ARCHIVES OF ORAL BIOLOGY 59 (2014) 461–469

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

Focussed microarray analysis of apoptosis in periodontitis and its potential pharmacological targeting by carvacrol

Fares Zeidán-Chuliá [a,*], *Mervi Gursoy* [b,d], *Ben-Hur Neves de Oliveira* [a], *Daniel Pens Gelain* [a], *Eija Könönen* [b,d], *Ulvi Kahraman Gursoy* [b,d,1], *José Cláudio Fonseca Moreira* [a,1], *Veli-Jukka Uitto* [c,1]

[a] *Centro de Estudos em Estresse Oxidativo, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*

[b] *Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland*

[c] *Department of Periodontology, Institute of Dentistry, University of Helsinki, Helsinki, Finland*

[d] *Bacteriology Unit, Department of Infectious Disease Surveillance and Control, National Institute for Health and Welfare (THL), Helsinki, Finland*

ARTICLE INFO

Article history:
Accepted 19 January 2014

Keywords:
Inflammation
Oxidative stress
Antioxidant
Antibacterial
Monoterpenoid phenol
Caspase-3
HaCaT cells

ABSTRACT

Objective: The objective of this study was to perform a landscape analysis of apoptosis-related genes/proteins and to study the differential gene expression by analysing array data from periodontitis patients and, second, to evaluate the anti-apoptotic effects of carvacrol, a monoterpenoid phenol, *in vitro*.

Design: A gene/protein interaction network model 'APOP' was developed by using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.05. Differential gene expression was determined by using the limma package from R and false discovery rate (FDR). With ViaComplex software, gene expression was plotted over the network. The anti-apoptotic effect of carvacrol was tested on sorbitol-treated HaCaT cells, by using a commercial kit for caspase-3 activity.

Results: The 'APOP' model characterised the landscape of interactions between apoptosis-related genes/proteins *in silico*. Forty-nine out of 70 genes from this model, such as *CSF2RB*, *NFKBIE*, *ENDOG*, *CASP10* and *CASP3*, were differentially expressed (corrected p-value < 0.05) in periodontitis samples when compared to those of healthy controls. In addition, carvacrol (0.43%) was able to inhibit the pro-apoptotic effects induced by sorbitol (0.3 M), as seen by the reduction in caspase-3 activity on HaCaT cells.

Conclusion: Our results suggest that caspase-3 can be a target protein to inhibit periodontitis-associated apoptosis of epithelial cells and that carvacrol has therapeutic potential as an anti-apoptotic agent.

* *Corresponding author at*: Centro de Estudos em Estresse Oxidativo, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Rua Ramiro Barcelos, 2600-anexo-Bairro Santana, 90035-003 Porto Alegre, RS, Brazil. Tel.: +55 51 3308 5577; fax: +55 51 3308 5535.
E-mail address: fzchulia.biomed@gmail.com (F. Zeidán-Chuliá).

[1] These authors jointly directed this work and should be considered as equal last authors.

Abbreviations: MMP, matrix metalloproteinase; EOs, essential oils; GEO, gene expression omnibus; FDR, false discovery rate; ROS, reactive oxygen species; IL, interleukin; CSF2, granulocyte–macrophage colony-stimulating factor.

0003–9969/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.archoralbio.2014.01.007

1. Introduction

Bioinformatics has established itself as an essential tool in science and the *in silico* analysis of gene expression/function has become an integral part of it by guiding the researchers towards the selection of the most relevant targets in the context of any disease.[1] Our group has successfully applied these tools in different areas of research such as pharmacognosy, neuroscience and toxicology.[2–4]

