

## Matrix Metalloproteinases in Orofacial Pain: A Review

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### ABSTRACT

Acute and persistent pain in the orofacial region are frequently and a major medical and social problem. Orofacial pain can have various aetiologies, but inflammation is associated with many of them. One of the most important developments in the study of matrix metalloproteinases (MMPs) is the recent finding that these enzymes are involved in nociception and hyperalgesia. Thus, the aim of this review is to evaluate the current state of knowledge regarding the relationship between MMPs and orofacial pain. Material and A search was performed in PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed>) by using the following key words (separately or combined): 'matrix metalloproteinases', 'dentistry' and 'pain'. MMPs are involved in some orofacial pain processes, where these MMPs are located in periapical, periodontal, temporomandibular and brain tissues. We know that these proteases modulate inflammatory mechanisms, however, the complete influence of MMPs on the orofacial pain process is not well understood and thus work in this area could help to elucidate mechanisms of chronic pain as well as to develop new therapies for this pain process. Interest in the relationship between pain and MMPs has been growing over the last decade, providing a new perspective for therapeutic applications.

### KEYWORDS

Hyperalgesia; Inflammation; Orofacial Pain; Metalloproteinases.

### INTRODUCTION

Orofacial pain is a very common problem in modern society and is one of the main reasons the general population seeks dental treatment [1]. The origins of orofacial pain conditions include pain associated with the hard and soft tissues of the face, head, neck and intraoral structures [2].

The inflammatory process is an important factor in the origin of pain in the orofacial region [3]. Taking into account the important role of inflammation, therefore, studies have shown that macromolecules in the extracellular matrix (ECM) can actively participate in this process. In fact, evidence shows that of the possible proteases, matrix metalloproteinases (MMPs) are essential in the physiological metabolism of collagen and other macromolecules during development and postnatal tissue remodelling and in pathological resorption, for example, the invasiveness of malignant tumours, structural resorption in periodontal disease and the destruction of joints in rheumatoid arthritis [4,5]. MMPs constitute a family of over 20 metalloenzymes that cleave various ECM components, including the interstitial and basement membrane collagen, fibronectin, laminin and proteoglycans, releasing fragments with distinct biological activities.

Interest in the relationship between pain and MMPs has been growing over the last decade, providing a new perspective for therapeutic applications. Because chronic pain mechanisms are not yet fully elucidated and, particularly those of orofacial origin, have difficult and inappropriate treatments, this literature review focuses on recent studies on the relationship between MMPs and pain.

### METHODS

A search was performed in PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed>) by using the following key words (separately or combined): 'matrix metalloproteinases', 'dentistry' and 'pain'. Selected papers, including reviews, were chosen on the basis of their content (quality and novelty). The main focus was on matrix metalloproteinases in orofacial pain. Papers that had no relation of the three keywords proposals were excluded from the study.

### RESULTS AND DISCUSSION

#### Matrix Metalloproteinases (MMPs)

Regarding pain of inflammatory origin, studies have demonstrated that the macromolecules of the extracellular matrix (ECM) can participate in the development of the inflammatory process [6,7]. Specifically, there is a locally secretion by cells of extracellular proteolytic enzymes, including the metalloproteinases and serine proteases that are involved [8]. In fact, of the possible proteases, the MMPs play a central involvement in the physiological metabolism of collagen and other macromolecules during development and postnatal tissue remodelling and in pathological resorption, for example, the invasiveness of malignant tumours, structural resorption in periodontal disease, joint destruction in rheumatoid arthritis and neurodegenerative diseases [9].

MMPs constitute a family of over 20 metalloenzymes that cleave various ECM components, including the interstitial and basement membrane collagen, fibronectin, laminin and proteoglycans, releasing fragments with distinct biological activities. The composition of MMPs is a prodomain, a prodomain rich in cysteine, a catalytic domain with the Zn<sup>2+</sup> binding site and a hinge region [10]. Furthermore, with the exception of matrilysins (MMP-7, -26), the others contain a hemopexin domain. Also, it is possible to observe other features, as MMP-2 and -9 contain a fibronectin domain with a strong compatibility for gelatin and the membrane-type MMP (MTMMPs) contain a transmembrane domain. MMPs are secreted as latent proenzymes and need activation by the cleavage of the prodomain rich in cysteine. In addition, their activity depends on the presence of zinc. MMPs are secreted as latent proenzymes and require activation by proteolytic cleavage of the amino-terminal domain rich in cysteine. In addition, its activity depends on the presence of zinc.

