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Differential effects of high-physiological oestrogen on the degeneration of mandibular condylar cartilage and subchondral bone

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ABSTRACT

The striking predilection of temporomandibular disorders (TMD) in women, especially during gonad-intact puberty or reproductive years, indicates that oestrogen plays an important role in the progression of TMD, but the underlying mechanism remains unclear. In this study, unilateral anterior crossbite (UAC) was used to create temporomandibular joint osteoarthritis (TMJ OA) models in rats, while 17β-estradiol (E_2) injections were applied to mimic patients with high-physiological levels of oestrogen. Micro-CT scanning, histological staining and real-time PCR assays were preformed to observe the degenerative changes in the mandibular condylar cartilage and subchondral bone. The results showed that obvious degradation was found in the condylar cartilage and subchondral bone of rats with UAC procedure, including decreased cartilage thickness, loss of extracellular matrix, increased apoptotic chondrocytes and expression of pro-inflammatory and catabolic factors, decreased bone mineral density and increased osteoclast activity. E_2 supplements aggravated the condylar cartilage degradation but reversed the abnormal bone resorption in the subchondral bone induced by UAC. Our results revealed that high-physiological oestrogen plays a destructive role in condylar cartilage but a protective role in subchondral bone at the early stage of TMJ OA. These dual and distinct effects should be given serious consideration in future OA treatments.

1. Introduction

The term "temporomandibular disorders" (TMD) encompasses a spectrum of clinical signs and symptoms involving the masticatory musculature, temporomandibular joint (TMJ) and associated structures [1]. It is estimated that over 50 million Americans are afflicted by TMD, which costs approximately 4 billion dollars annually for treatment in the US [2,3]. Pain and jaw movement limitation and sounds in TMJ are common symptoms of TMD which seriously impact on both physical and psychological condition of patients [1]. But due to a poor understanding of the aetiology of TMD and the lack of definitive diagnostic or therapeutic methods, patients must often tolerate symptoms, which substantially impact their quality of life [4]. As a degenerative disease, the most severe manifestation of TMD is temporomandibular joint osteoarthritis (TMJ OA) [5], which is characterised by chondrocyte apoptosis, cartilage degradation and abnormal subchondral bone remodelling [6]. However, the detailed mechanism underlying TMJ OA remains unclear.

Interestingly, most epidemiological studies have shown a striking predilection of TMD in women. The female-to-male ratio of patients seeking care has been reported as ranging from 3:1 to as high as 9:1 [7]. Unlike similar joint diseases that also have a greater female predilection but occur in postmenopausal women, a large proportion of women with TMD are between 18 and 45 years of age [1]. The specific age and gender predisposition indicate that hormone levels, particularly oestrogen, may play an important role in TMD [4].

With this clue, many clinical and basic research studies have been performed to explore the relationship between oestrogen and TMD. Landi et al. reported that female and male TMD patients had a higher serum 17β-estradiol concentration than healthy controls [8]. Through ovariectomy, increased condylar cartilage thickness and even degenerative changes have been observed, and oestrogen replacement can restore most of the changed histomorphometric parameters [9–11].

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Studies of the effect of exogenous oestrogen on cultured TMJ cartilage blocks or chondrocytes have also suggested that oestrogen played an important role in modulating the metabolism and morphology of TMJ [12,13]. The effect of oestrogen on TMJ has also been supported by the existence of oestrogen receptors (ERs) in TMJ [14]. So, it is suggested that oestrogen and selective oestrogen receptor modulators (SERMs) may represent future therapeutic options to treat joint diseases [15].

However, a growing body of epidemiological, clinical and experimental research focusing on the effect of oestrogen on TMD has produced conflicting results. For example, in a large-sample age-matched case-control survey, the odds of having TMD were approximately 30% higher among those patients receiving exogenous oestrogen compared to those not exposed, and a clear dose-response relationship was evident [16]. In another similar survey, use of oral contraceptives (OCs), which primarily contain oestrogen and progestin, was also associated with referral for TMD care, with an approximately 20% increased risk of TMD among OC users [16]. However, another study of a stratified random sample of 510 women has shown that the muscle and joint signs and symptoms of women taking or not taking exogenous oestrogen were not significantly different, and exogenous oestrogen use failed to distinguish women receiving relatively high and low scores on the craniomandibular index [17]. Interestingly, another study showed that there may be an inverse relationship between circulating oestrogen levels and joint pain [18], which was also supported by the report that TMD pain in women was highest at times of lowest serum oestrogen [19]. Obviously, the relationship between oestrogen and TMD is complex, and the specific underlying mechanism remains unknown.

In addition, OA is a complex disease of the entire joint. It affects the bones, cartilage and synovium, thereby providing multiple treatment targets [20]. Among these different structures, bone and cartilage health are tightly associated. There are multiple nutrients, biomechanical and biochemical molecular interactions between the cartilage and subchondral bone in osteoarthritic or healthy joints [21]. An increasing line of evidence suggests that cartilage and bone metabolism, particularly subchondral bone turnover and its interaction with the articular cartilage, are even more important than previously thought [20]. Thus, development of better OA interventions targeting the entire joint instead of a single tissue, including the cartilage-subchondral bone unit, is warranted [20,22].

