Immunohistochemical aspects of apoptosis in gingival mucosa with papilloma and condyloma acuminata

MONICA SCRIECIU¹, VERONICA MERCU⁴, RĂZVAN MERCUT², MARINA OLMPIA AMĂRĂȘCU³, SANDA MIHAELA POPESCU⁴, ANCA MIHAELA PREDESCU⁵, ILEANA MONICA BANIŢĂ⁵

¹Department of Prosthetic Dentistry, University of Medicine and Pharmacy of Craiova, Romania
²Department of Plastic Surgery, University of Medicine and Pharmacy of Craiova, Romania
³Department of Dental Technology, University of Medicine and Pharmacy of Craiova, Romania
⁴Department of Oral Rehabilitation, University of Medicine and Pharmacy of Craiova, Romania
⁵Department of Histology, University of Medicine and Pharmacy of Craiova, Romania

Abstract

The oral mucosa is a component of the oral ecosystem, which can be aggressed by corrosion products released from the dental alloys used in prosthetic dentistry therapy. The purpose of this study was to compare the in vivo effect of nickel and copper compounds on the oral mucosa cells, including their ability to induce cell death, by analyzing the cytochrome c (cyt. c) immunohistochemical expression. Gingival mucosa fragments obtained from the subjects with dentures manufactured by nickel or copper casting alloys were processed through the histological technique of paraffin inclusion. The sections obtained were stained by usually histological methods in order to highlight the histopathological lesions and also analyzed using the immunohistochemical technique in order to study the cyt. c expression. The papillomatosis lesions were observed in the gingival mucosa fragments obtained from the subjects with nickel-based alloy dentures and the condyloma acuminata lesions were observed in those obtained from the subjects with copper-based alloy dentures. The cyt. c immunohistochemical expression was different in the epithelial layer of two types of mucosal fragments but it was the same in their lamina propria connective tissue. We can conclude that the two types of metal alloys have different effects on the adjacent gingival mucosa.

Keywords: gingival mucosa, papilloma, condyloma acuminata, apoptosis, cytochrome c.

Introduction

The oral ecosystem contains an important heterogeneity of tissue types such as mucosa, the gums, the teeth and the tongue and also biological fluids such as the saliva and the crevicular fluid. Each of these represents ecological niches, which are available for colonization by oral microorganisms [1]. In the oral ecosystem, there are rapid changes in temperature, changes in the composition and pH of saliva, variations of microbial saprophyte flora. From time to time, these changes occur in the same individual, but differ from one individual to another.

The metal dental alloys used in prosthetic dentistry therapy are placed in the oral cavity for a long period of time, for years or even permanently. A protective oxide film is formed on the surface of the metal dental prosthesis inserted into the mouth to stop its corrosion [2]. The oxide film can be altered when changes occur in the oral ecosystem [3]. The metal alloys may suffer various forms of corrosion [4, 5] releasing elements such as nickel, chromium or copper [2] and [6]. The biochemical, bio- or microbiological corrosion of metal alloys is due to the metabolic activity of microorganisms using the metal as the culture medium. Bio-corrosion usually occurs in metallic structures, in contact with backwaters or implanted in living organisms [7].

The corrosion of dental metal casting alloys placed into the oral cavity in the watery saliva is a continuous one. Therefore, the release of corrosion products is carried out for a long period [8].

The adverse effects of the oral mucosa upon the dental metal alloys are probably due to the release of metal ions by corrosion [9]. The toxic and carcinogenic effect of nickel compounds, including their oxidative mechanisms on human and animal cells has been well documented [10, 11]. Many studies have shown that copper oxide nanoparticles can induce cytotoxicity, DNA alterations and apoptotic events in human cell cultures [12, 13].

Apoptosis is an essential (last) phase in the life cycle, a process that removes unwanted cells. The dysregulation of apoptosis highlights many diseases and physiopathological alterations.

When the cells detect the apoptotic stimuli, such as DNA damage or metabolic stress, the intrinsic apoptotic pathway is triggered and cyt. c is released from the mitochondria into the cytosol where are engaged to a strategic combat to promote or to counteract the activation of caspases and cell death [14].

