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Expression of parathyroid hormone-related protein in human inflamed dental pulp

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Abstract. – *Introduction:* **The aim of this study was to investigate the presence and the role of the parathyroid hormone-related protein (PTH-rP) in the inflamed pulp.**

Materials and Methods: **Thirty-four pulp tissue specimens (24 inflamed and 10 normal pulps) from extracted third molars were studied. The presence of PTH-rP was observed by using immunohistochemistry. Negative controls were performed using non immunized rabbit or mouse serum, omitting the primary antibody.**

Results: **The analysis of all the sections of normal pulps showed the presence of PTHrP positive cells only in the odontoblastic zone and in few fibroblasts. Instead all inflamed pulps showed PTHrP positive cells both in vascular zone and in pulp stroma, as well as in the odontoblastic and subodontoblastic zone.**

Conclusion: **Several works proved that this peptide plays a role even in angiogenesis process, but its function is controversial. It is possible to hypothesize that PTHrP stimulates angiogenesis, but it is recommended to further conduct research on this area.**

Key Words:

Dental pulp, Inflammation, Parathyroid hormone-related protein.

Introduction

Pulpitis is a dental inflammatory process; its principal cause is the action of pathogenic microorganisms[1]. Pulpitis can be reversible or irreversible. In reversible inflammation, the principal process is a modest increase in the activity of the blood vessels. In irreversible inflammation, pulpal tissues react to these challenges by increasing the activity of nerves, blood vessels and immune system, by chemotactic stimuli, and interstitial fluid turnover[2]. An angiogenesis role is hypothesized during the inflammation: the development of a new vascular network is essential for the onset and progression of many pathophysiological processes, like neoplasias and inflammation[3].

Many extracellular factors such as growth factors (cytokines), proteases, peptides and hormones are implicated in the inflammatory and immunological aspects of dental pulp inflammation and repair[4].

Parathyroid hormone-related protein (PTHrP) is a peptide revealed during a search for the circulating factor secreted by cancers in the humoral hypercalcemia of malignancy[5].

The structure of PTHrP gene is very similar to PTH gene. It appears from the nucleotide and amino acid homology that they share in the NH_2-terminal region.

In humans, the PTHrP gene is located on the short arm of chromosome 12, and the PTH gene is positioned on an analogous region of the short arm of chromosome 11. The gene codes for three separate isoforms through production of different 3' transcripts; 1-139, 1-142 and 1-173. The 1-173 isoform is present only in humans[6].

In adult life this hormone is present in many tissues: skin, mammary glands, uterus, oviduct, urinary bladder, gastrointestinal tract, vascular system, heart coronary circulation, lung, kidney, liver and biliary tree, central nervous system.

In particular, in the vascular system, PTHrP is a potent hypotensive peptide. The relaxant effect of PTHrP on vascular smooth muscle is endothelium independent. In fact this peptide has been repeatedly identified in arterial smooth muscle studying whole artery segments, endothelium-stripped arterial segments, and isolated vascular smooth muscle cells[7,8].

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Serum and vasoconstrictors such as angiotensin II, endothelin-1 bradychinin and thrombin can induce PTHrP mRNA and its protein. The major serum factor responsible for the induction of PTH-rP is angiotensin II. It has been reported that this factor induced PTHrP expression by transcriptional as well as mRNA stabilization mechanisms[9].

Angiotensin II plays also a role in the initial destabilization of endothelium during the angiogenesis. In fact Angiotensin II induces the expression of the Tie2 receptor ligand, angiopoietin-2 and important angiogenetic factor[10]. Even some pro-inflammatory cytokines, like the tumour necrosis factor α (TNF-α) and interleukin 1β (IL-1β), can increase the production of PTHrP.

Many studies supposed that PTHrP plays a role even in normal and tumor angiogenesis[11]. In fact, this protein, produced by tumour cells, influences homeostasis of their microenviroment, particularly targeting neighbouring endothelial cells, and promotes tumor neoangiogenesis. A study reported that PTHrP is produced by endothelial cells but its action was only on vascular smooth muscle[12].

The aim of this study was to investigate by himmunohistochemistry the presence of PTHrP in the dental pulp and its cellular localization, so to suppose a role of this peptide in the regulation of vascular compartment during pulp inflammation.

Materials and Methods

Subjects

Thirty-four third molars were extracted from 27 patients, 24-35 years old, at the Clinic of Dentistry of Catholic University of the Sacred Heart, Rome, (Italy). Medical histories of all patients in this study were non-contributory. Thirty-four pulp tissue specimens (24 inflamed and 10 normal pulps) were obtained. Inflamed pulp were selected from patients, with third molar caries, who showed spontaneous, pulsating, diffuse pain and lingering ache in response to cold and heat stimulus. Asymptomatic pulps were obtained from no carious teeth as normal controls.

