Abstract

Gingival overgrowth is a pathological manifestation related to some systemic diseases or to the administration of certain drugs, such as calcium channel blockers (nifedipine).

The aim of this study was to determine the role of CTGF, Cyr61, ornithine decarboxylase and NF-kB cell growth factors in nifedipine-induced GINGIVAL OVERGROWTH. A 50mg/body weight dose of nifedipine (Adalat) was administered to rats, by subcutaneous injections 6 days a week for 45 days. The collected tissues were examined both microscopically and immunohistochemically. The identified histological changes consisted in epithelial hyperplasia, enlarged gingival lamina propria with or without collagen deposition in the subepithelial lamina propria, decreased cellularity and rich microvascularization. The qualitative assessment of growth factors suggested that, in the development of the GO-associated tissue, changes in these factors are expressed in excess or inhibited (ornithine decarboxylase), as compared to the control tissues.

Keywords: gingival overgrowth, nifedipine, growth factors

INTRODUCTION

Gingival overgrowth is the accepted and used term to define the increase in the gingiva size, a common manifestation of several gingival disorders. It is a clinical finding, ignoring the pathological connotations of other terms, such as gingival hypertrophy or gingival hyperplasia. Hyperplasia is an exaggerated increase in size of a tissue, due to the increase in the number of cells, with no change in their size. Hypertrophy is the enlargement of a tissue, due to an increased size of its constituent cells. By its anatomic-pathological substratum and mechanism, gingival overgrowth can be considered a particular form of gingival disorders (1).

Various immunohistochemical studies have shown that nifedipine (calcium channel blocker) could be involved in gingival overgrowth, as type I collagen was found in excess in the gingival connective tissue of the tested animals (2). Other investigations have shown that GINGIVAL OVERGROWTH is not caused by the excessive synthesis of type I collagen, but by a decrease in its degradation in the gingival connective tissue, due to decreased phagocytosis at fibroblasts level (3), when their proliferation is not affected by nifedipine (3).

The pathogenesis of drug-induced gingival overgrowth is not well known, but it seems to be caused by a disbalance between collagen synthesis and degradation in the gingival connective tissue (4).

Recent studies have drawn attention on the involvement of some growth factors (Cyr61, CTGF) (5), enzymes (ornithine decarboxylase) (6), or proteins, acting as a transcription factor (nuclear factor kB-NF-kB) in cell proliferation and cell differentiation processes or in the inflammatory response, known as active mechanisms in gingival overgrowth (7).

The aim of the present study was to identify the growth factors CTGF, Cyr61, ornithine decarboxylase, and NF-kB in gingival overgrowth in the rats treated with nifedipine. The expression of ornithine decarboxylase, as an indirect measurement of polyamine synthesis, was investigated. The assumption is made that drug administration is a stress for any tissue which is not a therapy target, and that the increased ornithine decarboxylase cell levels, and implicitly, the polyamine levels, could represent an attempt made by the affected tissue of protecting itself.
MATERIALS AND METHOD

The study included 2 series of 5 adult, male Wistar rats (Rattus norvegicus albinus) of equal weight. Rats in series I received a subcutaneous injection of nifedipine (Adalat), at a dose of 50mg/kg body weight once a day, 6 days a week, for 45 days. Rats in series II were injected a saline solution. The rats were weighed every week, in order to adjust the nifedipine dosage according to weight.

When the nifedipine and saline solution administration interval was over, the animals were slaughtered by intraperitoneal administration of a sodium thiopental dose. From each animal, the mandibular area that supports the two lower incisors (the surrounding gingival tissue included) was collected. The fragments were processed according to the standard protocols for pathological examination. The microscopic sections were stained with HE for a primary qualitative assessment, then stained for immunohistochemistry.

The antibodies used in the “antigenic exposure” experiment were: rat anti-CTGF mice antibody, rat anti-Cyr61 rabbit antibody, rat anti-ornithine decarboxylase rabbit polyclonal antibody, rat anti-NF-kB rabbit polyclonal antibody, “Dako” antigenic exposure solution, Dako EnVision+Dual link System-HRP (DAB+) development kit.

RESULTS

Histologically, the changes identified in the gingival tissues of the animals treated with nifedipine consisted in epithelial hyperplasia, increased size of the gingival lamina propria, without deposition of collagen in the subepithelial lamina propria, a reduced number of cells and a rich microvascularization (fig. 1).

