status revealed significant differences for 8-iso-$PGF_{2\alpha}$ ≥4mm and CS vs. ≥4mm and FS or NS; 2.52±0.52pg/mg/min and 1.95±0.2pg/mg/min ($p=0.008$); <4mm and CS vs. <4mm and FS or NS; 3.1±0.4pg/mg/min and 2.19±0.83pg/mg/min ($p=0.004$), for 6-oxo-$PGF_{1\alpha}$ 1.3±0.38pg/mg/min and 3.22±0.98pg/mg/min ($p<0.001$); 1.88±0.65pg/mg/min and 2.55±1.08pg/mg/min ($p<0.05$) and for PGI_2 0.65±0.13pg/mg/min and 0.2±0.19pg/mg/min ($p<0.001$); 0.94±0.45pg/mg/min and 1.55±0.64pg/mg/min ($p=0.007$), but not for TXB_2 **(Figure 2)**.

The conversion rate of ^{14}C-AA was significantly different for 6-oxo-$PGF_{1\alpha}$ in CS vs. FS and NS, 0.25±0.12% and 0.73±0.19% ≥4mm ($p<0.001$); 0.32±0.27% and 0.71±0.42% <4mm ($p=0.007$) and for LOX-metabolites 3.03±0.89% and 2.31±0.44% ≥4mm ($p=0.05$) 3.31±0.69% and 2.7±0.58% <4mm ($p=0.014$) **(Figure 2)**. $PGF_{2\alpha}$ was significantly different in patients with granuloma <4mm and CS vs. <4mm and FS or NS; 0.08±0.04% and 0.17±0.12% ($p=0.008$), but not for granuloma ≥4mm, although the same trend was determined, 0.1±0.07% and 0.15±0.1%. The size of the granuloma neither influenced the synthetic profile of eicosanoids nor the conversion rate of ^{14}C-AA, whereas smoking status was responsible for significant differences.

The number of cigarettes smoked/day did not influence eicosanoid synthesis either via the LOX- or the COX-pathway. CS had significantly lower values for 6-oxo-$PGF_{1\alpha}$ and PGI_2 in comparison to FS and NS.

DISCUSSION

Our study supports the evidence of an increased synthesis of IPs, and an elevated LOX formation in patients with AP and arising granuloma with bone destruction. Furthermore, smoking status influences the synthesis of IPs and increases the amount of LOX products.

Periapical lesions are destructive inflammatory pathologies that are characterized by periradicular periodontal ligament and bone destruction. Different inflammatory mediators such as various interleukins, tumor necrosis factor α, granulocyte macrophage colony stimulating factor, nitric oxide, metalloproteinases, PGs, and cytokines producing T-lymphocytes have been associated with dental granuloma.[24,25,26] Thus, locally available eiscosanoids are extremely high in bone and play either a stimulatory or an inhibitory role in bone turnover depending on the physiological or pathological circumstances.[27] COX-1 is expressed in normal bone, while COX-2 expression is up-regulated during bone repair and under pathological conditions such as inflammation.

AA derived LOX-metabolites are potent pro-inflammatory mediators leading to tissue destruction in periodontal inflammation.[28] ^{14}C-AA in gingiva of patients with periodontal disease is mainly metabolized via the LOX-pathway.[29] As confirmed by our results extracted granuloma converted a significantly higher percentage of the total radioactivity to LOX-products as compared to PGs synthesized via the COX-pathway. The amount of products of the LOX-pathway were influenced by smoking. In CS LOX-metabolites were significantly higher than in NS or FS, which confirms current evidence that smoking is a significant risk factor in inducing and enhancing inflammation of the marginal periodontium and therefore it may be hypothesized that it would have a similar effect on the periapical periodontium.[8,9,30] Moreover, nicotine induces proinflammatory responses in macrophages and the aorta leading to acceleration of atherosclerosis and promotes enhanced angiogenesis and thrombosis.[31] Furthermore, it plays an active role in the development of coronary artery disease via oxidized LDL-cholesterol, originating from oxidation in the arterial wall by cell-associated LOX and/or myeloperoxidases.[4]

Tobacco smoking is the main risk factor associated with chronic destructive periodontal disease.[32] The harmful effects manifest themselves by interfering with vascular and immunologic reactions, as well as by undermining the supportive functions of the periodontal tissues.[8,9,13] Thus, chronic destructive periodontal disease in cigarette smokers is initiated and driven by smoking.