Immunohistochemistry

Every tooth was extracted after using anesthetic (mepivacaine 3%). Immediately after extraction, each dental apex was cut and the remaining part of each tooth was fixed with phosphate-buffered formalin, pH 7.2, for 4 to 18 hr. Then the teeth lengthways were halved using a carborundum disc under water irrigation. After this, we extracted carefully the dental pulp using an explorer and a dental forceps. Pulp tissue was paraffin-embedded according to standard procedures. Four-µm-thick tissue sections of the coronal pulp were collected on 3-aminopropyltriethoxy-silane (Sigma Chemicals; Milan, Italy) or on naturally charged slides (Dako; Milan, Italy), allowed to dry overnight at 37C to ensure optimal adhesion, dewaxed, rehydrated, and treated with 0.3% H_2O_2 in methanol for 10 min to block endogenous peroxidase. For antigen retrieval, the sections were microwave treated in 0.01 M citrate buffer at pH 6.0 for 10 min and allowed to cool for 20 min. The sections were incubated at room temperature with normal goat serum for 15 min and then for 1 hr with anti-human hPTHrP (dilution 1:50, clone 212-10.7; Oncogene, San Diego, CA, USA). Indirect immunostaining was achieved using the ABC-peroxidase technique. Endogenous biotin was saturated using a biotin blocking kit (Vector Laboratories; Burlingame, CA, USA). The peroxidase was developed using the DAB substrate kit (Vector Laboratories). Negative controls were performed using non immunized rabbit or mouse serum, omitting the primary antibody. Pulp stroma and odontoblastic zone were photographed with a Nikon Coolpix 950 digital camera (Nikon Corporation; Tokyo, Japan) both in inflamed and normal pulp specimens.

Results

The analysis of all the sections of normal pulps showed that the PTHrP immunoreaction was present both in the nucleus and cytoplasm of positive cells. PTHrP positive cells were localized in the odontoblastic/sub-odontoblastic zone. In the pulp core, PTHrP was expressed only by few scattered fibroblasts (Figure 1a). No immunoreactive peptide was present in the vascular compartment (Figure 1c).

In all inflamed sections, PTHrP was expressed, both in the odontoblastic/sub-odontoblastic cells (Figure 1b) and in the pulp inflammatory stroma (Figure 1d), particularly by pulpoblasts and by endothelial and vascular cells smooth muscle (Figure 1e,f).

