Attenuation of Pro-inflammatory Neuropeptide Levels Produced by a Cyclooxygenase-2 Inhibitor in an Animal Model of Chronic Temporomandibular Joint Inflammation

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Aims: *To study the neurogenic effects of a cyclooxygenase-2 (COX-2) inhibitor, rofecoxib, in an animal model of persistent inflammation.* ***Methods:*** *Arthritis was induced within the temporomandibular joint (TMJ) by placing complete Freund's adjuvant (CFA) within the superior joint space of the TMJ in adult male rats. The CFA animals were divided into 2 groups, with 1 group given the COX-2 inhibitor, rofecoxib, on days 21 through 28. Tissues were taken from experimental and control animals 4 weeks post-injection and analyzed by radioimmunoassay. The inflammatory-related neuropeptide, immunoreactive calcitonin gene-related peptide (CGRPi), was assayed from both the TMJ tissues and the trigeminal brain stem subnucleus caudalis.* ***Results:*** *CGRPi content was significantly increased in TMJ tissues within the untreated CFA group (72%) and was found to be effectively no different between the CFA/COX-2 group and controls. Trigeminal brain stem subnucleus caudalis CGRPi levels were not different between the groups.* ***Conclusion:*** *These results suggest that use of an inhibitor selective for the inducible form of cyclooxygenase enzyme, COX-2, may significantly attenuate the neurogenic component in an inflammatory TMJ animal model.*
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Key words: calcitonin gene-related peptide, temporomandibular joint, experimental arthritis, inflammation, complete Freund's adjuvant

Peripheral inflammation induces the metabolism of arachidonic acid by cyclooxygenase enzymes, which leads to the production of prostanoids such as prostaglandin E_2 (PGE_2). The prostanoids, in turn, cause the sensitization of local nociceptors, which enhance local stimulation (for review, see Fields[1]). Associated with the same peripheral inflammation and contributing to local hypersensitivity is the release of pro-inflammatory neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP).[2,3] Release of these same neuropeptides from the affected nociceptors centrally causes sensitization of the neighboring sensory neurons and leads to secondary hyperalgesia in the adjacent uninjured tissue.[4–6] Thus, an increased release of these neurotransmitters results in the exacerbation of inflammatory-related problems and has been suggested to play a role in the pathologic long-term or persistent sensitization of neurons within the nociceptor pathways.[3,7–11]

Inflammation can result from several sources such as infection, mechanical trauma, and disease conditions such as arthritis. Inflammation from these sources is also associated with some tem-

poromandibular disorders (TMD) and, as such, has been the source of a number of studies.[12–23] This is important because one of the more difficult TMD problems involves the patient with chronic pain complaints. Taken together, these previous studies concerning TMD patients[24–26] and related animal studies[20,27] involving inflammation of the temporomandibular joint (TMJ) have led to the following hypothesis: The peripheral inflammatory component of chronic pain patients with TMD may lead to central pathologic changes. Therefore, if the production of prostanoids could be blocked with a cyclooxygenase inhibitor, it might be possible to block the associated neurogenic cascade. This study was designed, therefore, to use the cyclooxygenase inhibitor rofecoxib to test for changes in a neurogenic pro-inflammatory indicator, CGRP, in a TMJ model of persistent inflammation.

Methods

Experimental Procedure

Twenty-four adult male Sprague-Dawley rats weighing approximately 250 g each were randomly placed into 1 of 3 groups and anesthetized with a solution of ketamine (87 mg) and xylazine (13 mg) at a dose of 0.1 mL/100 g body weight. Two groups of animals (n = 10 in each group) were injected on 1 side with 50 μL complete Freund's adjuvant (CFA; 50 μg of heat-killed *Mycobacterium tuberculosis,* 50 μL in paraffin oil; Sigma) within the superior joint space of the TMJ, while the third group (n = 4) served as the uninjected control. One of the groups that received an adjuvant-induced inflammation was given the oral cyclooxygenase-2 (COX-2) inhibitor rofecoxib 3 weeks post-injection. Each day, for 1 week, the animals were given rofecoxib (1.5 mg/kg twice daily)[28] with a 13-gauge feeding needle (Popper & Sons).

Food intake was monitored for the first 7 days and body weights were monitored for the entire study. All animal procedures followed the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Texas A&M University System Health Science Center, Baylor College of Dentistry.

