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Estradiol-potentiated cadherin-11 in synovial membrane involves in temporomandibular joint inflammation in rats

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ABSTRACT

Estrogen is involved in inflammation/pain of temporomandibular joint (TMJ), but the underlying mechanisms are largely unknown. Cadherin-11 plays an essential role in synovial inflammation. This study examined whether estrogen could potentiate cadherin-11 in synoviocytes and contribute to TMJ inflammatory pain. Female rats were ovariectomized, treated with increasing doses of 17β-estradiol for 10 days, and injected intra-articularly with complete Freund's adjuvant to induce TMJ inflammation. The expression of cadherin-11 in synovial membrane was evaluated. TMJ pain was blocked with intra-articular injection of anti-cadherin-11 antibody and evaluated by head withdrawal threshold. Primary TMJ synoviocytes were treated with estradiol and tumor necrosis factor (TNF)-α or blocked with anti-cadherin-11 antibody to assess the expression of cadherin-11, interleukin (IL)-6, cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS). We observed that estradiol potentiated the inflammation-induced expression of cadherin-11 in the synoviocytes of synovial membrane from inflamed TMJ. Estradiol induced cadherin-11 expression in a dose- and time-dependent manner in primary synoviocytes and further potentiated the induction of cadherin-11 by TNF-α in synoviocytes. Furthermore, an estrogen receptor antagonist or a NF-κB inhibitor partially blocked the effects of estradiol on cadherin-11 induction in the synovial membrane. Blocking cadherin-11 partially reversed the TMJ inflammatory pain and estradiol-potentiated proliferation of synovial lining cells accompanied with iNOS expression. In addition, blocking cadherin-11 reversed TNF-α-induced and estradiol-potentiated transcription of IL-6, COX-2, and iNOS in primary synoviocytes. These results suggest that estrogen aggravated TMJ inflammatory pain partially through cadherin-11-mediated release of proinflammatory cytokines and enzymes in the synoviocytes.

1. Introduction

Pain in the temporomandibular joint (TMJ) or masticatory muscles is one of the chief complaints of patients with temporomandibular disorders (TMDs) [1]. Similar to rheumatoid arthritis, TMDs are approximately twice as prevalent (and more severe) in women than in men [2,3]. Sex hormones, particularly estrogens, are involved in TMD pain [2,4,5]. Joint inflammation is believed to be the major cause of pain in patients with TMDs [6,7]. We have previously reported that estrogen aggravates TMJ inflammation/pain by inducing proinflammatory cytokines in the synovial membrane [8]. However, the underlying mechanism remains to be elucidated.

The synovial membrane in normal TMJ consists of a lining layer of condensed cells, one- to three-cell thick, that overlies the loose connective tissue of the synovial sublining, and the lining layer is composed of fibroblast-like synoviocytes and macrophages [9]. The synovial membrane of TMD patients often shows inflammatory changes, including synovial lining hyperplasia and infiltrated inflammatory cells [10,11]. Synoviocytes producing inflammatory factors are believed to play pivotal role in the process of joint inflammation [12].

Cadherin-11 is recently reported to have an essential function in synovial inflammation and arthritis pathology

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[13]. Cadherin-11 is a classical cell adhesion molecule responsible for tissue morphogenesis and architecture [14]. High levels of cadherin-11 expression are mainly found in synoviocytes in rheumatoid arthritis and osteoarthritis tissues [15]. Furthermore, cadherin-11-deficient mice show significantly less synovial inflammation; treatment with anti-cadherin-11 antibody displays reduced severity of arthritis in the mouse model [16]. This finding suggests the key function of cadherin-11 expressed by fibroblast-like synoviocytes in joint inflammation. However, the mechanism underlying the involvement of cadherin-11 in joint inflammation, especially in TMJ inflammation, remains unknown.

Estradiol can regulate cadherin-11 expression in human endometrial stromal cells [17] and neurons of macaques [18], indicating that estradiol is an important regulator of cadherin-11 on stromal cells. Estrogen receptors are expressed on synoviocytes [19], suggesting that synovial cells could be the target of estrogens. Therefore, we hypothesized that estradiol could aggravate TMJ inflammation or inflammatory pain by regulating cadherin-11 in the synovial membrane.

This study investigated whether estrogen could upregulate cadherin-11 expression in the synoviocytes and, if so, whether estradiol-potentiated TMJ inflammation could be reversed by blocking cadherin-11.

2. Materials and methods

2.1. Animals

Adult female Sprague-Dawley (SD) rats weighing 180–200 g were used. The experimental protocols were approved by the Animal Use and Care Committee of Peking University and were consistent with the Ethical Guidelines of the International Association for the Study of Pain.