In the literature, few in vivo studies have focused on the effect of oestrogen on the prognosis of TMJ OA, particularly in gonad-intact animals or concurrently on condylar cartilage and subchondral bone. The purpose of the present study was to investigate the effects of high-physiological oestrogen on the degeneration of mandibular condylar cartilage and subchondral bone in gonad-intact rats.

2. Material and methods

2.1. Experimental animals

Two hundred and sixteen 6-week-old female SD rats (weight 140–160 g) were provided by the Animal Centre of the Fourth Military Medical University. All procedures and animal care were approved by the University Ethics Committee and performed according to the institutional guidelines. All animals were randomly assigned to 3 groups according to the experimental time points (2, 4 or 8 weeks), and each group was divided into 6 subgroups: the control (C) group, group with oestrogen injection at 0.2 mg/kg/day (0.2E), group with oestrogen injection at 0.5 mg/kg/day (0.5E), unilateral anterior crossbite (UAC) group, unilateral anterior crossbite with oestrogen injection at 0.2 mg/kg/day (UAC + 0.2E) group and unilateral anterior crossbite with oestrogen injection at 0.5 mg/kg/day(UAC + 0.5E) group. With12 rats in each group, all rats were housed in a pathogen-free room and fed sterilised food and redistilled water during the study.

In the UAC, UAC + 0.2E and UAC + 0.5E groups, the UAC model was established in the rats as previously described [23]. Briefly, under deep anesthetised with 1% pentobarbital sodium (0.35 ml/100 g weight), a small metal tube (length = 2.5 mm, inside diameter = 3 mm) was bonded to the left maxillary incisor with zinc phosphate cement, and another metal tube (length = 4.5 mm, inside diameter = 2.5 mm) was bonded to the left mandibular incisor in the rats. The end of latter tube was bent to a 135° angle to guide the mandible forward to create a UAC relation of the left side incisors. The rats in the C, 0.2E and 0.5E group were subjected to the same procedure but without maintaining the tubes on the incisors. Each operation was completed within 3 min, and all efforts were made to minimise suffering. During the entire experimental period, no detachment of the metal tube was found. From one week before the UAC operation, the rats in 0.2E, 0.5E, UAC + 0.2E and UAC + 0.5E group were injected with 17β-estradiol (E_2, ab120657, Abcam, Cambridge, MA, UK) subcutaneously daily in the morning, at a dose of 0.2 mg/kg/day (0.2E and UAC + 0.2E group) and 0.5 mg/kg/day (0.5E and UAC + 0.5E group) until the end of the experiment. E_2 was dissolved in DMSO and diluted to 0.2 mg/ml in saline immediately before administration. The other groups received saline injections. The experiment schedule and design are shown in Fig. 1A.

At each time point (2, 4 and 8 postoperative weeks), the body weights of the rats were measured respectively. Then, experimental rats were sacrificed with a single intraperitoneal injection of overdose pentobarbital sodium. Our previous studies have demonstrated that no significant difference was noticed in the manifestation of OA phenotype between the right and left TMJ cartilage and subchondral bone induced by UAC [23]. Thus, for each subgroup (12 rats), 12 TMJs from 6 rats were divided into 2 groups, 6 left TMJs were used for paraffin section staining (n = 6), and six right TMJs for micro-CT scanning (n = 6). While the condylar cartilage and subchondral bone from 12 TMJs of the other 6 rats were divided randomly into three separate samples (n = 3) for real-time PCR assays. Finally, the uterus was dissected integrally and weighed to assess the effect of E_2 supplement.

2.2. Tissue preparation

The 12 tissue blocks for staining and micro-CT which containing intact TMJs from 6 rats in each subgroup were dissected and fixed in 4% paraformaldehyde (pH 7.4) at 4 °C for 24 h. Then, 6 right TMJ blocks were sent to scan with micro-CT immediately, while the other 6 left TMJ blocks were continued to be decalcified in 10% EDTA for 1 month, followed by being embedded in paraffin wax and cut into 5 μm-thick serial mid-sagittal sections.

2.3. Histochemical and immunohistochemical staining

To observe the changes in morphology, catabolic factors, proliferation of chondrocytes and expression of extracellular matrix in condylar cartilage, H&E, Safranin O and IHC staining were performed, as previously reported [24]. In IHC staining, anti-Col II (diluted 1:100, sc7763, Santa Cruz, Dallas, TX, USA), anti-TNFα (diluted 1:200, ab199013, Abcam, Cambridge, MA, UK), anti-MMP-13 (diluted 1:200, 18165-1-AP, Proteintech, Wuhan, Hubei, China) and anti-Ki67 (diluted 1:200, ab11580, Abcam, Cambridge, MA, UK) primary antibodies were used, according to standard avidin-biotin complex (ABC) method. The H&E, Safranin O and IHC stained sections were analysed under a light microscope (Leica DM 2500), and images were acquired using a Leica DFC490 system (Leica, Wetzlar, Germany), as previously reported [25].

The total cartilage thickness measurements and image analysis were performed in a blinded manner, without knowing the treatments of animals. Under the images acquired by Leica DFC490 system, the surface of the cartilage was firstly equally divided into the anterior, centre and posterior thirds regions between the anterior and posterior attachment positions of the condyle to the disc. Then, the thickness of the whole layer of condylar cartilage was measured at quarter points of the centre and posterior regions in each section (Fig. 1B) using the Leica Q-win system (Leica Microsystem Imaging Solutions Ltd, Cambridge, UK), and the