Elevated PGE_2 in periapical exudates was associated with the presence of clinical symptoms that reflected an acute inflammation in the periapical lesion.[33] In contrast, a significantly negative association of decreased periapical exudates-PGE_2 with increasing size of radiolucent areas was demonstrated. As shown in our results PGE_2 was detected in all granuloma. In NS with pain, PGE_2, a marker of disease activity and inflammation, was significantly elevated vs. NS without pain, whereas there was no significant difference for CS, which may be due to a decreased PGE_2-synthesis in smokers as shown by Kibayashi et al.[34]

However, Tanaka et al.[35] suggested that nicotine stimulates the formation of osteoclast-like cells via an increase in macrophage colony-stimulating factor and PGE_2 production by osteoblasts. This could be confirmed by Shoji et al.[36] There are contradictory results in PGE_2 synthetic rate, cigarette smoking and bone loss.[37,38] In comparison to the mentioned studies, we were measuring PGE_2 indirectly via the conversion rate of ^{14}C-AA and not by ELISA which may yield different findings.

IPs, stable metabolites of in-vivo lipid peroxidation, are free radical catalyzed products of AA. Excessive free radical generation is thought to contribute to tissue injury in a broad spectrum of diseases, serve as an indicator of in-vivo oxidative stress, and has been successfully used as a marker for chronic inflammation.[39] Apparently, individuals who smoke cigarettes or consume alcohol exhibit dose-dependent increments in 8-iso-$PGF_{2\alpha}$ excretion.[40,41] Furthermore, passive cigarette smoking significantly increases in-vivo oxidation injury with an increase of isoprostanes favoring the development and/or progression of associated diseases.[42] The cigarette smoking associated with *in-vivo* oxidation injury almost completely disappears within 4 weeks of smoking cessation.[43,44] Salivary 8-iso-$PGF_{2\alpha}$ was significantly higher in CS compared to NS.[45,46] Oxidative stress, as reflected by elevated salivary 8-iso-$PGF_{2\alpha}$ levels, is associated with the extent of periodontal disease and is significantly aggravated by concomitant tobacco use. Chronic inflammation and smoking have been associated with the development of atherosclerosis. The results of Wolfram et al.[46] and our results indicate that salivary IPs can reliably assess the degree of oxidative stress, and smoking and periodontal disease are two modifiable cardiovascular risk factors that are able to potentiate each other. In addition, CS showed significantly lower 6-oxo-$PGF_{1\alpha}$ levels, the stable metabolite of PGI_2 due to increased free radicals in smokers immediately inactivating prostacyclin synthase.[47] RIA measurements and bioassay technique showed differences in the amount of synthesis of PGI_2. The produced substance is measured by RIA, whereas the

amount of PGI_2 synthesized in bioassay technique is time dependent, much lower, and depends on the actual available biological substance during testing.[48]

One limitation of our study is that hs-CRP, a marker of inflammation, was not measured. Elevated CRP levels are associated with an increased risk for adverse cardiovascular events in healthy individuals and patients with known coronary artery disease. Therefore, identifying conditions that contribute to CRP elevations may have significant prognostic and therapeutic implications in patients with cardiovascular disease. Oxidative stress is assumed to be the key underlying factor in the pathogenesis of many common diseases. The influence of other oxidative injuries resulting in 8-iso-$PGF_{2\alpha}$ synthesis were not excluded. In addition, we did not determine acute and chronic AP; however 54.3% of patients' suffered from pain, which would be clinically classified as acute periodontitis.