2.2. Estradiol administration and induction of TMJ inflammation

17β-estradiol (E2) administration and TMJ inflammation induction were carried out as previously described in detail [8]. In brief, rats were randomly divided into five groups with six rats for each group: control, sham, and three ovariectomized groups (0 μg-E2, 20 μg-E2, and 80 μg-E2 groups). Ovariectomized rats were subcutaneously injected with E2 (Sigma) at doses of 0, 20, or 80 μg per rat daily for 10 days. On the 10th day of E2 treatment, TMJ inflammation was induced by injecting 50 μl of complete Freund's adjuvant (CFA; Sigma) (1:1 oil:saline emulsion) or saline into the upper compartment of bilateral TMJs.

2.3. Intra-articular injection of anti-cadherin-11 antibody

Following the same E2 administration schedule, additional two sham-ovariectomized groups and two 80 μg-E2 groups ($n = 5$ for each group) of rats were intra-articularly injected twice with isotype IgG or anti-cadherin-11 antibody (10 μg; sc-30314, Santa Cruz) 24 and 0.5 h before the induction of TMJ inflammation, respectively. The anti-cadherin-11 antibody used as antagonist was described previously [16,20].

2.4. Application of NF-κB inhibitor and estrogen receptor antagonist

Pyrrolidine dithiocarbamate (PDTC), an NF-κB-specific inhibitor, and ICI 182,780, an estrogen receptor-specific antagonist, (all from Sigma) were administrated as described in our previous study [8]. Briefly, another control group and four 80 μg-E2 groups ($n = 3$ for each group) of rats were intraperitoneally injected twice with vehicle or PDTC (10 or 30 mg/kg body weight) or ICI 182,780 (500 μg per rat) 24 h before and immediately before the induction of TMJ inflammation.

2.5. Measurement of head withdrawal threshold

Head withdrawal threshold, which was negatively associated with TMJ pain, was measured as described in detail in our previous study [21].

2.6. Hematoxylin–eosin and immunohistochemistry staining

TMJs were removed en bloc and fixed in 4% paraformaldehyde, demineralized in 15% EDTA, and embedded by paraffin. TMJ blocks were sectioned (5 μm) and used for hematoxylin–eosin and immunohistochemistry staining. Immunohistochemical staining was performed with a two-step detection kit (Zhongshan Golden Bridge Biotechnology, Beijing, China) as described previously [21]. The primary antibodies against rat cadherin-11 (1:100, sc-6463, Santa Cruz) and inducible nitric oxide synthase (iNOS, 1:100; ab15323, Abcam) were also used.

2.7. Cell culture and treatments

Fibroblast-like synoviocytes were isolated from the synovial membrane of TMJs from six-week-old rats following previously described methods [22] and used for experiment between passages 4 and 6. At the passages used for stimulation, the medium was changed to phenol red-free DMEM/F12 Nutrient Mix (Gibco) containing 15% charcoal-stripped FBS (Hyclone). Cells were treated with indicated concentrations of E2 or tumor necrosis factor (TNF-α; T 5944; Sigma). Anti-cadherin-11 antibody (1 μg) was added to the media 0.5 h before treatment with TNF-α (10 ng/ml).

2.8. Western blot and quantitative real-time PCR

Twenty-four hours after the induction of TMJ inflammation, the TMJ synovial membrane was bilaterally harvested for RNA and protein extraction. The protein expression of cadherin-11 in synovial membrane and synoviocytes was assessed by western blot following the detailed method previously described [23].

Total RNA was isolated from the synovial membrane or synoviocytes with Trizol reagent (Invitrogen) according to manufacturer's instructions. Reverse transcription and real-time PCR were performed as previously described [21]. The efficiency of primers for rat β-actin, IL-6, iNOS, and cyclooxygenase 2 (COX-2) was confirmed previously [8]. The primer was designed using Primer Premier 5.0 software and commercially synthesized as follows: rat cadherin-11 sense/antisense, 5′-TCCAACCAGCCAATAGTTACAGT-3′/5′-ATCACAATGGGCAGGAGGTAGAG-3′. The efficiency of the newly designed primers was confirmed by sequence analysis.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 13.0. All data were presented as mean ± SD and assessed by ANOVA. Value of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Cadherin-11 was upregulated and further potentiated by estradiol in the hyperplastic synovial membrane of inflamed TMJ

The efficiency of E2 administration was evaluated previously and showed that the plasma levels of E2 in the ovariectomized groups increased dose dependently [8] and remained within the physiological level of the estrous cycle in normal female rats [24].