The morphological aspects have revealed stratified, parakeratinized squamous epithelium, areas of hyperplasia alternating with normal areas, and lamina propria with a chronic inflammatory infiltrate with mononuclear elements of lympho-plasmocytic type. At the level of the epithelio-connective interface, deep and interdigitate epithelial crests (“glove fingers”), determined by the proliferation of the epithelial basal layer, that invade lamina propria, going deep into the connective tissue, were found. Also present was a chronic inflammatory infiltrate occupying the entire lamina propria, suggesting a micronodular organization (fig. 2).

The gingiva of nifedipine-treated animals was characterized by an enlarged lamina propria, highly reduced cellularity (fig. 3), absence of collagen deposition in the subepithelial area and a rich vascular network.

Compared to the control animals (fig. 4), the tissues collected from the nifedipine-treated rats presented evident changes, characteristic to the occurrence of epithelial hyperplasia.

CTGF was identified both in the gingival epithelium and at the level of lamina propria in all nifedipine-treated rats (fig. 5). The immunohistochemical findings show that the molecule was located within the cell, disposed diffusely in the epithelial and endothelial cells and fibroblasts, but also in the extracellular matrix, probably associated to its components, deposited in excess during the fibrosis process. The cells of the gingival epithelium are positive for CTGF expression, and the intensity of staining varies from case to case in the same animal series. Differences in the intensity of staining were even found in the epithelial cells of the same tissue, suggesting the heterogenicity of these molecules expression.
The Cyr61 expression of the series here under study partially replicates that of CTGF. It was found out that the epithelial and endothelial cells and only a small number of fibroblasts were positive for Cyr61. The gingival epithelium of the control rats was slightly positive or negative for this growth factor (fig. 6), while the intensity of staining in nifedipine-treated rats was significantly increased (fig. 7). However, the absence of signals at the level of extracellular matrix suggests that this growth factor has only an intracellular location, which differentiates it from CTGF. The Cyr61 expression mainly involves the endothelial cells, an aspect present in all analyzed animals, thus supporting the literature data suggesting an autocrine intervention of Cyr61 on vascularization. The major effect of this action is stimulation of endothelial growth and formation of new vessels, the direct result being cell proliferation at this level (epithelial, endothelial, fibroblasts, inflammatory cells). This accounts for the rich vascularization observed in the gingival lamina propria of nifedipine-treated rats, compared to the controls.
The presence of ornithine decarboxylase in the gingival epithelium and in the endothelial cells and fibroblasts of lamina propria (fig. 8) in the control rats was determined immunohistochemically. The obtained signal demonstrates the cytoplasmic location of ornithine decarboxylase.

The gingiva of nifedipine-treated rats showed varied immunohistochemical aspects. The epithelial cells have a negative immunomarking, their number varying. The endothelial cells in the gingival lamina propria of all investigated animals express ornithine decarboxylase in a number significantly varying from one case to another (fig. 9).

The number of positively immunomarked endothelial cells in the nifedipine-treated rats is distinctly more reduced than in the controls.

Identification of the NF-kB expression in the experimental animals revealed interesting aspects. The tissues of the control rats showed an intense epithelial staining, while the cytoplasmic location of the signal suggested that the molecule was trapped into the cytoplasm in its inactive form, being linked to IkBs inhibitors. Few cells of the normal gingival epithelium show nuclear staining (fig. 10), which demonstrates both that most epithelial cells are not activated, and also the presence of a small number of cells proliferating in the physiological process of epithelial renewal.

The nifedipine-treated rats presented NF-kB expression in the nucleus, which suggests that, in these cells, the molecule was translocated into the nucleus following cell activation. Staining involved a relatively high number of epithelial cells, while the presence of this molecule in the nucleus suggested an activated status of the epithelium (fig. 11).
DISCUSSION

CTGF and Cyr61 molecules are cysteine-rich proteins, belonging to the growth factors that play an active role in the regulation of apoptosis, angiogenesis, and cell proliferation; also, CTGF seems to stimulate the synthesis of the extracellular matrix constituents, such as type I collagen (7). Numerous studies have demonstrated CTGF overexpression in the disorders accompanied by fibrosis (12).

Variation - in the presence of CTGF - in the gums of the control rats is extremely high. Thus, four of the five rats in the control series did not present immunofixation at gingival epithelium level, but only at the level of some capillary endothelia in lamina propria while, in the remaining rat, a weak signal was obtained in both gingival epithelium and endothelium. On the other hand, CTGF was identified in both the gingival epithelium and lamina propria in all nifedipine-treated rats.