Periodontitis is an infection-induced chronic inflammatory disease of tooth-supporting tissues. Destruction initiates from the soft tissues (epithelium and gingival connective tissue) and continues to the hard tissues (alveolar bone) of the periodontium. Different bacteria, such as *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola*, have been associated with both the initiation and regulation of soft and hard tissue destruction during periodontal inflammation.[5] In periodontal tissues, the balance between cell death and survival of resident cells, provided by physiological programmed cell death (*e.g.*, autophagy and apoptosis), is critical for tissue homeostasis.[6] Autophagy is considered a self-degradative process, critical for the balance of energy sources at specific times as it removes aggregated proteins and/or damaged organelles as well as eliminates intracellular pathogens.[7] For instance, mitochondria play an important role in pro-inflammatory signalling and generation of reactive oxygen species (ROS), and it is regarded as an important activator of inflammasome-mediated inflammation.[8] Thus, the turnover of non-functional mitochondria (or mitophagy) would become essential in inflammatory scenarios, such as periodontitis, in order to maintain tissue homeostasis. As a matter of fact, increased levels of autophagy gene expression and high levels of mitochondrial ROS production in peripheral blood mononuclear cells from patients with periodontitis have already been reported.[9] Pathogenic bacteria induce several changes in resident cells of the gingiva (epithelial cells and fibroblasts), including the stimulation of cytokines and production of antimicrobial peptides, modulation of matrix metalloproteinase (MMP) secretion and induction of apoptosis.[10–12] Periodontitis-associated bacteria induce apoptosis in different ways: *A. actinomycetemcomitans* induces apoptosis in a caspase-3-dependent manner,[13] *P. gingivalis* initiates anti-apoptotic mechanisms in early infection and stimulates pro-apoptotic mechanisms in continuing infection[14] and *F. nucleatum* triggers apoptosis in epithelial cells by inducing caspase-3 expression; however, such an effect seems to be strain-dependent.[15]

Inhibition of tissue destruction is one of the main goals in host modulation therapies for periodontal inflammation by suppressing bacterial challenge and regulating tissue response.[16] Essential oils (EOs) have been proposed as natural therapeutic agents due to their antibacterial and anti-gelatinolytic properties.[2] *Satureja hortensis* L. EO, with carvacrol as its main active component, has been shown to exhibit strong antibacterial effects on periodontopathogenic bacteria and to decrease their cytotoxicity and excessive MMP production by epithelial cells *in vitro*.[17,18] In fact, in our recent study, it was demonstrated that 0.05% of *S. hortensis* L. EO is able to inhibit cell death and increase the proliferation of human epithelial cells.[18] These events could be a result of anti-apoptotic properties induced by *S. hortensis* L. EO. In the present continuation study, we hypothesised that its anti-apoptotic effect depends on carvacrol, which is the main compound of *S. hortensis* L. EO. Our aims were (1) to characterise the apoptotic landscape in periodontitis with an *in silico* approach and a pathway-focussed (apoptosis) microarray analysis and (2) to analyse the effect of carvacrol as an anti-apoptotic agent *in vitro*.

2. Materials and methods

2.1. Network development

The apoptosis interaction network model 'APOP' was developed by associating a total of 70 apoptosis-related genes/proteins (Table 1), provided by the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database (http://www.genome.jp/kegg/pathway.html; Apoptosis: map04210). The network was generated by using the database resource search tool Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.05 (http://string-db.org/) for the retrieval of interacting genes[19] with 'Experiments' and 'Databases' as input options and a confidence score of 0.400. STRING is a well-known public database with information about direct and indirect functional protein–protein interactions. The genes/proteins belonging to the 'APOP' model were identified by the Human Genome Organisation (HUGO) Gene Symbol[20] and Ensemble protein ID. Once they were selected, the links between two different nodes (genes/proteins) were provided by the STRING database and saved in data files to be handled in the Medusa interface.[21]

2.2. Differential gene expression analysis

Microarray data were downloaded from the international repository (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/; GEO accession No.: GSE10334; Platform: GPL570, Affymetrix), an experiment that is composed of 247 human samples (64 healthy and 183 diseased gingival samples).[22] In order to perform the differential gene expression analysis, normalised data of periodontitis samples *versus* controls were analysed by using the limma package from R and false discovery rate (FDR)[23,24] for statistical assessment of the microarray data (corrected p-values < 0.05 were considered significant). The means of gene expression in diseased samples were plotted *versus* the expression found in healthy ones to be visualised with ViaComplex.[25] This software plots gene expression over the Medusa network (in a colour scale) by overlapping functional input data (microarray expression data) with interaction information ('APOP' model) and distributes the microarray signal according to the coordinates of the nodes and its interactions in the network of interest.

2.3. Chemicals and concentrations

Carvacrol (282197) was purchased from Sigma. For every test performed on epithelial cells, a concentration of carvacrol of