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Perspective Article

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Does the relaxin, estrogen and matrix metalloproteinase axis contribute to degradation of TMJ fibrocartilage?

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Introduction

Temporomandibular joint disorders (TMJDs) is a term encompassing a spectrum of clinical signs and symptoms which involve the masticatory musculature, the temporomandibular joint (TMJ) and associated structures. These symptoms occur in approximately 6 to 12% of the adult population in the United States[1,2]. While the relative contributions of the muscles of mastication and the joint to TMJD remain unknown, in a substantial number of patients, these disorders result secondary to TMJ diseases such as osteoarthritis, internal joint derangement and joint hypermobility[3,4]. Due to a poor understanding of the etiopathogenesis of these diseases, and the lack of definitive diagnostic or therapeutic approaches, patients often have to tolerate symptoms, including debilitating pain, that substantially impacts on their quality of life over extended periods of time. Identification of specific factors causing or predisposing to TMJ diseases is necessary to enable early recognition and specific therapeutic management of these diseases. Whereas the determination of causative factors remain elusive, some concepts on the potential etiologic and predisposing factors for TMJ diseases can be developed by examining the epidemiology of these diseases.

Women comprise a significantly high proportion of the patients seeking treatment for TMJDs

The epidemiologic predilection of TMJD in women is striking; In the general population, TMJDs are 2 times more prevalent in women then in men, whereas in patient populations these diseases have a female-to-male preponderance as high as 10:1[2,5,6]. Furthermore, unlike similar diseases of other joints that also have a greater female predilection but occur postmenopausally, a large proportion of women with TMJDs are between 18 and 45 years of age[7]. The reasons for this marked sexual dimorphism and age distribution remain unclear. While several hypotheses have been proposed, some investigators have focused on the potential role of female reproductive hormones, particularly estrogen in the etiology of these diseases. Thus, attempts have been made to immunolocalize estrogen receptors in baboon or pathologic human TMJs, but have produced conflicting results[8-11]. Similarly, there is conflicting epidemiologic evidence on the role of exogenous hormones, such as those in oral contraceptives, in TMJD pain[12,13]. Despite some positive findings on the potential association between TMJDs and estrogen[8,9,11,13], no direct evidence exists linking female reproductive hormones to TMJ disease or defining the mechanisms by which these hormones may cause joint disease. Additionally, the potential role of lesser known, but important female reproductive hormones in TMJ disease has not been examined. Of particular interest in this regard is the hormone relaxin, which is found systemically in cycling and pregnant women but not in men and which modulates matrix remodeling in various tissues. Our studies focus on determining the mechanisms by which relaxin with or without β-estradiol mediates degradation of fibrocartilage which is the predominant tissue within the TMJ. Additional studies aim to determine if asymptomatic and symptomatic women demonstrate significant differences in the systemic levels of these hormones. Together, these studies may suggest a potential contribution of relaxin and β-estradiol to the pathogenesis of TMJ diseases.

Relaxin and estrogen are potent mediators of matrix remodeling activity in female reproductive tissues and in skin

In humans relaxin exists as three gene products, H1, H2 and H3[14]. Relaxin H2 is the major stored and circulating

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form of relaxin in humans, and appears to have long-term effects on connective tissues by altering the turnover of collagen and proteoglycans. Because of the known effects of relaxin H2 on matrix turnover and because of the association of relaxin with joint hypermobility, the remaining discussion focuses on the H2 form of this hormone. Relaxin H2 is a 6-kDa polypeptide belonging to the insulin family of structurally related hormones, but its activities are distinct from other members of this family[15-18]. In humans, relaxin is produced by the corpus luteum, decidua, chorion and breast[19,20].

Although, the best-characterized biologic activities of relaxin are those that relate to various tissues and organs of the mammalian reproductive system, more recently relaxin's effects on other systems particularly the central nervous and cardiovascular systems have received attention[21]. In most reproductive tissues including the cervix, uterus, ovary, breast and decidua, relaxin mediates tissue-remodeling activities through partially characterized mechanisms. In these tissues, relaxin produces a net change in matrix composition and organization by modulating the synthesis of matrix macromolecules[22,23] or by altering the expression of matrix-degrading enzymes[24,25] or both[23]. For the matrix degradative component of the remodeling cycle, relaxin appears to act primarily by modulating the induction of several matrix metalloproteinases (MMPs) including collagenase and stromelysin[24,25]. The remodeling activities are not limited to reproductive tissues, but have also been reported for skin and lung alveolar fibroblasts[26,27]. In the former cell type relaxin increases the expression of procollagenase, and concomitantly decreases that of tissue inhibitor of metal metalloproteinase (TIMP) and type I collagen. However, despite the implied relationship between relaxin-mediated increases in MMP expression and loss of matrix macromolecules, no study has conclusively demonstrated a link between relaxin's induction of MMPs and tissue degradation.

