Effect of tobacco smoking on human gingival and periodontal fibroblasts. A systematic review of literature

Wpływ palenia tytoniu na fibroblasty jamy ustnej. Przegląd literatury

The biological plausibility of an association between tobacco smoking and periodontitis was founded on broad effects of multiple tobacco-related substances on cellular structure and function. Smoking has been shown to affect the vasculature, the humoral and cellular immune responses, cell signaling processes, and tissue homeostasis [1-4]. Nicotine has been shown to be absorbed rapidly through the skin and mucous membranes, and to be present even in the tooth structures of periodontally-diseased teeth in smokers [5]. The epithelial cells, acting as mechanical barrier, can reduce but not completely eliminate the deleterious effect of nicotine on gingival and periodontal fibroblasts [6].

In a healthy periodontium, gingival fibroblasts are one of the most copious stromal cell types [7], regulating matrix synthesis and remodeling, injury-site-directed migration, and attachment to extracellular matrix [8]. Fibroblasts are very sensitive, particularly to changes in the surrounding matrix, growth factors, and cytokines and they respond to these signals. Exposure of human fibroblasts, derived from the periodontium, to nicotine affects cell growth as well as attachment [9,10]. Mechanisms by which smoking leads to loss of attachment are, however, still not well understood.

In view of summarizing the effect of tobacco smoking on oral cavity fibroblasts, a Medline literature search was performed on the effect of tobacco and their factions on: human gingival fibroblasts, human periodontal fibroblasts and gingival fibrosis. Inclusion criteria: full length articles, articles written in English, in vitro and in vivo study. Exclusion criteria: abstracts of articles, articles written in languages other than English, animal study.

It was found that smokers exhibit decreased concentrations of salivary and serum antibodies especially to Prevotella intermedia and Fusobacterium nucleatum [11], and it cannot be attributed to poorer oral hygiene. Both smokers and nonsmokers have a similar composition of subgingival periopathogens, however they colonize the shallow pockets in smokers [12].

Therefore a hypothesis that nicotine and other tobacco ingredients may affect the host response was considered. Some functions, for example chemotaxis and phagocytosis of oral and peripheral neutrophils, may be impaired by tobacco ingredients [13]. It was found that human gingival fibroblasts rapidly take up and accumulate high levels of nicotine in vitro, most of which remains inside the fibroblasts where it can affect cell metabolism or other functions [14]. In addition, nicotine inhibits the proliferation, attachment, alkaline phosphatase production and migration of human periodontal ligament fibroblasts, suggesting that it may impair the host defense system against the progression of periodontitis [15].

Cell migration is a key process of wound healing and it is highly regulated by a variety of signaling pathways. One of the possible reported ways in which cigarette smoke influences fibroblast migration is by deregulating the ATP production pathway [16]. Also the small G protein, Rac, is necessary for cell migration. Nicotine decreased human gingival fibroblast (HGF) migration by 50% in serum-free conditions and decreased Rac activation while up-regulating PAK1/2 and erk 1/2. The authors conclude that these signaling pathways may be important modulators which can be regulated to increase oral wound healing in smoking patients [17].

Other studies noted the adverse effect of cigarette smoke on gingival fibroblast cell migration through alterations of fibroblast integrity by a decreased production of critical cell junction proteins, such as E-cadherin [18]. Gingival fibroblasts exposed once to whole cigarette smoke also exhibited a significant inhibition of cell adhesion, a decrease in the number of β1-integrin-positive cells, increased lactate dehydrogenase (LDH) ac-
tivity in the target cells, and reduced growth. Integrins, crucial cell migration mediators, are essential throughout the wound healing process [19]. The inhibitory effect on cell adhesion may occur through a deregulation of expression of adhesion proteins, including β1-integrin, α5-integrin and β1-integrin being to a large integrin family - a ubiquitously expressed and highly conserved group of adhesion receptors involved in the cell-ECM physical link, as well as cell-to-cell adhesion [20]. Smoke-exposed fibroblasts were also not able to contract collagen gel matrix and migrate following insult [21, 22]. These results demonstrate that a single exposure to whole cigarette smoke produced significant morphological and functional deregulation in gingival fibroblasts [23]. It compromises PDL cell adhesion to root planed surfaces, and might affect periodontal regeneration following therapy [24]. Furthermore, vacuolization of human fibroblasts and disturbance of microtubules and actin filaments in keratinocytes due to nicotine and other ingredients of tobacco smoke is also documented [9]. The tubular and filament-associated microtubules (VIFs), two major cytoskeletal elements of examined HGF, are disrupted by dose-dependent acroleine and acetaldehyde - toxic aldehydes from cigarette smoke. Arecoline is cytotoxic to human gingival fibroblasts at a concentration higher than 50 μg/ml by depleting intracellular thiols and inhibiting mitochondrial activity. The HGFs displayed a marked arrest at G2/M phase in a dose-dependent manner [25], resulting in decreased cell adhesion and morphological changes in HGFs [26]. Cyttoplasmic organelles are also influenced by those aldehydes [27, 28]. It can be suggested that nicotine acts on lysosomes by lowering the inner pH or by enlarging them due to endocytosis in gingival fibroblasts [9].

