Karolinska Institutet

Department of Dental Medicine

Prostaglandin E synthases in periodontitis-affected gingival tissue and in gingival fibroblasts

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i sal 4V, plan 4, Institutionen för odontologi, Alfred Nobels Allé 8, Huddinge

Fredagen den 10 juni 2011, kl 09:30

av
Tove Båge
Civilingenjör

Huvudhandledare:
Docent Tülay Yucel-Lindberg
Karolinska Institutet
Institutionen för odontologi
Enheten för pedodonti

Bihandledare:
Professor Emeritus Thomas Modéer
Karolinska Institutet
Institutionen för odontologi
Enheten för pedodonti

Fakultetsopponent:
Professor Ulf Lerner
Umeå universitet
Institutionen för odontologi
Avdelningen för molekylär parodontologi

Betygsnämnd:
Professor Ralf Morgenstern
Karolinska Institutet
Institutet för Miljömedicin
Enheten för biokemisk toxikologi

Professor Göran Andersson
Karolinska Institutet
Institutionen för Laboratoriemedicin
Avdelningen för patologi

Docent Afshin Ahmadian
Kungliga Tekniska Högskolan
Skolan för Bioteknologi

Stockholm 2011

Abstract

Periodontitis is a chronic inflammatory disease resulting in the destruction of the tissue and alveolar bone supporting the teeth and leading ultimately to tooth loss. Prostaglandin E_2 (PGE_2) is an important inflammatory mediator in the pathogenesis of periodontitis. The biosynthesis of PGE_2 is catalysed by three groups of enzymes acting sequentially: phospholipase A_2 (PLA_2), cyclooxygenases (COX-1 and COX-2) and prostaglandin E (PGE) synthases, which catalyse the final step of PGE_2 synthesis. Three PGE synthase isoforms have been identified: i) the inducible microsomal membrane-associated and glutathione-dependent PGE synthase, mPGES-1, ii) the constitutively expressed cytosolic PGE synthase, cPGES, and iii) the glutathione-independent, membrane-associated mPGES-2. The aim of this thesis was to investigate the expression of PGE synthases in gingival tissue from periodontitis patients, as well as to study their expression and regulation in relation to PGE_2 production in gingival fibroblasts.

In periodontitis-affected gingival tissue, we demonstrated *in vivo* protein expression of mPGES-1, mPGES-2 and cPGES, as well as COX-2 in fibroblasts, endothelial cells, smooth muscle cells, epithelial cells and immune cells. We further showed that, in cell cultures of gingival fibroblasts and smooth muscle cells, the inflammatory cytokines tumour necrosis factor α (TNFα) and interleukin-1β (IL-1β), or co-culture with lymphocytes, markedly induced mPGES-1 and COX-2 expression, accompanied by an increase in PGE_2 production. In cultured endothelial cells, only TNFα was found to increase PGE_2 production, via enhanced COX-2 expression. In mast cell cultures, basal levels of PGE_2 were detected, but no increase was observed in response to TNFα or IL-1β.

To elucidate the impact of mPGES-1 inhibition on mPGES-2 and cPGES expression, as well as on PGE_2 production we used knock-down of mPGES-1 expression by small interfering RNA (siRNA). The cytokine-induced protein expression of mPGES-1 was reduced by up to 79% by siRNA silencing, without affecting mPGES-2 or cPGES expression. Moreover, mPGES-1 siRNA did not affect the cytokine-stimulated PGE_2 production, whereas levels of the downstream prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were enhanced.

Using inhibitors and activators of various signalling pathways, we demonstrated that cytokine-induced mPGES-1 expression in gingival fibroblasts did not involve protein kinase C, p38 mitogen-activated protein kinase or tyrosine kinase pathways, in contrast to COX-2 expression. We further observed a possible positive feedback loop in which PGE_2 and $PGF_{2\alpha}$ increased the expression of mPGES-1. Furthermore, cytokine-induced mPGES-1 expression and PGE_2 production were reduced after the inhibition of the upstream enzyme PLA_2 and increased after the addition of arachidonic acid, the product of PLA_2. The proposed anti-inflammatory prostaglandin 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2), reduced mPGES-1 expression but not COX-2 expression or PGE_2 production.

To further explore the pathways involved in increased PGE_2 synthesis in TNFα-stimulated gingival fibroblasts, a global gene expression profile was established using a microarray platform. Enrichment analysis of the gene expression data led to further investigation of nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK) signalling pathways, revealing that these pathways are involved in the signal transduction of TNFα-induced mPGES-1 and COX-2 expression.

In conclusion, all three PGE synthases are expressed in gingival tissue from patients with periodontitis. The isoenzyme mPGES-1 is the main PGE synthase involved in cytokine-induced PGE_2 production in gingival fibroblasts. The cytokine-increased expression of mPGES-1 involves the signal pathways JNK and NF-κB. Furthermore, the prostaglandins PGE_2 and $PGF_{2\alpha}$ increase mPGES-1 expression, which may create a positive feedback loop. Collectively, these results suggest that inflammation-induced production of PGE_2 by gingival fibroblasts, mediated by the increased expression of mPGES-1 and COX-2, may contribute to chronic inflammation in periodontitis. The results provide new insights into the expression and regulation of mPGES-1 in gingival fibroblasts and gingival tissue.

Keywords: c-Jun N-terminal kinase, cyclooxygenase, cytokines, gingival fibroblasts, gingival tissue, inflammation, interleukin-1β, nuclear factor-κB, mPGES-1, periodontitis, prostaglandin E_2, prostaglandin E synthase, tumour necrosis factor α

ISBN 978-91-7457-392-3