A differentiation between a cyst and a granuloma was necessary without a histopathological work up knowing that exploratory surgery and/or histopathological study of the lesion is the only test that guarantees the diagnosis. A histopathological work up of the extracted teeth including the granuloma could not be performed as further assessments of our study parameters would not have been possible. Another limitation of our study was the location of the granuloma, in 5 patients (10%) furcation and not apical granuloma were present. However, statistical analysis revealed no significant differences between furcation and apical granuloma.

In conclusion, our data demonstrate that in CS with granuloma due to periodontitis products of lipid peroxidation such as 8-iso-$PGF_{2\alpha}$ and products of the LOX-pathway increase. The size of granuloma did not influence the amount of synthesized eicosanoids, IPs or LOX-metabolites converted from ^{14}C-AA, whereas cigarette smoking was an influencing and modifiable risk factor.

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Variables	**All** (n=46)	**CS** (n=20)	**FS** (n=12)	**NS** (n=14)	**p-value**
Males (n)	29	13	10	6	
Females (n)	17	7	2	8	
Age (mean±SD)	47±15	38±12	53±13	55±16	*
Diabetes (n)	1	0	0	1	n.s.
ASA (n)	5	1	3	1	n.s.
Clopidogrel (n)	1	0	0	1	n.s.
Oral anticoagulation (n)	2	0	1	1	n.s.
Statins (n)	1	0	1	0	n.s.

Table 1. Patient characteristics, prescribed medication and smoking status CS indicates current smokers; FS, former smokers; NS, non-smokers; ASA, acetylsalicylic acid; n, number of patients, *significant for CS vs. FS and CS vs. NS ($p \leq 0.001$); n.s., non significant

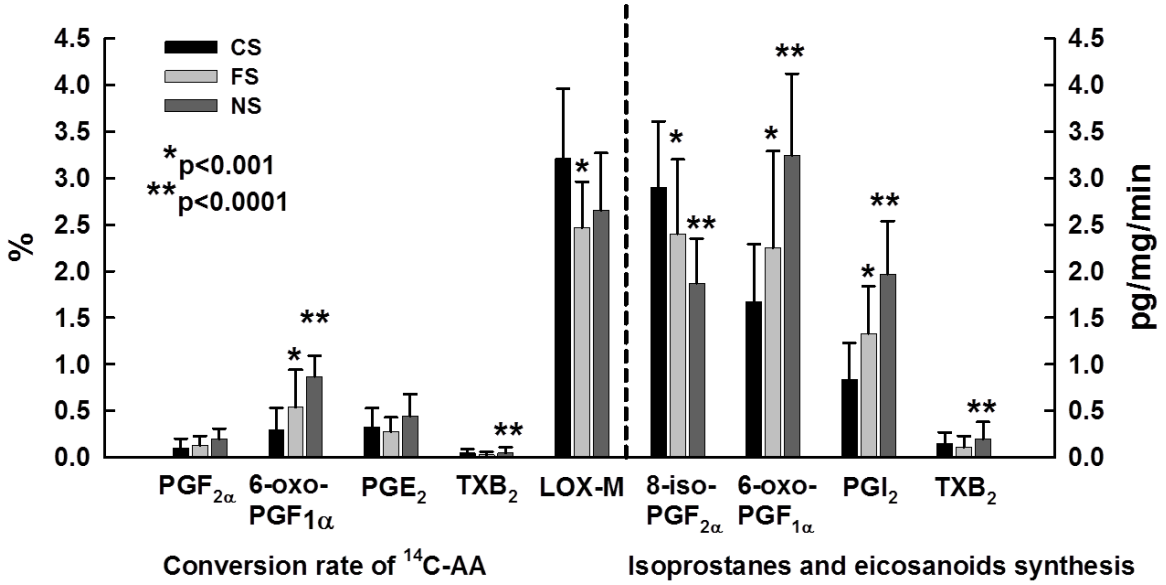

Figure 1.

Smoking status and synthesis of eicosanoids, isoprostanes and LOX-metabolites.

In relation to smoking status 8-iso-$PGF_{2\alpha}$ and metabolites of the LOX-pathway were significantly increased, whereas 6-oxo-$PGF_{1\alpha}$, PGI_2 and $PGF_{2\alpha}$ were significantly decreased in CS vs. FS and NS. PGE_2, and TXB_2 revealed no significant difference.