The major cell producing MMPs are polymorphonuclear leukocytes, keratinocytes, monocytes, macrophages, fibroblasts and mesenchymal cells. Those cells are able to respond to growth factors and cytokines, including Interleukin -1 (IL-1), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and transforming growth factor- $\alpha$  (TGF- $\alpha$ ). In the presence of these growth factors and cytokines, these cells release the MMPs of specific storage for extracellular region [11].

The subclasses of MMPs have been identified and classified according to substrate specificity, including gelatinases, collagenases, elastases, stromelysins and MTMMPs12. Thus, the collagenases MMP -1, -2 and -3 are MMP-1, -8 and -13, respectively. These MMPs degrade interstitial type I, II and III collagen, and digest other ECM molecules. The MMPs gelatinase A (MMP-2) and B (MMP -9) mainly degrade denatured collagen (gelatine) and type IV collagen, while MMPs 3 and 10 are stromelysins and can degrade fibronectin and proteoglycans [12].

Although MMPs are classified according to substrate whereby have affinity, it is noted that there is overlap between subclasses, signifying MMPs do not have necessarily a defined substrate. In this perspective, MMP-1 have as substrate collagen I, II, III, VII, VIII and X; gelatins; aggrecan and tenascin. MMP-2 have collagen I, IV, VII, X, XI; gelatins,

elastin and aggrecan. About MMP-3, substrate are collagen I, III, IV, V, VIII, XI and IX; aggrecan, elastin, tenascin, proteoglycanases, activated procollagen. MMP-7 have gelatins and fibronectin as substrates; MMP-8, collagen I, II, III, VII and X; and aggrecan; MMP-9, collagen I, III, IV, V, VII, X, XI, elastin, gelatin and aggrecan. MMP-12 has elastin as substrate, among others MMPs [13].

In relation of specific substrate of MMPs in brain regions, in pathological conditions particularly, glial cells and neurons, as well as inflammatory cells forming the main substrates for the activity of these proteases.

The proteolytic activity of MMPs is primarily modulated by tissue inhibitors of metalloproteinases (TIMPs). These inhibitors are found in a large variety of tissues and they form complexes with MMPs through the binding to the highly conserved zinc site of these proteases. This mechanism permit the inhibition and regulation of the enzymatic activity of MMPs [14]. Currently, four TIMPs are known, and their inhibitory action is selective, reversible and occurs in a balanced way. The organized regulation of MMPs and TIMPs directs the cleavage and release of important cell surface receptors and growth factors [6]. The expression of TIMP is observed during physiological tissue remodelling, contributing to the maintenance of metabolic and structural balance of the ECM. Changes in the homeostasis between MMPs and TIMPs have been identified in diseases related with imbalance ECM remodelling, such as arthritis, cancer, cardiovascular disease, nephritis, neurological disorders and fibrosis, where consequently there is greater degradation of ECM [15]. TIMP -type 1 (TIMP-1) and type 2 (TIMP-2) represent well-characterised members of this family of inhibitors and have inhibitory activity against active forms of the entire family of MMPs [16].

Matrix metalloproteinases participate in a large number of physiological processes in oral cavity, such as collagen remodeling in periodontal tissues, dentin mineralization processes, tooth eruption, among others. In human metabolism, they participate also in pathological processes as disorders of the temporomandibular joint, periodontal tissue destruction, root caries lesions and metastasis in tumors.

In addition, MMPs can be expressed in various brain regions, especially under the pathological conditions in which glial cells and inflammatory cells form the main substrates for the activity of these proteases. Investigations of cerebral ischemia in rats and mice note the expression and activation of MMP-2 specifically localised to neurons, glial cells and endothelium, while MMP-9 was found at high levels in neurons and glial cells, as well as beams myelinated fibres, and MMP-3 were detected in neurons [17]. The expression of MMPs was studied in two large-scale structures, the cerebellum and hippocampus. Using the zymography technique, lower levels of MMP-9 and higher levels of MMP-2 were found in the adult rat cerebellum [18]. A more extensive analysis of the expression of MMP-2 and MMP -9 in the hippocampus indicated that while MMP-2 seems to have a mostly glial

origin, MMP -9 is mainly expressed in neurons.

When a synaptic remodelling injury is induced, an elevated expression and enzymatic activity of MMP-3 in the hippocampus of adult mice is observed [19]. Both, neurons and glial cells express MMP-3 and this metalloproteinase, in turn, can activate other MMPs, such as gelatinases. It is known that synaptic remodelling induced by MMPs can be blocked by NMDA receptor antagonists, indicating that the effects of MMPs are also mediated through the activities of these receptors [20].