MMPs contribute significantly to loss of tissue matrices in joint diseases

The MMP family of enzymes contributes substantially to joint tissue degeneration during rheumatoid arthritis and osteoarthritis[28]. MMPs are a family of enzymes that are characterized by their extracellular matrix substrate specificity, zinc-dependent activity, extracellular inhibition by TIMP, secretion as a zymogen and sequence similarities[29,30]. Currently 23 MMP genes have been identified and include collagenase-1 (MMP-1), stromelysin-1 (MMP-3), 72-kDa gelatinase (MMP-2) and 92-kDa gelatinase (MMP-9) whose activities range from specific to broadly reactive. Although the altered expression of MMPs and their inhibitors in joint fibroblasts and chondrocytes in arthropathies has largely been attributed to proinflammatory cytokines[31,32], it is plausible that relaxin has the ability to directly modulate MMPs in fibrocartilaginous tissues of the TMJ. Indeed, relaxin causes a decrease in collagen in the pubic symphysis fibrocartilage[33,34] and has been linked to hypermobility of the pelvic joint in women during pregnancy and parturition[19]. Because of these reasons and relaxin's known induction of MMPs in many tissues, it could be postulated that synovial joints, particularly those such as the TMJ that contain large quantities of fibrocartilage, could be target sites for the tissue remodeling activities of relaxin— a hypothesis that we are testing through our ongoing laboratory, animal and clinical studies.

Relaxin induces MMPs in TMJ disc fibrocartilaginous cells but not in synoviocytes, which is accompanied by loss of disc matrix molecules

In our initial studies[35] we determined the effects of relaxin on the expression of MMPs and TIMPs in unprimed and β-estradiol-primed TMJ disc fibrocartilaginous cells. Using cells from the fibrocartilaginous TMJ disc, we demonstrated that relaxin dose-dependently induces the MMPs collagenase-1 and stromelysin-1 with minimal modulation of TIMP-1 and -2 expression. Priming of these cells with β-estradiol potentiated their MMP-inductive response to relaxin such that the maximal expression of collagenase-1 and stromelysin-1 occurred at 10- to 100-fold lower concentration of relaxin in primed than in unprimed cells. β-estradiol alone did not alter the expression of these proteinases. β-estradiol's potentitation of relaxin's matrix-degradative effects has also been reported for various reproductive tissues[24,36]. Although, the basis for this increased responsiveness of estrogen-primed tissues to relaxin is not fully understood, it may occur through the upregulation of relaxin receptors[15,16] and/or an intersection of the relaxin and estrogen signaling pathways[37]. We also found that the induction of collagenase and stromelysin was specific to fibrocartilaginous cells and was not observed in TMJ synoviocytes. In further defining the basis for relaxin's induction of collagenase-1, we identified and characterized the degree of involvement of specific collagenase-1 promoter sequences that are regulated by relaxin in fibrocartilaginous cells[38]. These studies demonstrate that collagenase-1 induction by relaxin requires both the AP-1 and PEA-3 promoter sites. Further studies showed that c-fos and c-jun, which together form the AP-1 heterodimer, and the Ets-1 transcription factor, that regulates the PEA-3 site, are upregulated in fibrocartilaginous cells exposed to relaxin or β-estradiol plus relaxin.