The purpose of this study was to compare in vivo effect of nickel and copper compounds on the oral mucosa cells, including their ability to induce cell death, by studying immunohistochemical expression of cyt. c.

Materials and Methods

Subjects

The participants in the study were selected from those who came in the Prosthetic Dental Clinic within the Faculty of Dentistry, University of Medicine and Pharmacy of...
Craiova, Romania, between July 2013 and June 2014. They were informed about the study and signed an informed consent. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova. The inclusion criteria: adult subjects, aged over 21 years, who had fixed dentures older than five years. The fixed dentures of the study participants were made of nickel-based metal alloys and copper-based metal alloys.

The exclusion criteria: there were excluded the smoking participants, those with allergic disorders, those with associated diseases of the oral mucosa, those with general diseases and those who worked in a toxic environment.

Nine participants of both sexes aged between 47 and 63 years were selected from rural and urban areas. The period of using fixed dentures in the studied subjects was between 7 and 15 years. Three-study participants worn nickel-based alloy fixed dentures and six subjects worn copper-based alloy fixed dentures. The selected subjects presented diseases at least one tooth of a fixed denture. From the subjects that required the extraction of one of the fixed denture teeth, the fragments of gingival mucosa were obtained through excision. The excision of gingival mucosa was performed to smooth the edges of the extraction wound and to prevent the extraction complications. The gingival mucosa was in contact with the metal of the fixed dentures before being removed.

Methods

The gingival mucosa fragments were fixed in 10% formalin and then were processed through the histological technique of paraffin embedding.

For the histological examination, the obtained sections were stained with Hematoxylin–Eosin (HE) and examined by light microscopy.

Immunohistochemistry

Serial sections were dewaxed and incubated for 30 minutes with 0.3% hydrogen peroxide in methanol, in order to block the endogenous peroxidase. Blocking of non-specific binding was made with non-immune serum 1:75 diluted in phosphate-buffered saline (PBS) for 20 minutes at room temperature. Sections were incubated with the primary mouse monoclonal antibody anti-cytochrome c 1:50 (Santa Cruz Biotechnology Inc.), in a moist Chamber for 12 hours at 40C.

With the primary antibody, the fragments of gingival mucosa were obtained through excision. The excision of gingival mucosa was performed to smooth the edges of the extraction wound and to prevent the extraction complications. The gingival mucosa was in contact with the metal of the fixed dentures before being removed.

Table 1 – Cytochrome c immunohistochemical expression in gingival mucosa with papillomatosis

<table>
<thead>
<tr>
<th>No.</th>
<th>Histological localization</th>
<th>Antigen type</th>
<th>Highlighted cells</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gingival epithelium</td>
<td>Cytochrome c</td>
<td>Apoptotic cells</td>
<td>• absent reaction in the superficial and basal epithelial layers; • positive reaction in the intermediate epithelial layer with increased intensity in the upper areas.</td>
</tr>
<tr>
<td>2.</td>
<td>Lamina propria</td>
<td>Cytochrome c</td>
<td>Apoptotic cells</td>
<td>• inconstant positive reaction in the deep lamina propria.</td>
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Table 2 – Cytochrome c immunohistochemical expression in gingival mucosa with condyloma acuminata

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<tr>
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Figure 1 – Gingival mucosa papillomatosis. HE staining, ×100.

Figure 2 – Gingival mucosa condyloma acuminata with koilocytes in an epithelial area located in the connective tissue. HE staining, ×100.

Figure 3 – Papillomatosis gingival mucosa with apoptotic anucleate cells, ×200.

Figure 4 – Papillomatosis gingival mucosa with positive apoptotic reaction in the connective tissue of papillae and in the superficial lamina propria, ×200.
Anucleated cells with positive reaction for cyt. c and localized in the intermediary layer were found in the epithelium of papillomatosis gingival mucosa fragments. The number of those cells was higher towards the top of the intermediary epithelium layer (Figure 3). The apoptotic intensely positive reaction was observed in the connective tissue of the papillae and in the superficial lamina propria. An unsteady apoptotic positive response and numerous angiogenesis phenomena were observed in the deep lamina propria (Figure 4).

