Journal of Dental Sciences (2017) 12, 83–90

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

ORIGINAL ARTICLE

# Response of the temporomandibular joint tissue of rats to rheumatoid arthritis induction methods

**Ding-Han Wang [a], Mu-Chen Yang [a], Wun-Eng Hsu [b], Ming-Lun Hsu [a], Ling-Ming Yu [c*]**

[a] *School of Dentistry, National Yang-Ming University, Taipei, Taiwan*
[b] *Department of Stomatology, Veterans General Hospital, Taipei, Taiwan*
[c] *Department of Dentistry, Chang Gung Memorial Hospital, Taipei, Taiwan*

Received 27 October 2016; Final revision received 2 December 2016
Available online 11 February 2017

**KEYWORDS**
collagen-induced arthritis;
rheumatoid arthritis;
temporomandibular joint arthritis

**Abstract** *Background/purpose:* The pathogenesis of rheumatoid arthritis (RA)-related temporomandibular joint (TMJ) disorder remains unclear. Studies have reported the change of the TMJ after complete Freund's adjuvant (CFA) injection, which is consistent with osteoarthritis. However, few studies have reported that the tissue response of the TMJ in collagen-induced arthritis (CIA) can mimic RA. The present study was aimed to investigate the TMJ response in rat models by CFA-induced arthritis and CIA to verify the proper RA-related TMJ arthritis rat model.

*Materials and methods:* In total, 24 rats were randomly divided into four groups: (1) control group; (2) type I collagen injection group; (3) CFA-induced arthritis group; and (4) CIA group. Drugs were injected on Day 0, and the rats were sacrificed on Days 7 and 35. Next, TMJ tissue was collected for hematoxylin and eosin staining, and inflammatory gene (*IL-1β and MMP3*) expression was investigated.

*Results:* Compared with the control group, the type I collagen injection group confirmed the negative inflammatory response through hematoxylin and eosin staining and *IL-1βand MMP3* expression. Although CFA-induced arthritis and CIA groups showed inflammatory response ($P < 0.05$) compared with the control group, histological changes were different. The 7-day CFA-induced arthritis group showed adaptive changes and partly recovered after 35 days of induction. In contrast, 7- and 35-day CIA groups underwent a degenerative process.

*Conclusion:* Considering the study limitations, the CIA method is a proper method to study the mechanism of RA-related TMJ arthritis.



* Corresponding author. Department of Dentistry, Chang Gung Memorial Hospital, 199 Tung-Hwa Road, Taipei, Taiwan.
*E-mail address:* b101092037@gmail.com (L.-M. Yu).

http://dx.doi.org/10.1016/j.jds.2016.12.001

## Introduction

Temporomandibular joint (TMJ) arthritis induced by rheumatoid arthritis (RA) or osteoarthritis (OA) are common in clinical settings. Inflammatory response of patients may damage the joints and affect their normal function.[1] TMJ arthritis may limit jaw movement or cause chewing difficulty, TMJ disc internal derangement, and even bone destruction. Some of the studies reported that OA is the most common type of arthritis in human body that can also affect the TMJ function.[2–4] There were 42.6% of patients with TMJ disorder presenting with radiographic evidence of TMJ-OA changes.[5] The destructive process of TMJ can be observed in OA patients. Their bony surfaces of condyle and fossa become altered because of inflammatory response; radiographic images may show flattening of the condyle.[6,7] Furthermore, osseous changes of condyle also occur in response to TMJ-OA and lead to disc displacement, frequently with longstanding disc displacement without reduction.[8] On the other hand, Lin et al[9] reported a higher prevalence of TMJ abnormalities in patients with RA, as determined through physical (85.7%) and radiological (74.5%) examinations. Studies indicated that RA is derived from a complex interaction between genes and environment, leading to a breakdown of immune tolerance, synovial inflammation, and autoantibody production. Distinct mechanisms may induce joint swelling and tenderness and ultimately bone destruction and joint deformity.[10–12] With damage to the joint tissues, severe osseous changes can occur in the TMJ-RA patients. TMJ-RA can cause painful symptoms of the joint and cause destruction of the temporomandibular articular surfaces.[13–15]