**Tissue Inhibitors of Metalloproteinases:** Regulation of extracellular proteolysis by MMPs is crucial for survival of organisms and the interaction with various inhibitors is responsible for this control [21]. The main inhibitor is tissue inhibitor of metalloproteinase (TIMP), an endogenous inhibitor, which consist of four families that decrease MMP activity and consequently, restrict ECM breakdown [22]. The four TIMPs are 1984-1994 amino acids long and maintain two domains with three conserved disulphide bonds each one. The N-terminal is responsible for binding into the active-zinc site of MMPs to inhibit these proteins [23]. Some findings have clarified multifunctional characteristics for these proteins and not only inhibition of MMPs. The first known biological function of TIMPs was the collagenase's inhibition and now, almost all MMPs can be inhibited by all 4 TIMPs, besides there are reports that indicate differences in binding affinity [22]. The expression of TIMPs is observed during physiological tissue remodeling, contributing to the maintenance of metabolic and structural balance of ECM. Changes in the homeostasis between MMPs and TIMPs have been identified in diseases associated with uncontrolled remodeling of ECM such as arthritis, cancer, cardiovascular diseases, nephritis, neurological disorders and fibrosis [24].

TIMP type 1 and type 2 represent well-characterized members of this family inhibitors and exhibit inhibitory activity against active forms of the entire family of MMPs, while TIMP-1 forms preferentially complex with MMP-9, and TIMP -2 acts on MMP-2. TIMP-3 has the broadest inhibition spectrum and it also differs from the others in being tightly bound to the extracellular matrix [25]. In addition to their metalloproteinase inhibitory activity, TIMPs have various biological activities including modulation of cell proliferation, pro- and anti-apoptotic, anti-angiogenic and synaptic plasticity activities, but many of them have been shown to be independent of MMP inhibition [26].

## Pain

The word pain has a mean of penalty or punishment according the derivations of the Latin and the Greek, which are "peone" and "poine", respectively. Aristotle considered it to be a feeling, specifically a passion, where the heart is the source and centre of its processing. The idea of the heart as the centre of sensations lasted for many centuries and received support from leading thinkers. In contrast, there were also many who defended the idea of the brain as the centre of sensations. Anatomical and physiological studies by

Descartes demonstrated the existence of nerves capable of receiving sensory information from the periphery and taking them to the brain [27,28].

The actual concept for pain is given by the International Association for the Study of Pain, which defines this process as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [29]. Thus, pain is a complex experience, which not only involves the transduction of noxious stimuli coming from the environment but also cognitive emotional processing, carried out by the central nervous system (CNS) [30]. In this way, pain is a subjective experience with sensory and affective components. Indeed, nociceptive and emotional behaviours are related and widely represented with overlapping neural pathways, which may form substrates that allow emotions to modulate pain [31,32].

Early indications of specialised receptors that captured nociceptive stimuli were described in 1906 by Sherrington, who proposed that primary afferent fibres contain nociceptors (receptors for noxious stimuli), which are activated by stimuli capable of causing tissue injury [30]. These afferent fibres respond to different types of stimuli (polymodal nociceptors) and stimuli with specific properties (thermal and mechanical stimuli) [33]. They are found in both somatic structures (bone, skin, and muscle tissue) and visceral structures (pancreas, intestine) [34]. There are two types of afferent fibres responsible for transmitting the pain information, A-delta fibres ( $A\delta$ ), which are myelinated and therefore a higher calibre responsible for the rapid conduction of pain, and C fibres, which are not myelinated, with a lower calibre and responsible for the slow conduction of pain. The activation of  $A\delta$  fibres causes a pain sensation described as sharp and well localised, whereas the activation of C fibres produces a burning sensation without a precise location.

Regarding nociceptive transmission, nociceptive impulses are generated by primary afferent neurons peripherally directed to grey matter regions of the spinal cord, which can be divided into 10 laminae according to cytoarchitectonic characteristics [35]. Communication between the primary and secondary afferent neurons occurs through a variety of substances involved in the central transmission and modulation of nociceptive information, including neuropeptides and excitatory amino acids [36].

