ARCHIVES OF ORAL BIOLOGY 58 (2013) 1812–1817

Available online at www.sciencedirect.com

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Short communication

Beta2-adrenergic receptor agonists reduce proliferation but not protein synthesis of periodontal fibroblasts stimulated with platelet-derived growth factor-BB

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ARTICLE INFO

Article history:
Accepted 24 September 2013

Keywords:
Periodontal therapy
Stress
Beta2-adrenergic receptor
PDGF-BB
Proliferation

ABSTRACT

Objective: Catecholamines released from β-adrenergic neurons upon stress can interfere with periodontal regeneration. The cellular mechanisms, however, are unclear. Here, we assessed the effect of catecholamines on proliferation of periodontal fibroblasts.

Methods: Fibroblasts from the gingiva and the periodontal ligament were exposed to agonists of the β-adrenergic receptors; isoproterenol (ISO, non-selective β-adrenergic agonist), salbutamol (SAL, selective β_2-adrenergic receptor agonist) and BRL 37344 (BRL selective β_3-receptor agonist). Proliferation was stimulated with platelet-derived growth factor-BB (PDGF-BB). Pharmacological inhibitors and gene expression analysis further revealed β-adrenergic signalling.

Results: Gingiva and periodontal ligament fibroblast express the β_2-adrenergic receptor. ISO and SAL but not BRL decreased proliferation of fibroblasts in the presence of PDGF-BB. The inhibitory effect of β-adrenergic signalling on proliferation but not protein synthesis in response to PDGF-BB was reduced by propranolol, a non-selective β-adrenergic antagonist.

Conclusions: These results suggest that β_2-receptor agonists can reduce the mitogenic response of periodontal fibroblasts. These data add to the compelling concept that blocking of β_2-receptor signalling can support tissue maintenance and regeneration.

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0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2013.09.005

1. Introduction

Stress, including psychological, mechanical, and oxidative forms, can promote periodontal diseases, stimulate tissue destruction, and compromise wound healing.[1–7] Stress related molecules in the sulcus and saliva are suggested to predict the onset and progression of periodontal diseases.[8] The molecular mechanisms linking stress and periodontal diseases are, however, not fully understood. Among the stress meditators are catecholamines such as epinephrine and norepinephrine. Catecholamines induce β-adrenergic signalling by binding to their respective transmembrane adrenergic receptors.[9,10] Adrenergic signalling represents a possible target to interfere with the compromising effects of stress.[11–15]

Under stress, the periodontal tissue, being highly innervated with nerves of the sympatic system, is assumably exposed to increasing levels of catecholamines. A possible link between nerve activation and the progression of periodontal diseases is suggested by preclinical studies e.g. in rodents showing that chemical sympathectomy inhibits periodontal disease and enhances bone regeneration.[16,17] The impact of β-adrenergic signalling on osteogenic cells that maintain the alveolar bone has been shown in rodents.[18] Moreover, propranolol, a non-selective β-adrenergic receptor antagonist, in rats prevented bone loss under occlusal hypofunction[19] and in an inflammation model.[20] Catecholamines can also impair soft tissue healing.[21] Respective models on periodontal regeneration have not been reported.

Periodontal regeneration requires a process where fibroblastic cells proliferate and produce matrix. These steps are governed by growth factors including those released from platelets and macrophages such as PDGF-BB.[22] The approval of recombinant PDGF-BB for periodontal regeneration support the importance of these growth factors in the clinic.[23] The cellular response to PDGF-BB may, however, be compromised by adrenergic signalling as suggested by in vitro studies with fibroblasts and vascular smooth muscle.[24,25] Yet, the impact of adrenergic signalling on the responses of fibroblasts to PDGF-BB is unclear.