TMJ inflammation is a key factor of OA and RA; however, the detailed degeneration process in the inflamed TMJ remains unclear. In studies regarding TMJ-OA, complete Freund's adjuvant (CFA)-induced arthritis has been widely used in physiological, biochemical, and histopathological studies.[16–18] CFA is a solution of antigen emulsified in mineral oil and consists of inactivated and dried mycobacteria. It has been injected into the intra-articular space of the joint. Edema and pain are produced within 12 hours. After that, articular cartilage destruction and bone erosion may persist for several weeks. In CFA-induced arthritis model, higher level of proinflammatory cytokines, such as TNF-$\alpha$, IL-1$\beta$, and IL-6, can be detected in the serum and synovial fluid. Proinflammatory cytokine expression in the inflamed synovial membrane of arthritis leads to an imbalance in bone and articular cartilage formation.[19]

However, the pathogenesis of CFA-induced TMJ arthritis in an animal model is more similar to OA than to RA. OA and RA are different types of arthritis.[20] The development of OA is driven by inflammatory processes and finally leads to joint degeneration. RA is an autoimmune disease which will lead the body's immune system to attack its own tissues. These two diseases share some similar characteristics, but each has different symptoms and requires different treatment modalities.[21] Therefore, the animal model of CFA-induced TMJ arthritis is not appropriate for research in TMJ-RA. Among the induced method of RA animal models, Bolon et al[22] used different arthritis-inducing methods, such as adjuvant-induced arthritis, collagen-induced arthritis (CIA), and streptococcal cell wall-induced arthritis, to evaluate the appropriateness of these methods for inducing RA in knee joint. Results indicated that the immunological change of CIA was similar to that of RA. CIA has been widely used in experimental model of RA by the injection of heterologous collagen emulsified in CFA.[23,24] Collagen is highly conserved between different species, and the pathogenesis of CIA is due to heterologous collagen eliciting cross-reactive autoimmune responses to endogenous collagen and then both CD4 T-cells activation and the production of anticollagen antibody, leading to CIA.[25] The role of anticollagen antibodies is as direct mediators of tissue pathology in the spontaneous autoimmune disease. The anticollagen antibody reaction which will lead to complement activation and cause inflammatory response.[26] However, most of the RA studies were focused on the knee joint, but few studies have discussed animal models of RA-related TMJ arthritis. This means that the experience of CIA in knee joint of RA animal model may help to clarify the RA-related TMJ arthritis. Based on the above researches, the aim of this study was to investigate the response of the TMJ tissue by CFA-induced arthritis and CIA. The experiments were designed to clarify: (1) Did CIA have the same inflammatory response as CFA-induced arthritis? (2) Did CIA have the same TMJ change as CFA-induced arthritis? (3) Was CIA more appropriate than CFA-induced arthritis in RA-related TMJ arthritis research?

## Material and methods

### Rat TMJ injection technology

All animal protocols were performed in accordance with the protocol of the Institutional Animal Care and Use Committee of National Yang-Ming University. The animals were housed in a temperature controlled environment under a 12-hour light/dark cycle. In this study, 8-week-old female Sprague-Dawley rats weighing 250—320 g were used. Furthermore, 50 $\mu$L of alcian blue dye was injected into the superior space of the TMJ to demonstrate the TMJ injection technology. Next, the rats were sacrificed to evaluate the accuracy of TMJ injection technology.

### Drug-induced TMJ arthritis model

In total, 24 rats were randomly divided into four groups. The first group was the control group, which did not receive any drug injection. The second group was the collagen I group, which received 50 $\mu$L of type I collagen (sc-29009; Santa Cruz; Dallas, USA), to determine whether type I collagen alone leads to rat arthritis. The third group was the CFA-induced arthritis group; the induced method of this group was followed by other previous studies.[16,17,26] This group was injected with 50 $\mu$L of CFA (F5881; Sigma; St. Louis, USA). The fourth group was the CIA group, which received 50 $\mu$L of type I collagen and CFA (1:1). The injection dose was as followed in previous studies in knee joint and TMJ.[16,22] The drugs were injected on Day 0, and the rats were sacrificed on Days 7 and 35. After the rats were sacrificed on Day 7, the TMJ tissue was collected to