The pain ascending pathways form two phylogenetically different systems. The first runs through the middle region of the brainstem and is formed by the spinothalamic, spinoreticular, spinomes-encephalic, spinoparabrachial-amygdaloid, hypothalamic-spinoparabrachial, spino-hypothalamic and neospinothalamic pathways. These transmissions are projected directly to brain structures and are involved in the transmission of rapid or acute pain; and, in general, these pathways have few synapses and are directly connect to the primary somatosensory cortex. The second system occupies the lateral region of the brainstem and consists of the paleospinothalamic, spinocervical and

postsynaptic dorsal horn pathways of the dorsal horn. These pathways have multiple relay stations in the bulbar, pontine and midbrain structures before reaching the prosencephalic structures [37]. In general, the fibre axons of second-order neurons connect with thalamic third-order neurons that project into the primary somatosensory cortex and the limbic system, giving pain its cognitive and emotional properties.

In the orofacial region, however, the anatomical arrangement is slightly different. Trigeminal first-order neurons have their cell bodies located in the trigeminal ganglion, also called the Gasser ganglion. The axons of these neurons enter the brainstem directly at the pons region. These trigeminal fibres, in the brainstem, descend to the spinal cord, forming the spinal trigeminal tract [38]. The spinal trigeminal tract is divided into three subnuclei, the oral, interpolar and caudal subnuclei. Trigeminal first-order neurons synapse with the fibres of the second-order neurons in the caudal and interpolar subnuclei. The axons of these second-order neurons cross the midline and ascend contralaterally to the thalamus and cortex, via the trigeminal lemniscus. These fibres are the trigemino-thalamic tract [39].

Depending on its characteristics, pain can be classified as acute or chronic. Chronic pain is characterised by persistent pain whose duration exceeds the period of recovery from the damage and / or injury to the nerve. It may be spontaneous (not elicited by external stimuli), caused by the presence of hyperalgesia (increased pain that is triggered by low intensity noxious stimuli) and / or allodynia (pain elicited by non-noxious stimuli). Chronic pain has no obvious physiological role, i.e., no adaptive value. Unlike chronic pain, acute pain has the function of protecting the body from harmful external influences, through the phasic activation of nociceptors by noxious stimuli. Thus, it evokes protective responses such as the flight reaction and / or physical withdrawal to cease exposure to the noxious stimuli, thereby eliminating it [35,40].

It is important to note that chronic inflammatory disease induces neuronal plasticity. Thus, inflammation resulting from tissue injury occurs, initially recruiting immune cells that release at least three important mediators tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and factor nerve growth factor (NGF) into the injured tissue. These factors are able to activate and sensitise peripheral and central nociceptive neurons, thereby contributing to the maintenance of pain and hyperalgesia [41]. Studies by Kim report that damage to sensory neurons induces the expression of TNF-  $\alpha$ , IL-1 $\beta$ , and interleukin-6 (IL-6), and promotes the synthesis of nitric oxide in glial cells in the spinal cord [42]. These factors also mobilise cytoplasmic mediators from TLRs (Toll-like receptors) thus promoting the dimerisation and autophosphorylation of PKR [43-46]. TLRs are transmembrane signalling proteins that are expressed by the cells of the innate immune system; there are more than ten different TLRs with distinct, specific ligands [47]. CNS neurons, astrocytes and microglia were shown to express several TLRs, including TLR2 and TLR4, which was expected due to the role of innate immune system cells in

the CNS. In a rat model of neuropathic pain, it was reported that TLR2 is required for the maximal induction of pain hypersensitivity after transection of the spinal nerve [48].

Currently, there are several modulators and mediators of the pain transmission circuits, showing the complexity of the process of nociception in both primary peripheral neurons and spinal and supraspinal central neurons [35]. Thus, it is increasingly clear that these new mediators are also produced and released by non-neuronal cells, predominantly glial cells and immune cells. Experimental models have further revealed that chronic pain of inflammatory origin is mainly the consequence of neurochemical, physiological and morphological changes in sensory neurons, both peripheral and central, that dramatically and chronically change their phenotypic characteristics and their synaptic activity [49]. These changes cause a reduction in the inhibitory activity and an increase in excitatory events in the sensory neurons of the spinal cord, while lowering the levels of excitability of central neurons resulting in sensitisation and the emergence of permanent or recurrent pain [35,50-54].

From a physiological point of view, pain can be classified into three types, nociceptive, inflammatory and neuropathic. Nociceptive pain is a defence mechanism that protects the body from potential or imminent harm and along with its physiological consequences, is produced for the central nervous system (CNS) and peripheral nervous system (PNS). In contrast, inflammatory pain and neuropathic pain are mainly reflections of the pathologically altered functioning of these two systems [55]. Inflammatory pain is characterised by spontaneous pain sensation and hyperalgesia when inflammatory mediators are present, whereas neuropathic pain is a chronic condition that occurs or persists after a primary lesion or dysfunction in the CNS or PNS [56,57].