Tissue Preparation

Animals were sacrificed by decapitation after 4 weeks. The skin was reflected and the superficial temporalis and masseter muscles were removed, freeing the TMJ tissues from the zygomatic arch and underlying condyle. The retrodiskal tissue, disk, and anterior attachments were subsequently removed. To assay the trigeminal brain stem subnucleus caudalis, the brain was removed and the caudalis tissue was dissected from approximately –4.0 mm interaural to the C1/C2 spinal cord level.[29] All tissue was immediately frozen with liquid nitrogen and stored at –80°C.

Radioimmunoassay

Routine radioimmunoassay (RIA) techniques were used to assay for immunoreactive CGRP (CGRPi). Briefly, tissue was placed in buffer containing peptidase inhibitors, heated for 10 minutes at 96°C, and homogenized. One hundred microliters of each sample were taken and assayed with an RIA kit for CGRPi (Phoenix Pharm). In addition, protein content was determined by a standard Lowry assay,[30] and the results were normalized to 1 mg of protein per sample. Data were then compared in a Student *t* test, with significance indicated when $P \leq .05$.

Results

Acute inflammatory changes were monitored indirectly by measuring food intake during the first week. Food intake and body weight initially dropped in the CFA-injected animals; however, all animals had all regained their normal daily eating patterns after 2 days. Their body weights also showed normal weight gains beginning 2 days post-injection (data not shown), and this weight pattern following adjuvant-induced TMJ inflammation effectively supported earlier observations.[21]

After 10 days, the CFA-related inflammation typically begins to take on characteristics of a chronic inflammation,[22] which is well established by 4 weeks.[31,32] Results from the assay support these previous observations, as TMJ tissue from the CFA-treated group exhibited a significantly greater CGRPi content than either the control or CFA/COX-2–treated groups (Table 1). Analysis of these same tissues also demonstrated that the COX-2–treated TMJ tissues had similar CGRPi content when compared to control TMJ tissues (Fig 1). However, all 3 groups exhibited similar levels of CGRPi content within the trigeminal brain stem subnucleus caudalis (Table 1).

Table 1 CGRPi Content in Different Groups

Group	Mean	SEM
TMJ		
CFA/COX-2	180.3	17.47
CFA only	276.8*	19.19
Control	199.7	31.86
Brain stem		
CFA/COX-2	485.8	68.91
CFA only	525.2	59.26
Control	502.2	24.32

CGRPi is measured in pmol/mg protein with *significance set at $P \leq .05$.
SEM = Standard error of the mean.

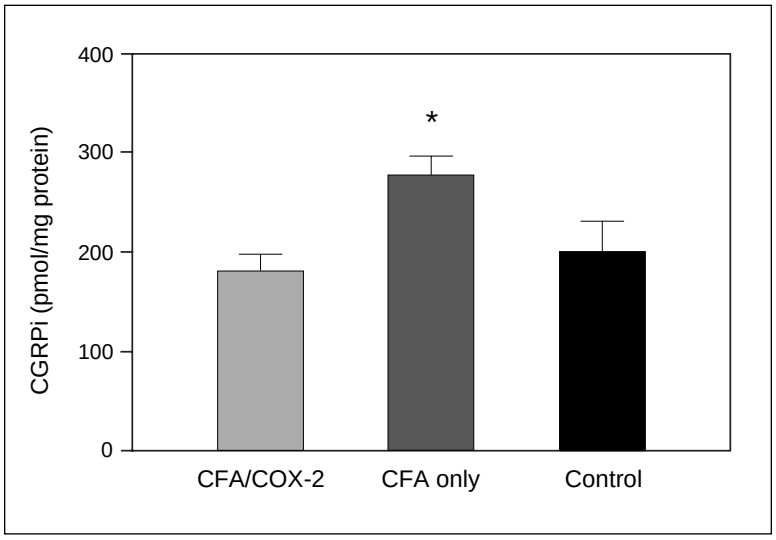

Fig 1 Bar graph illustrating the TMJ content of CGRPi following 4 weeks of CFA-induced inflammation. *$P \leq .05$.