The signals obtained by immunohistochemistry indicate an intracellular location of the molecule disposed diffusely in the cytoplasm of the epithelial and endothelial cells and fibroblasts. At the level of the extracellular matrix, CTGF is probably, associated to its components, deposited in excess during the fibrosis process, a result agreeing with the findings of other authors (13). The cells of gingival epithelium were positive for CTGF expression, the intensity of immunomarking varying from animal to animal even within the same animal series.

One of the possible mechanisms involved in the development of fibrotic pathology could be the process known as epitheliomesenchimal transition (7), (14).

Gingival overgrowth is accompanied by the "glove finger" histopathological aspect characteristic to hyperplasia, demonstrating the invasion of lamina propria by epithelial cells.

Related to our findings, but also with the conclusions of other authors (7), (13) on the overexpression of CTGF in the epithelium of the gums affected by gingival overgrowth, the above-observation suggests that the epitheliomesenchimal transition process could be involved in gingival fibrosis. CTGF is able to interact with various types of cells, causing different effects.

CTGF also stimulates the synthesis of extracellular matrix molecules, such as type I collagen and fibronectin, evidencing the very important role it plays in the enlargement of gingival lamina propria and in the occurrence of fibrosis (15, 16). In conclusion, it may be ascertained that CTGF plays an extremely important role in the pathology of gingival overgrowth.

As, under physiological condition, the Cyr61 molecule is not detected in epithelial but only in endothelial cells, fibroblasts, and smooth muscle cells, it comes out that gingival overgrowth is a pathological condition associated with the epithelial expression of Cyr61, and that this might be due to some cytokines, such as TGF-ß1, the secretion of which is known to be amplified by nifedipine administration (17).

The inflammatory reaction responsible for the tissue changes associated with nifedipine-induced gingival overgrowth does not seem to be accompanied by increased ornithine decarboxylase expression levels, as expected according to literature data (18). Moreover, several studies demonstrated the stimulating effect of TGF-ß1 on ornithine decarboxylase and polyamine expression (19).

Identification of NF-kB expression in the study on rats revealed interesting aspects. The tissues of the control rats presented and intense
immunohistochemical reaction in the epithelium, while the cytoplasmic location of the signal suggests that the molecule was entrapped in the cytoplasm in its inactive form, linked to IkBs inhibitors. Few cells in the normal gingival epithelium present nuclear staining. This aspect demonstrates the absence of activation of most epithelial cells and the presence of a small number of proliferating cells during the physiological process of epithelial renewal (20).

The nifedipine-treated rats present NF-κB expression at nuclear level, which suggests that, in these cells, the molecule was relocated into the nucleus following cellular activation. Immunofixation involves a relatively high number of epithelial cells, and the presence at nuclear level of this molecule suggests an epithelial activated status.

Based on these results, we could speculate that, possibly, in gingival overgrowth, too, the main cell activator, and, implicitly of NF-κB, is TGF-β cytokine. High TGF-β levels, nifedipine-induced in gingival tissues, are responsible for the uncontrolled activation of the epithelial cells and fibroblasts. Excess proliferation of these cells, mediated by NF-κB, could be accompanied by its stimulating effect upon their survival. Cummulated NF-KB effects could be responsible, at least in part, for the tissue changes associated to gingival overgrowth.

CONCLUSIONS

The results of the present study suggest that during events leading to the occurrence of tissue changes associated with gingival overgrowth, the molecular factors are expressed in excess, compared to the control tissues (CTGF, Cyyr61, NF-κB). An exception is ornithine decarboxylase molecule, the expression of which is inhibited, so that ornithine decarboxylase remains in inactive form within the cytoplasm.

By their mediated effects, the investigated molecules seem to be directly or indirectly involved in the pathogenesis of this condition.

Thus, we could state that the gingival tissue changes in gingival overgrowth associated to nifedipine administration are the result of some perturbations in the physiological mechanisms involved in preserving tissue homeostasis at this level. Such perturbations can be induced directly by the administered drugs or may occur as indirect effects.

The observed heterogeneity of the expression of the molecules under study, even in the same animal series, demonstrates that the perturbations at tissue level may have different intensities, probably as a result of some individual particularities, which could result in the occurrence of some clinical manifestations that might explain the varying percentages of the individuals developing gingival overgrowth following drug administration.

References