**Orofacial Pain:** The orofacial region contains several unique structures, and also facial skin, intraoral mucosa, tooth pulp, oral/nasal mucosa, intracranial meninges and vessels, and musculoskeletal tissue, and so, it has unique physiologic characteristics [58]. Various types of acute and persistent pain in the orofacial region are very frequently and they have an important social and medical problem. Collectively, orofacial pain has a high prevalence rate, large symptoms of pain and often an unpleasant influence on quality of life. Considering these factors, the correct diagnosis and therefore the efficient treatment of orofacial pain represent a significant topic in the field of health [59].

Neuropathic pain can also be found in the orofacial region. These types of pain disorders include trigeminal neuralgia and glossopharyngeal neuralgia [60]. The etiologic factors to the development of neuropathic pain disorders appear in a large number and can include the trigeminal nerve's injury or compression; inflammatory process, with glial contributions; or infection by herpes virus [60]. The responsiveness of orofacial neuropathic or neuralgic pain conditions to different treatments have been studied by some clinical reports [60-62]. The neuropathic pain disorders are not always

consequence of all injuries to the trigeminal nerve; indeed, the neuropathic pain after injury by dental treatment occur in a low incidence [63]. These dental treatments can be facial trauma, orthognathic surgery, tooth extraction, or dental implants surgery [64-67].

The trigeminal nociceptive system is responsible for the transmission of pain information in orofacial region. Some studies have described differences in the electrophysiological, anatomical, or pharmacological characteristics of trigeminal afferents [68-70]. Together, these studies support the idea that guide trigeminal neuron terminals interactions with tissue, through neurotrophins or integrin binding to extracellular matrix molecules, regulate neuronal proteins through their expression or signalling pathways [71-74]. Therefore, the differences of these neurons's responsiveness may be due to the existence of unique tissues innervated by the trigeminal afferents [59].

In orofacial pain, the afferent neurons are usually A- $\beta$  and A- $\delta$  fibres, which are myelinated with their termini in areas of orofacial tissues with complex structures that respond to light mechanical stimuli such as touch, or proprioceptive stimuli, such as stretch or muscle tension. Others fibres are primary afferent nerve fibres A- $\delta$  or C, which are demyelinated with their termini in orofacial tissue as free nerve endings. Through the trigeminal ganglion, all these fibres send afferent impulses to the periphery. There are three parts of the trigeminal nucleus, the superior oral subnucleus, the medium interpolar subnucleus and in the lower part, the caudal subnucleus. Most synapses are pain fibres in the caudal subnucleus [39].

Some studies have evaluated the trigeminal system and acute inflammatory injury. These investigations use pain models that have been developed as a clinical method for analysing preferred anaesthesia, stimulation of endogenous analgesic systems, local release of inflammatory mediators, other physiologic mechanisms, or the association of genetic targets with pain [75-80]. These studies have increased the evaluation of analgesics, anxiolytics and anaesthetics. Also, the chronic inflammation has received attention in the orofacial area. By this way, works with irreversible pulpitis in teeth have demonstrated that this inflammatory process caused by bacteria promotes significant changes in ion channels, receptors, and neuropeptides [81-83]. Besides that, it is known that an inflammation of a unique tooth in patients is sufficient to generate a central sensitisation [84]. Still, experimental studies regarding chronic inflammation in the trigeminal region have demonstrated differences in sensitisation according to the target location and differences in neural activities in the different sexes [85].

For musculoskeletal pain, temporomandibular disorder (TMD) is a dysfunction that includes clinical problems in masticatory musculature, temporomandibular joint (TMJ) and associated structures. The pain in the masticatory muscles, in the TMJ, and in the associated hard and soft tissues accompany these disorders [86]. There are included as symptoms of TMD, the decreased mandibular motion, pain in the muscles of

mastication, temporomandibular joint (TMJ) pain and joint noise associated with function, generalised myofascial pain, and a functional limitation or deviation of the jaw opening [87]. The prevalence of TMD is thought to be greater than 5% of the population [88]. A broad prevalence peak of TMD symptoms happened between 20 and 40 years of age, and there is a lower prevalence in older and younger people [89]. The higher prevalence of TMD in women is characteristic of this disorder.<sup>86</sup> the reasons to the sexual disequilibrium during TMD are not entirely clear, but some have suggested a hormonal influence [90].