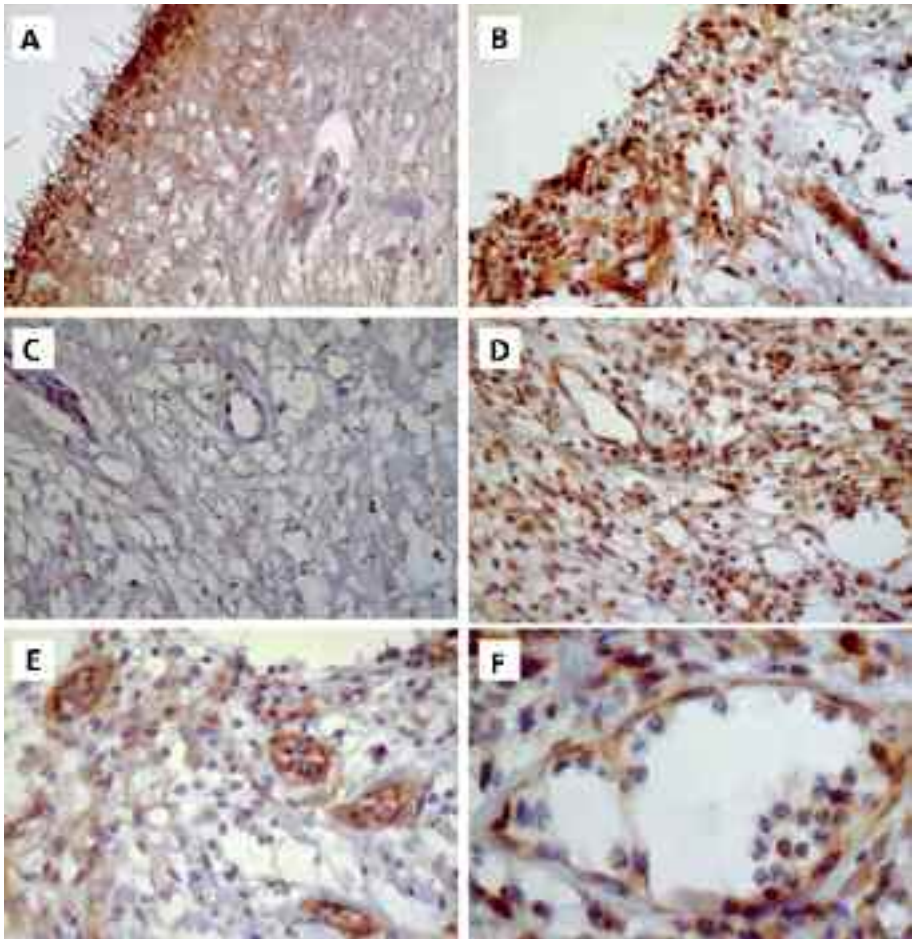

Figure 1. Sections of dental pulp stained with anti-PTH-rP monoclonal antibody. ***A,*** The image shows PTHrP immunostainings only in the odontoblastic zone of a normal pulp (original magnification 100 ×). ***B,*** In inflamed pulp, PTHrP was expressed by odontoblastic and subodontoblastic cells (original magnification 100 ×). ***C,*** In the core of normal pulp, PTHrP is expressed only by scattered fibroblasts. ***D,*** In the core of inflamed pulp, PTHrP positive cells were localized in the vessels, in the pulpal stroma and in inflammatory cells around pulp vessels (original magnification 200 ×). ***E,*** PTHrP positive neoangiogenetic cells in inflamed pulp (original magnification 300 ×). ***F,*** Inflamed pulp section, at high magnification, showing a vessel with PTHrP positive endothelial cells. Note the inflammatory cells around and into the vessel lumen (original magnification 400 ×).

Discussion

Our data revealed, by immunohistochemistry, the presence and the localization of PTHrP in normal and inflamed pulp. Previous studies have indicated that this peptide regulates cell growth, development, migration, differentiation, and survival in many organs[5,13].

Inflammation seems to favour a cell phenotype reorientation and to promote the transdifferentiation of some inflammatory cells into odontoblast-like or osteoblast-like progenitors[14]. In the present research we showed the presence of PTHrP in the subodontoblastic zone both in normal and inflamed pulps. Some experiments conducted on human and animal pulps, especially bovine and rat, demonstrated a regulatory role of PTH/PTHrP receptor in the differentiation process of odontoblasts[15]. We observed that PTHrP was expressed in the odontoblastic cells of normal pulp. This finding is consistent with the observation that the activation of PTH/PTHrP receptor, PTH1R, regulates the osteoblastic and odontoblastic cells during the maturation of the teeth. The activation of this receptor is involved also in mediating the later mesenchymal-epithelial interactions that are necessary for terminal odontoblastic and ameloblastic cytodifferentiation[16]. Moreover, at the end of the maturation process of the teeth, PTH1R is present in the odontoblastic

zone and its activation is implicated in the continuous process of differentiation of the odontoblast-like cells, during the production of secondary dentin[17]. This finding could explain the PTHrP positivity observed in the subodontoblastic zone, both in normal and in the inflamed pulps.

In all inflamed pulps we found a great PTHrP positivity in pulpoblasts. Many studies demonstrated that pulp fibroblasts undergo a phenotypic conversion into osteoblast/odontoblast-like progenitors implicated in reparative dentin formation. Many peptides and cytokines are involved in this conversion[18]. Our findings suggest that also PTHrP could play a similar role in the inflamed pulp.

Interestingly, the vascular component expresses PTHrP in the inflamed but not in the normal pulp. PTHrP production is increased by many cytokines, such as the tumour necrosis factor α (TNF-α) and interleukin 1β (IL-1)[19]. TNF-α and IL-1β are the principal pro-inflammatory cytokines, and are involved in many processes, called "endothelial activation". The main role of these molecules is the synthesis of endothelial adhesion factors and chemical agents, like growth factors and others cytokines[20].

It has been demonstrated that TNF-α and IL-1β significantly stimulated the production of PTHrP in a time and dose-dependent manner. Furthermore, the induction of PTHrP mRNA by TNF-α and IL-1β was observed within 2 hours and remained detectable after 4 and 12 hours, respectively.

An important function of PTHrP in the vascular compartment is to activate PTH1R and to function in a local paracrine and/or autocrine mode to regulate vascular smooth muscle cell tone. In this study we showed that, in the inflamed pulp, PTHrP is highly expressed both in endothelial and vascular smooth muscle cells. The presence of this peptide in the vascular zone suggested that PTHrP may be involved in the vasodilative process.

Many works proved that PTHrP plays a role in the angiogenesis, although its function is still unclear[21,22].

The angiogenesis is present in inflammatory processes: it starts with vasodilation, and our findings suggest that PTHrP is involved in this process. In this context, it is noteworthy that the principal pro-inflammatory cytochines, such as TNF-α and IL-1β, have been reported to increase PTHrP production[19].

In conclusion our study suggests that PTHrP carries out an important role in inflamed pulp through the regulation of odontoblast differentiation, vasodilatation and neoangiogenesis. However, it is necessary to get more insight into the mechanisms of PTHrP regulatory actions in inflamed pulp.

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