Acanthosis and an intense positive reaction for cyt. c were observed in the upper intermediary epithelium of gingival mucosa fragments with condyloma acuminata. Apoptotic cells were distributed throughout the thickness of the epithelium (Figure 5). The apoptotic cells with different morphological aspects have been observed in the same epithelial layer: cells with cytoplasmic negative reaction for cyt. c, cells with peripheral chromatin condensation process, cells with degradation of the nuclear membrane and with apoptotic intracytoplasmatic corpuscles and cells with diffusely aspect. Regarding the shape, round, oval and fusiform apoptotic cells were observed. The cells with positive reaction for cyt. c, have had variable sizes, especially those of cells with fusiform shape. There were highlighted large round cells with cytoplasmic response for cyt. c and small round cells with intense reaction for cyt. c in the cytoplasm and nucleus. An intense response for cyt. c was observed in the connective tissue of the papillae (Figure 6).

Discussion

Exposure of the gingival mucosa to high concentrations of nickel or copper ions and corrosion products is a mechanism of aggression.

It has been demonstrated that nickel ions can penetrate the cells in a variety of ways [15, 16], to mix with certain biological components [10] in order to alter the function, morphology and ultrastructure of certain cells [17].

In contrast to the uptake of nickel by inhalation and ingestion, the dermis absorption is neglected because nickel ions do not penetrate the intact skin. Soluble nickel salts produced because of corrosion of nickel based-metal alloys may cause dermatitis, by percutaneous absorption [18]. Because both dentures and their adjacent gingival mucosa coexist in the oral cavity, the aggression elements of mucosa are most likely due to bio-corrosion compounds of dental metal alloys. Wataha et al. (2001) showed that the distribution of nickel in the tissues was better correlated with pronounced tissue inflammation [19]. Oral keratinocytes are the first tissue target of elements released from dental metal alloys [20]. Maintaining permanent fixed prosthesis in the oral cavity allows the accumulation of these compounds into the surrounding tissues. Therefore, all gingival mucosa structures can be altered. Released nickel ions from the dental metal alloys can have toxic, carcinogenic or immunological effects on the soft tissue of the mouth, due to the placement of these alloys in the oral cavity for a long period of time [21]. Numerous studies have investigated the in vivo biocompatibility of fixed orthodontic appliances, assessing the presence of metal ions in the cells of the oral mucosa, cytotoxicity and their potential genotoxic effects. One of these studies confirmed that nickel and cobalt released from fixed orthodontic appliances can induce DNA damage in the oral mucosa cells [22]. Other studies have shown that the corrosion products released from nickel-based alloys could not modify the morphology and viability of cultured human gingival fibroblasts. However, these products caused reductions of cell proliferation. The accelerated corrosion potential and local and systemic exposure of tissue to high levels of corrosion products raises the biocompatibility of these alloys [23]. According with results of this study, similar morphological alterations of the gingival paraprosthetic mucosa were mentioned in our previous studies [24].

Regarding the cytotoxic effect of copper on fibroblasts, Cao et al. showed that they become round and eventually detached from the surface of TCP due to the toxicity of copper [25]. Copper-based dental alloy or elements released from these are involved in some allergic reactions such as hypersensitivity reactions or in local chronic toxic reactions [26, 27]. These toxic effects may occur due to a repeated or continuous influence of toxic agents in low concentrations for long periods of time. These reactions are most often located in the area of contact with...
the toxic agent [28]. The occurrence of the corrosion phenomena of copper-based alloys induces an in vivo toxicity, and the metal surface becomes rough with pits and cracks [29].

Corrosion products of copper aluminum alloys are easily removed from the oral cavity through teeth brushing. Benatti et al. reported that copper-aluminum alloys suffered corrosion in sulfide solutions and in the oral cavity with poor oral hygiene [30]. Therefore, poor oral hygiene promotes bio-corrosion of copper alloys.

The comparative study of gingival mucosa fragments with papillomatosis and condyloma acuminata showed that the distribution of koilocytes was different in the epithelium layers. Tovaru [31] and Staniceanu et al. [32] have noted that the last years, research increasingly emphasized the role of human papillomavirus (HPV) in the appearance of condyloma acuminata lesions. Tovaru [31] indicated that the types 6 and 11 of HPV are responsible for the appearance of oral condyloma acuminata.