Approximately 80 percent of patients with TMDs have signs and symptoms of joint disease, including disc instability and inflammatory process [91]. The exact cause of this disease is not completely understood but is believed to involve structural, physiological, behavioural and environmental factors. During a degenerative TMJ disease, it is possible to observe an imbalance in the synthesis and destruction of matrices that occur in the cartilage and fibrocartilages of the joint through chondrocytes and fibrochondrocytes, resulting in a continuous loss of extracellular matrix (ECM) components of the articular cartilage [86]. The ECM alterations can occur by genetic problems or by hormones and other factors that interfere the ECM remodelling in TMJ [86]. Members of the matrix metalloproteinases (MMPs) enzyme family are the major ECM-degrading proteinases that are involved in tissue turnover processes. Thus, the alterations of the ECM by the MMPs is a possible mechanism for degenerative TMJ diseases [92].

**Matrix Metalloproteinases and Orofacial Pain:** Because much of orofacial pain is of inflammatory origin, it is possible that MMPs participate actively in the orofacial pain processes, (Table 1) [93]. In fact, the degeneration of matrix proteins is one of the phenomena that occur during pulpal inflammation. Additionally, the MMPs are expressed in inflamed pulps and periapical lesions, which suggests that MMPs play an important role in pulpal and periapical inflammation, especially in the symptomatic pulpitis [94-97]. This suggestion was based on the fact that inflammatory cells, polymorphonuclear leukocytes, macrophages, plasma cells and lymphocytes may secrete MMP-1 and -3.

COX-Cyclooxygenase; MMP-Matrix Metalloproteinases; WB-Western Blot Analysis; IF-Immunofluorometric assay; F-Fluorescent Assay; IHC-Immunohistochemistry; HT-Histometry; RT-PCR, Real-time polymerase chain reaction; GZ-Gelatinase Zymography; ISZ-in situ Zimography; BA-Biomarker analysis; ELISA-Enzyme Linked Immuno Sorbent Assay.

Additionally, in regards to the dental origin causes of orofacial pain, Tjaderhane et al [98]. Suggested that many MMPs are activated by bacterial acids and are involved in caries by the destruction of dentin. By an endodontic perspective, MMPs have been observed in inflamed dental pulp, cystic fluid and periapical tissue [94].

| Authors                         | Tissue Investigated      | Methods        | Conclusion   |
|---------------------------------|--------------------------|----------------|--|
| Avellan <i>et al.</i> , 2005    | Pulp tissue              | WB and IF      | Local elevations in MMP-8 levels induced by pulpal pain  |
| Zehnder <i>et al.</i> , 2011    | Dentinal fluid           | F              | Higher MMP-9 levels in dentinal fluid of patients with irreversible pulpitis   |
| Tsuji <i>et al.</i> , 2009      | Periapical tissue        | IHC and HT     | Increased MMP-9 in destructed periapical tissue induced at 7 days after pulpal exposure  |
| Wang <i>et al.</i> , 2006       | Oral mucosa              | RT-PCR         | Inhibition of COX-2 results in an increase of the expression of genes MMP-1 and MMP-3 in oral mucosa   |
| Nascimento <i>et al.</i> , 2013 | Trigeminal ganglion      | GZ and ISZ     | Increased MMP-2 and MMP-9 expression in trigeminal ganglion during TMJ inflammation  |
| Israel <i>et al.</i> , 1998     | Temporomandibular tissue | WB             | Reduction in collagenase activity in synovial fluid of patients with a long history of TMJ pain symptoms                                       |
| Ishimaru <i>et al.</i> , 2000   | Temporomandibular tissue | IHC            | Synovial fluid concentration of MMP-3 was significantly increased in the painful patients with TMD   |
| Kanyama <i>et al.</i> , 2000    | Temporomandibular tissue | GZ and WB      | Higher concentration of MMP-1, MMP-2, MMP-3 and MMP-9 in TMJ osteoarthritis patients   |
| Ishimaru <i>et al.</i> , 2003   | Temporomandibular tissue | IHC            | MMP-3 decreased in synovial fluid of patients with severe pain associated with TMJ disorders treated with arthrocentesis and COX-2 inhibitor   |
| Ge <i>et al.</i> , 2009         | Temporomandibular tissue | RT-PCR         | Increased levels of MMP-1, MMP-3, MMP-9, and MMP13 in TMJ condylar chondrocytes during arthritic pathology                                     |
| Planello <i>et al.</i> , 2011   | Temporomandibular tissue | RT-PCR         | Association between MMP-1 polymorphism and TMJ degeneration  |
| Wang <i>et al.</i> , 2012       | Temporomandibular tissue | RT-PCR and IHC | Increased MMP-3 expression in condylar head of rats submitted to intra-TMJ injection of monosodium iodoacetate                                 |
| Nordstrom <i>et al.</i> , 1999  | Saliva                   | WB             | Collagenase activities measured from saliva were significantly reduced in rheumatoid arthritis patients  |
| Mirrielees <i>et al.</i> , 2010 | Saliva                   | BA             | Elevated salivary levels of MMP-8 in periodontal disease group of patients and unaffected levels of this MMP in rheumatoid arthritis patients. |
| Kyrkanides <i>et al.</i> , 2000 | Periodontal tissue       | RT-PCR and GZ  | COX inhibition resulted in exacerbation MMP-9 production and activity by rat endothelial cells culture of periodontal tissue                   |
| Avellan <i>et al.</i> , 2006    | Periodontal tissue       | IF             | Local elevations in levels and activation of MMP-8 in gingival crevice fluid under capsaicin stimulation of alveolar mucosa                    |
| Avellan <i>et al.</i> , 2008    | Periodontal tissue       | IF             | Significant elevations of MMP-8 levels in gingival crevice fluid during experimental tooth pain  |
| Shin <i>et al.</i> , 2011       | Periodontal tissue       | ELISA          | Periradicular inflammation of endodontic origin elevated MMP-8 levels in gingival crevicular fluid   |
| Ahmed <i>et al.</i> , 2013      | Periodontal tissue       | HT and IHC     | Expression of MMP-9 was significantly elevated in patients with symptomatic apical periodontitis   |