Discussion

Arthritis-Related Inflammation

Arthritis is generally described as an inflammatory process affecting joints, typically beginning within the body's smaller joints and eventually spreading to include larger joints such as the knee or hip. Rheumatoid arthritis is considered one of the arthritides and is an inflammatory disease of the connective tissues. CFA has been characterized as inducing a rheumatoid-like arthritis,[18,22,33–35] which may include over time inflammation of the surrounding tissues.[22] Diagnostic criteria for identifying rheumatoid arthritis typically include, but are not limited to, swelling in 3 or more joints for more than 6 weeks, morning stiffness for more than 1 hour for more than 6 weeks, and positive rheumatoid factor.[36] Unfortunately, the problem for dental professionals is how to effectively treat patients whose TMJs have become involved. Although there is some disagreement on the specific clinical criteria that may indicate a TMD, the more significant criteria that have been used for the diagnosis of rheumatoid arthritic involvement of the TMJ are radiographic signs of erosion of the articular cortical bone, bilateral joint involvement, and the presence of a significant monocyte and plasma cell population.[37] Therefore, it would be of some significance if there was a treatment that might address at least some of these arthritic-related problems.

Adjuvant-Induced Inflammation

This laboratory has been involved with the characterization of an animal model that will mimic not only a typical acute inflammatory process, but also

one that mimics very closely the chronic inflammation observed in rheumatoid arthritis.[21,22] Therefore, in this study, we relied on a model of persistent inflammation to test a new class of non-steroidal anti-inflammatory drugs, the COX-2 inhibitors. The animal model is created by the introduction of an endotoxin, a heat-killed *Mycobacterium tuberculosis* in paraffin oil (ie, CFA), into the superior joint space of the TMJ. As a result of the adjuvant-induced inflammation, inflammatory-related peptides become elevated in the peripheral tissues of the TMJ[15,22,38] as well as in the central nervous system.[22,32] Our data reflect a peripheral elevation in this chronic model of inflammation; however, in contrast to previous studies, we were unable to demonstrate any such elevation in the trigeminal subnucleus caudalis, which was unfortunate, as we were effectively unable to demonstrate any central effect that may have been attributable to the COX-2 inhibitor. Although the contradictory data may reflect some methodologic difference between the studies, the resolution of this disparity might result if we were to use a larger number of animals in the future. Nevertheless, the inflammatory-related neuropeptide, CGRP, was clearly elevated within the adjuvant-injected animals, and there was an equally clear reduction of CGRPi following treatment with the COX-2 inhibitor.

COX-2 Inhibitors

Cyclooxygenase was originally identified in the early 1970s[39,40] as the enzyme necessary for the production of prostaglandins. However, with the identification of 2 enzymes, cyclooxygenase-1 (COX-1) and a second cyclooxygenase enzyme (COX-2), as the inducible isoform needed for the production of the pro-inflammatory prostaglandins (eg, PGE_2),[41–43] came the potential for producing a drug designed specifically for inhibiting the COX-2 enzymes. Non-selective COX inhibitors such as acetylsalicylate (aspirin) or ibuprofen have long been associated with a beneficial reduction in inflammation, pain, and fever as well as the unwanted side effects of gastric erosions and ulcers. With the advent of the COX-2 inhibitors, it was predicted that there would still be a beneficial reduction of inflammatory symptoms, while the toxic side effects associated with inhibiting the COX-1 enzymes would be avoided. Previous pharmacologic studies have described COX-2 anti-inflammatory drugs as highly selective for their inhibition of the COX-2 isoform. Specifically, rofecoxib has been reported to be 1,000 times more selective for the inhibition of COX-2 in Chinese hamster cells expressing human COX-2 compared to COX-1 inhibition.[28]

It is important to note that CGRPi levels were significantly reduced in the inflamed/rofecoxib–treated group (CFA/COX-2) and approximated control levels. Therefore, in this study, our findings support the hypothesis that the COX-2 inhibitor rofecoxib can help reduce or eliminate the signs and symptoms of a clinically relevant TMJ-related inflammatory disorder. This also suggests that rofecoxib was able not only to reduce the prostanoid levels, but also to attenuate the neurogenic inflammatory cascade during the chronic phase of an adjuvant-induced inflammation. Moreover, the underlying assertion is that reducing the prostanoid-related neurogenic response may potentially eliminate any central pathologic changes.

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