Nicotine influences fibroblast viability and growth, depending on the concentration as well as duration of cell exposure. [29,30] In fact, nicotine by itself is toxic only at concentrations higher than that found in plasma and crevicular fluid of heavy smokers (more than 15 cigarettes per day) it is suggested that the low concentration of nicotine (800 ng/ml) could increase the toxicity of some periodontal anaerobes, and it is probably in synergistic action with some substances that nicotine can develop a toxic effect [29]. Heme-oxygenase 1 (HO-1) was found to be up regulated by nicotine in HGFs. Thus, HO-1 expression might be a signal pathway linked to the induction of stress response protein by cigarette smoking. In addition, the regulation of HO-1 expression induced by nicotine is critically dependent on the intracellular glutathione (GSH) concentration [31]. The sulfhydryl-group-containing tripeptide constitutes a first defense intracellular antioxidant that serves many important biological functions. It helps maintain membrane integrity, optimal transport of amino acids, and enzyme activity. In addition, GSH has been documented as having regulatory effects on cell proliferation [33]. Exposure of HGFs to cigarette smoke decreased cellular protein thiols and rapidly depleted intracellular GSH, with a minimal increase in the intracellular levels of glutathione disulfide and G-glutathionylated proteins, as well as total glutathione levels. Mass spectrometric analyses showed that total GSH consumption is due to the export by the cells of GSH-acroleine and GSH-crotonaldehyde adducts [34]. It is suggested that GSH depletion could be a mechanism for cigarette smoke induced cytotoxicity [30, 34], and could be prevented by addition of extracellular GSH [35] or by factors which are able to induce GSH synthesis by human PDLF. It was demonstrated that 2-o xoethoxazolidine-4-carboxylic acid (OT2) and buthionine sulfoximine (BSO) pretreatment reduced c-fos mRNA levels after exposure to nicotine. These results suggest that intracellular thiol pools can modulate c-fos gene induction by nicotine [30]. These findings may be used for further chemoprevention of cigarette smoking-related periodontal diseases. [35]

In addition, GSH is able to inhibit the isomerase enzyme responsible for converting prostaglandin H2 (PGH2) to prostaglandin E2 (PGE2) [36], Prostaglandin (PG) products as a result of cyclooxygenase-2 (COX-2) expression are an important factor in the pathogenesis of the chronic inflammatory disorder. This might partly explain why nicotine cytotoxicity is not directly mediated by its induction of COX-2 [37]. GSH also plays a role in cellular protection from damage produced by free radicals and electrophiles. Nicotine may play a role in smoking associated periodontitis via the activation of COX-2, which is augmented by oxidative stress and mediated by extracellular signal regulated protein kinase signaling [38]. Two major phases were identified in whole cigarette smoke, a complex mixture of over 7000 chemical compounds: a tar phase and a gas phase. Both phases are rich in reactive oxygen species (ROS) and reactive nitrogen species [39]. It was estimated that a single cigarette puff contains approximately 1014 free radicals in the tar phase and 1015 radicals in the gas phase [40]. Nicotine might possess anti-proliferative activity and be a potential contributor to cell death in HGF through ROS generation and caspase-3 dependent pathway [41].

Nicotine also increases chromogranin A (ChGA) production by human periodontal ligament-derived fibroblasts (HPdLF) [42], which is a stress marker produced by neuroendocrine system cells in response to mental stress [43]. ChGA is a physiologically active substance, serving as an index of autonomic nerve system activity. It is also related to the systemic and local immunomodulation systems, being involved in antimicrobial peptide production, mast cell migration, leukotriene and prostaglandin production, and histamine release [44]. ChGA is also associated with periodontal and smoking-related airway diseases, and is abundant in saliva and the blood of patients [45]. Nicotine increased the production of connective tissue growth factor (CCN2/CTGF) protein in both gingival, and periodontal fibroblasts and promoted via this effect the periodontal fibrosis [46]. Clinically, it has been widely recognized that fibrosis is frequently observed in the smoker’s gingiva. The elevated COX-2 expression is well recognized as having a potential role in contributing to the progress of head and neck cancer through several biological pathways [47].

Betel nuts contain several alkaloids, of which arecoline is the most abundant. It is suggested that arecoline-induced Interleu kin-1 beta (IL-1β) from the oral mucosa may enhance more COX-2 induction in human gingival fibroblasts via Ca2+ mobilization in long-term betel quid chewing [48]. People who combine habits of betel nut chewing with cigarette smoking could be more susceptible to damage of the periodontium than faces of nut chewing alone [49]. The synergistic effects of alcohol drinking, betel chewing, and cigarette smoking on the risk of oral precancerous lesions, such as leukoplakia and erythroplakia was shown [50]. This may explain the higher predisposition of tobacco users to oral infections and diseases such as cancer.

In conclusion, the exposure of human oral fibroblasts to nicotine and some other tobacco compounds, as well as to whole tobacco smoke, influences fibroblasts function, including proliferation and adhesion to root surfaces, and can potentially be cytotoxic. This may explain the increased incidence and severity of periodontal diseases in chronic tobacco users, but also indicates possibilities for prevention and treatment of the side effects of tobacco smoke in periodontium in the future.

References