**Table 1.** Summary of the studies performed to determine the association of various MMPs with orofacial pain

The MMP-1 and MMP-8 have been the basis of periodontal studies. This fact has been happened because these collagenases may collaborate to physiological and pathologic collagen degradation in periodontal tissues [99-102]. In addition, elevated MMP-9 and MMP-8 have been established in gingival crevicular fluid (GCF) during the advanced stages of periodontal disease [100,103-105]. This pathology also increases hs-CRP and IL-6, systemic inflammatory mediators that, on the other hand, increase the expression of systemic inflammatory markers, some of which participate of the upregulation of MMP expression [106-108].

In orofacial pain of non-dental origin, the TMDs stand out. Degenerative changes in the TMJ can be due an imbalance between the synthesis and the destruction of ECM components [92,109]. By this way, these events have been related with the inflammatory process of TMJ because some modulators are found in increased levels in synovial fluid during TMDs. Among them, it was revealed the presence of cytokines, cartilage matrix catabolites, nitric oxide and proteinases, including matrix metalloproteinases (MMPs) [110-112]. In fact, MMPs derived from fibrocartilage and cartilage cells are considered the essential enzymes for TMJ ECM breakdown [86,113-116]. There is an important correlation between increased MMP-3 in synovial fluid and patients with a painful TMJ [117]. Therefore, it is possible associate the painful joints with synovial inflammation. In this inflammatory condition, synoviocytes and macrophages, and inflammatory cytokines, including tumour necrosis factor and interleukin-1 secret proMMP-3 into the synovial fluid [118].

Srinivas et al. showed the presence of the gelatinases MMP-2 and -9 and of the collagenases MMP-1, -8, and -13 in the synovial fluid of the patients with internal derangement of the TMJ [110]. In vitro studies have been shown an increased production of gelatinases in the synovial cells, fibroblasts and chondrocytes of the joints with osteoarthritis and rheumatoid arthritis [111]. MMP-9 is more expressed by inflammatory cells, in comparison with MMP-2, which is more expressed in fibroblasts [111]. Thus, Nascimento et al. showed the increased expression and activity of MMP-2 and MMP-9 in the trigeminal ganglion during TMJ inflammation in rats, and treatment with an MMP inhibitor attenuated the increases in mechanical allodynia and orofacial hyperalgesia induced by intra-TMJ injection of CFA [119].