These findings suggest a potential mechanism by which relaxin, by targeting joint fibrocartilage, may predispose to degradative remodeling of this tissue, potentially predisposing women to musculoskeletal diseases afflicting joints containing fibrocartilaginous tissues. However, these studies in cell culture do not necessarily imply similar responses of the cells when they are in the context of their natural matrix environment. More importantly, the findings do not prove that the relaxin-mediated induction of MMPs causes a loss of matrix macromolecules. Thus, using TMJ disc fibrocarti-

laginous explants, we found that as in cell cultures, relaxin increased collagenase-1 and stromelysin-1 expression in these tissues (Naqvi et al., manuscript submitted). In contrast to our previous findings on isolated cells showing a slight decrease in MMPs by β-estradiol, this hormone-induced collagenase and stromelysin expression in disc explants. We also found that while the relaxin- or β-estradiol plus relaxin-mediated increase in MMPs were paralleled by a statistically significant loss in disc GAGs and collagen, the induction of MMPs by β-estradiol was not accompanied by changes in disc GAG or collagen. Disc explants cultured in the presence of the synthetic pan-MMP inhibitor GM6001 showed an inhibition of relaxin- and β-estradiol plus relaxin-induced collagenase and stromelysin activities to control baseline levels that were accompanied by the maintenance of collagen or GAG content at control levels. The findings show that the relaxin and β-estradiol plus relaxin-mediated increases in MMP activity contribute directly to the loss of disc collagen and GAGs.

By modulating the expression of MMPs and matrix macromolecules, relaxin with or without β-estradiol may cause or predispose to joint disease by at least two possible modalities. First, since relaxin affects the composition of collagen[39] and induces collagenase and stromelysin in ligaments (S. Kapila, unpublished observations), it could increase the laxity of the supporting structures of the joint, leading to hypermobility and subsequent osteoarthritis. This mode of action is likely to affect most joints and may be one of mechanisms by which systemic joint hypermobility occurs in women. Second, fibrocartilaginous tissue that, amongst synovial joints, is unique to the TMJ, may serve as a specific target for the matrix remodeling actions of relaxin and β-estradiol. This latter mechanism may explain the distinct age and gender distribution of TMJDs in women as opposed to the distribution of diseases of other joints.

While MMPs are known to contribute substantially to tissue degeneration in inflammatory joint diseases including rheumatoid arthritis and osteoarthritis[40-42], our findings show the direct effects of a reproductive hormone in modulating MMP expression and causing matrix loss in fibrocartilaginous tissues from a synovial joint. Since even subtle alterations in collagen and GAG composition impact upon the structural properties and the ability of joint tissues to withstand normal function, this modulation of MMPs and resulting matrix loss in the fibrocartilaginous TMJ disc by relaxin may contribute to TMJ disease. Furthermore, the findings of the present study have an implied physiologic relevance because the induction of collagenase-1 and stromelysin-1 and the loss of collagen and GAGs occurred at concentrations of relaxin found systemically in cycling women[43-45]. While the ability of systemic relaxin to access the TMJ and reach the avascular disc remains to be determined, our recent *in vivo* findings showing relaxin-mediated decreases in GAG concentration in the TMJ discs of ovariectomized rabbits lends credence to the hypothesis that this systemic hormone can indeed access the TMJ disc and contribute to degradation of this tissue (Hashem and Kapila, manuscript in preparation). Additional cohort studies on women with and without symptoms of TMJ disease are being performed with the aim to further define whether relaxin and β-estradiol are associated with this disease in a subset of symptomatic women.

Summary

The findings on the modulation of collagenase-1, stromelysin-1 and TIMPs by relaxin in TMJ disc fibrocartilaginous cells are the first to demonstrate the ability of relaxin to induce MMPs and cause matrix loss in connective tissues from synovial joints. Priming of these cells with β-estradiol increases their responsiveness to relaxin in cell cultures but not in tissue explants. This matrix-degradative response to relaxin is cell-type specific, since this hormone does not increase the expression of collagenase or stromelysin in TMJ synoviocytes. MMPs are known mediators of tissue degradation and have been strongly, albeit indirectly, implicated in joint degeneration during rheumatoid arthritis and osteoarthritis. Our studies, together with the age and gender distribution of TMJDs indicate a potential for relaxin to initiate or aggravate TMJ disease. An additional aspect in understanding the biology of relaxin on TMJ tissues is to identify its sites of action through localization of relaxin receptors. Until recently no relaxin receptors had been identified, thus limiting the use of radiolabeled relaxin to demonstrate the presence of high affinity relaxin-binding molecules on cells in the pubic symphysis, uterine myometrium, the cervix, the placenta, breast, heart and brain. The recent discovery of two receptors for relaxin[46] has opened a broad area for future inquiries including those aimed at defining the role of relaxin in TMJ degeneration.

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