Rihet et al. [33] showed that the HPV role in causing nuclear damage to the host cells has not been yet defined. The authors showed that HPV viral proteins can bind and inhibit the function of tumor suppressor genes such as p53 proteins. These phenomena can lead to disruption of the cell cycle and so, there results instability of the cellular genome of the host cells.

Apoptosis or programmed cell death is a normal physiological process, in order to maintain tissue homeostasis [34]. There are two types of apoptosis: extracellular apoptosis, determined by extracellular factors, and intracellular apoptosis due to the intracellular factors. Intracellular induced apoptosis is a reaction initiated by the cell in response to stress [35, 36]. Chronic irritation of the cells produces increased levels of various reactive oxygen species (ROS). ROS increases the expression of NADPH oxidase, considered to be a proapoptotic enzyme. Moreover, increased ROS causes the release of apoptotic intracellular signals and formation of apoptotic proteins targeting the mitochondria and the cyt. c releasing into the cytoplasm. Therefore, cyt. c leads to the activation of caspase-3, a protein that plays an important role in the execution of apoptosis and cancer incidence [37–39]. The mitochondrial membrane permeabilization (MMP) was proposed as a "point-of-no-return" during various types of cell death [40]. Both the outer mitochondrial membrane and the inner mitochondrial membrane contribute to MMP [41] and cause the release of cyt. c, apoptosis inducing factor (AIF) and G endonuclease, which are normally located in the mitochondrial intermembrane space [42]. Recent studies showed toxic effects of nickel oxide nanoparticles (NiO NPs) on bacteria and microalgae [43, 44]. NiO NPs induce intracellular production of ROS in dose-dependent manner [45]. Cyt. c increasing in the cell cytosol after nickel treatment was demonstrated by Western blot analysis of cytosolic and mitochondrial fractions. Simultaneously, it was observed a decrease of cyt. c in the mitochondrial fraction indicating a dose-dependent release of cyt. c [46]. Also, a linear increase in the apoptotic cell population was observed with increasing concentrations of copper ions in cultured fibroblasts [25].

In this study, a highly positive apoptotic reaction was observed in the superficial epithelial intermediary layer, in the connective tissue of papillae and in the superficial lamina propria, for both types of gingival mucosal fragments.

Anucleated cells with cytoplasmic positive reaction for cyt. c were observed in the epithelium gingival mucosa with papillomatosis. It is significant that in this case the apoptotic stage of mitochondrial membrane permeabilization is not based on the presence of the nucleus. These data suggest that the phases of decision on life/death cell are clearly different from those that determine cell cycle progression [47]. The process of apoptosis is asynchronous in studied gingival fragments with condyloma acuminata. This idea is supported by the fact that there were detected cells with a negative reaction for cyt. c, cells with positive reaction for cyt. c and high variability of sizes and shapes and various nuclear damage. The same aspects were pointed out in our previous study [48].

These observations lead to the idea that although the apoptosis process is triggered by the same mechanisms, in the same areas of the gingival mucosa, the speed of the apoptotic events is different. If in the papillomatosis of gingival mucosa, in the upper intermediate epithelial layer, the apoptotic cells do not present a nuclear structure, in the case of gingival mucosa fragments with condyloma acuminata there were observed koilocytes and apoptotic cells with various stages of nuclear degradation.

Conclusions

In the gingival mucosa adjacent to fixed dentures made of nickel or copper-based alloys there were observed papillomatosis or condyloma acuminata lesions. In the gingival mucosa fragments with papillomatosis, there was developed a nuclearly independent apoptotic process. In the gingival mucosa, fragments with condyloma acuminata there were highlighted apoptotic cells with variable morphological aspects and various sizes in different stages of evolution. In both types of gingival mucosa fragments, the apoptotic reaction was intensely positive in the connective tissue of the papillae and in the superficial areas of lamina propria.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All authors have equally contributed to this paper.

References


Corresponding author
Monica Scrieciu, Associate Professor, MD, PhD, Department of Prosthetic Dentistry, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40723–516 539, e-mail: scrieciu_monica@yahoo.com

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