Significantly, MMPs produced by ganglion cells due to various stimuli, such as the inflammatory process, can be transported from the neuronal soma to the periphery, promoting their effects both within the ganglion and peripherally [120]. Previous studies have shown that MMP-9 and MMP-2 are involved in persistent pain [7,121,122]. The gelatinase MMP-9 seems to be responsible for the activation of IL-1 $\beta$  and other bioactive molecules, such as tumour necrosis factor (TNF- $\alpha$ ) and pro-neurotrophins, such as proNGF and proBDNF, in the initial phases of the inflammatory process, while MMP-2 is implicated in maintenance of persistent pain [7, 122,123]. Additionally, MMP-9 may participate in the

induction, proliferation and remodelling of satellite glial cells (SGCs) [124]. Studies have shown that in models of chronic or persistent pain, communication between SGCs and the sensory neurons of the ganglion is greatly increased, due to the facilitation of gap junctions between cells, the increase in sodium currents and the suppression of potassium currents [125-127]. A recent investigation demonstrated that the blockade of MMP-9 abolishes the activation of SGCs and the expression of IL-1 $\beta$  [128]. These events could be correlated with the hyperalgesia and allodynia observed in inflammatory conditions [125].

It is important to clarify that MMPs can cleave other substrates and interfere in other mechanisms that are also critical for neuronal sensitisation [7,120,129]. Thus, recent studies have demonstrated that the rapid upregulation of MMP-9 in primary sensory neurons in the DRG can mask opioid analgesia without interfering in opioid-induced hyperalgesia [128]. According to Berta et al. it is possible that neuronal MMP-9 expression after acute morphine administration promotes the SGCs and IL-1 $\beta$  activation, thereby reducing the analgesic effect of the opioid [128].

Expanding the functions of MMPs, these proteases were shown to be involved in the modulation of neuropathic pain [7]. In this study, the intrathecal administration of an acute or prolonged form of a non-specific MMP inhibitor (GM6001) significantly reduced mechanical allodynia assessed using the von Frey Test on the plantar surface of the hind paw after an injury of the spinal nerve. Considering these findings, the authors suggest the possible involvement of MMPs in the development of neuropathic pain due to demyelination that occurs through the degraded myelin basic protein in CNS [130]. Corroborating this hypothesis in MMP-9 knockout mice, demyelination did not occur even after 10 days of sciatic nerve injury [124]. Additionally, Kawasaki et al. showed that the MMP-2 and MMP-9 gelatinases are expressed in the spinal ganglia in response to the progress of neuropathic pain induced by nerve clamping in rats. In this study, there was increased expression of MMP-9 in the early stages of inflammation, whereas the increased expression of MMP-2 was observed in the late phase. Thus, it is suggested that MMP-9 participates in the initial stage of inflammation, whereas MMP-2 was responsible for the maintenance phase of persistent or chronic inflammation. This finding supports the other research on gelatinases in the trigeminal ganglia of rats during persistent temporomandibular inflammation [119]. The expression and activity of MMP-9 in the ganglion was higher in the initial stages of inflammatory pain (1 to 3 days), whereas MMP-2 increased in later periods of the experiment (7 and 10 days).

One of the most important developments in MMP research is the new finding that these enzymes are involved in nociception and hyperalgesia [128,131]. Although evidence for the direct influence of MMPs on peripheral nociceptors is still sparse, these proteases may participate in the origin of the inflammatory pain and nerve injury through

the involvement of cytokines, growth factors, adhesion molecules and chemokines with nociceptors. With respect to neuropathic pain, the astrocytic and microglial activation and the glial production of pro-inflammatory cytokines controlled by mitogen-activated protein kinases (MAPKs), are possible new therapeutic targets that are being studied in recent years [132,133].

To date, the metalloproteinase 2 and 9 (MMP-2 and MMP-9) are most related to the origin and maintenance of neuropathic pain. In the first days after spinal nerve injury in animal models, MMP-9 is upregulated in the neurons of the dorsal root ganglion (DRG), where it is required for the cleavage and activation of IL-1 $\beta$  [131]. From this ganglion, this MMP is transported to central terminals of the dorsal horn and activate microglia. Contrary, MMP-2 is induced by satellite cells and persists in the DRG and spinal cord astrocytes in the later stages of the generation of neuropathic pain. The MMP-2 is important in the activation of IL-1 $\beta$ , astrocytes and extracellular signal regulated kinase (ERK). IL-1 $\beta$ , in turn, creates positive feedback for both MMP-2 and ERK [131].

## CONCLUSION

MMPs are involved in various orofacial pain processes. We know that these proteases modulate inflammatory mechanisms, however, the complete influence of MMPs on the orofacial pain process is not well understood [7,120]. In this context, it is valuable to investigate the physiological and pathological mechanisms of these extracellular proteolytic enzymes in nociceptive conditions. There is great innovative potential for the treatment of orofacial pain through new pharmacological targets, because only difficult treatments exist for chronic disorders in the dental clinic at present.

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