CS, current smokers; ^{14}C-AA, ^{14}C labelled arachidonic acid; FS, former smokers; LOX, lipoxygenase; NS, non-smokers; PG, prostaglandin; TX, thromboxane.

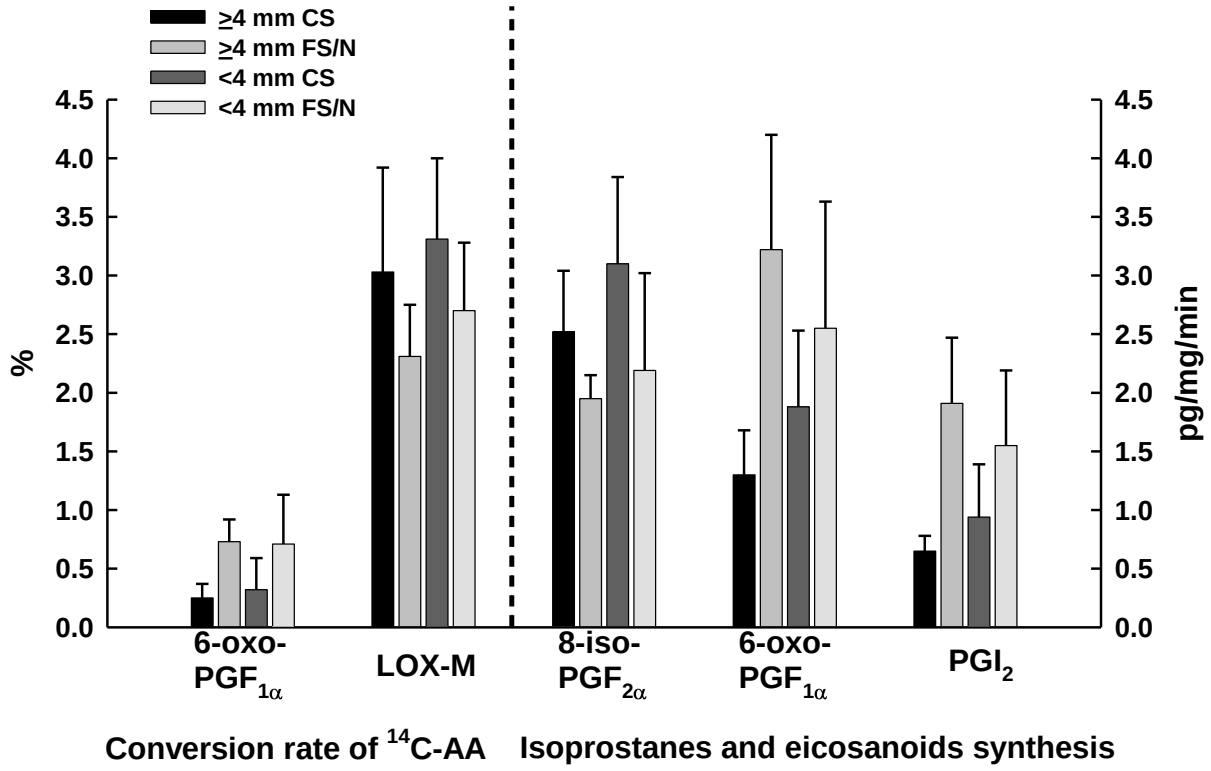

Figure 2.

Smoking status is responsible for significant differences in synthesis and composition of eicosanoids and the conversion rate of ^{14}C-AA independent of the size of the granuloma. Only significant results of eicosanoids, IPs and LOX-M, comparing CS vs. FS/NS with granuloma <4mm or ≥4mm, are shown. There were no significant differences in CS with granuloma <4mm vs. ≥4mm or in FS/NS with granuloma <4mm vs. ≥4mm.

CS, current smokers; ^{14}C-AA, ^{14}C labelled arachidonic acid; FS, former smokers; IPs, isoprostanes; LOX, lipoxygenase; NS, non-smokers; PG, prostaglandin.