Here, we assessed the impact of β-adrenergic receptor agonists on the cellular response to recombinant PDGF-BB by assessing the impact on proliferation and protein synthesis in vitro. Fibroblasts from the periodontal ligament and the gingiva were stimulated with the β-adrenergic receptor agonists isoproterenol, salbutamol, and BRL 37344. We also included the blockers metoprolol, propanolol, and atenolol in the study. Our study can provide insights into the effect of the β-adrenergic system on the mitogenic response of periodontal cells to growth factors during periodontal healing.

2. Material and methods

2.1. Cell culture

Fibroblasts from the gingiva (GF) and the periodontal ligament (PDLF) were prepared from extracted third molars after informed consent was obtained from the patients routinely treated at the BGZMK and the study was approved by the Ethics Committee of the Medical University Vienna, Ek-Nr.: 631/2007. The donors were selected based on the absence of previous history of periodontal inflammation. Gingiva fragments from the tooth neck and of the periodontal ligament fragments from the tooth root were scraped off the extracted teeth. Explant cultures from gingival and periodontal ligament tissue fragments were performed separately. GF and PDLF were cultivated in a humidified atmosphere of 5% CO_2 at 37 °C in α-Minimum Essential Media (αMEM Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 10% foetal calf serum (FCS, PAA Laboratories, Linz, Austria), penicillin and streptomycin (Invitrogen Corporation). Cells from passage 3–9 were used for the experiments and seeded at 50,000 cells/cm^2. Cells were stimulated with or without PDGF-BB (R&D Systems, Minneapolis, MD, USA) at 30 ng/ml, and with or without the β-AR agonists isoproterenol (ISO), salbutamol (SAL), BRL 37344 (BRL) (Sigma Aldrich) at concentrations of 100 nM. To reveal the involved β-AR, specific antagonists of β-adrenergic signalling were used: Metoprolol (MET), propanolol (PRO), and atenolol (ATE) all at 100 nM (Sigma–Aldrich).

2.2. Proliferation, protein synthesis and MTT assay

GF and PDLF were pulse-labelled with ^{3}[H]thymidine and ^{3}[H]leucine (both 0.5 μCi/well, Hartmann Analytic, Braunschweig, Germany), respectively, for the last 6 h of exposure to the β-AR agonists. The plates were subjected to liquid scintillation counting using Microscint 0 on a Top Count NXT system (Perkin Elmer, Minnesota, USA). Data were normalized to untreated controls. For MTT assay, GF and PDLF were treated with the β-AR agonists for one day and then incubated with 1 mg/ml MTT (Sigma, Saint Louis, MO, USA) for 2 h at 37 °C. MTT and the medium were removed and formazan crystals were solubilized with dimethyl sulfoxide. Optical density was measured with a photometer (Spectra max 384 PLUS, Molecular Devices, LLC, CA, USA).

2.3. Reverse transcription-PCR analysis

Total RNA of fibroblasts from the gingiva and the periodontal ligament was isolated using RNeasy mini kit (Qiagen, Hilden Germany) and treated with DNAse according to the manufacture's protocol. Reverse transcription was performed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Life Technologies Corporation, NY, USA). For the PCR, the Taq DNA Polymerase Kit (Invitrogen Corporation) was used according to the instructions of the manufacturer. In brief, PCR was performed with 2 u of Taq polymerase (Invitrogen Corporation), in 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 0.2 mM dNTP mix, 1.5 mM MgCl. The following primers were used (5′–3′) at 0.5 μM each[26]: β_2-AR forward ACCCACCAGGAAGCCATCAACTGCT, β_2-AR reverse GCCTATCCAATTTAGGAGGATGTAAACTTCC, beta-actin forward AAAGACCTGTACGCCAACACAGTGCTGTCTGG, beta-actin reverse CGTCATACTCCTGCTTGCT GATCCACATCTGC. The tubes were incubated in the thermal cycler at 94 °C for 3 min following 30 cycles of 94 °C for 45 s, 55 °C for 30 s, and 72 °C for 1 min 30 s. The products were evaluated by performing gel electrophoresis on a 2% agarose gel. Pictures were taken using a UV transilluminator (Bio-Rad, CA, USA)