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Intra-articular Injection of IL-1 β Facilitated Formalin-induced Temporomandibular Joint Pain in Freely Moving Rats

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The present study was performed to investigate the effects of intra-articular injection of interleukin-1 β (IL-1 β) on the formalin-induced temporomandibular joint (TMJ) pain. Under anesthesia, a 30-gauge needle was introduced into the right TMJ region for injection of formalin. Microinjection of 50 μl of 5% formalin significantly produced noxious scratching behavioral response, and the scratching behavior lasted for 40 min. Although the responses produced by formalin injection were divided into two phases, the response of 1st phase did not significantly differ from the scratching behavior response in the saline-treated group. We examined the effects of intra-articular injection of IL-1 β on the number of noxious behavioral responses produced by 50 μl of 5% formalin injection. Intra-articular injection of 100 pg and 1 ng of IL-1 β significantly increased the number of behavioral responses of the 2nd phase, while 10 pg of IL-1 β did not change the formalin-induced behavioral responses. To investigate whether IL-1 receptor was involved in the intra-articular administration of IL-1 β-induced hyperalgesic response, IL-1 receptor antagonist (IL- ra, 50 ng) was administrated together with IL-1 β injection. IL-1 β receptor antagonist blocked IL-1 β- induced hyperalgesic response in the TMJ formalin test. These results suggest that intra-articular injection of IL-1 β facilitated the transmission of nociceptive information in the TMJ area.

Key Words: Cytokines, Hyperalgesia, IL-1 β, TMJ, Formalin test

INTRODUCTION

Peripheral tissue injury or inflammation increases responsiveness to noxious stimuli or exaggerates pain behavior, including hyperalgesia. Interleukin-1 β (IL-1 β) is released from activated macrophages and monocytes during infection and plays an important role in acute inflammatory responses (Dinarello, 1998). Several studies have demonstrated involvement of IL-1 β in pain modulations. Systemic administration of IL-1 β produced a potent hyperalgesic response and enhanced pain reflexes (Ferreira et al, 1988). Peripheral or subcutaneous injection of IL-1 β also produced thermal hyperalgesic response in rats (Maier et al, 1993; Watkins et al, 1994; Safieh-Garabedian et al, 1995) and pmechanical allodynia in the orofacial area of rats (Ahn et al, 2004). These results indicate that peripheral IL-1 β plays an important role in the pain processing in the orofacial area.

Although pain in the temporomandibular joint (TMJ) is one of chief complaints of patients with temporomandibular disorders, underlying mechanisms of TMJ pain remain poorly understood. This is due in part to the limited experimental models available to study this condition. Therefore, the development of experimental models to study the underlying mechanisms of TMJ pain conditions and different pharmacological approaches that can efficiently be used to treat TMJ pain have great clinical relevance. Recently, TMJ pain model has been developed by injection of formalin into the TMJ region of rats: Intra-articular injection of formalin into the TMJ significantly produced noxious scratching behavioral response (Roberoni et al, 2001).

Injury to the TMJ can cause inflammation of the TMJ and surrounding tissues. IL-1 β is released in the synovium of TMJ during antigen- and CFA-induced arthritis (Harper et al, 2001; Tominaga et al, 2001). The concentration of IL-1 β is high in synovial fluid from the TMJ of human patients with rheumatoid arthritis (Rooney et al, 1990; Kubota et al, 1997; Alstergren et al, 1998; Nordahl et al, 1998; Takahashi et al, 1998; Costello et al, 2002). These results indicate that IL-1 β seems to be one of the key mediators in the inflammatory process and contributes to destruction of cartilage in inflammatory joint diseases such as rheumatoid arthritis (Arend & Dayer, 1990). Furthermore, although these reports suggest that IL-1 β is involved in nociceptive response in the TMJ region, direct behavioral evidence for participation of IL-1 β in the processing of TMJ pain has been lacking.

The present study was performed to investigate the role of peripheral IL-1 β in the processing of TMJ pain. For this purpose, effects of intra-articular injection of IL-1 β on the formalin-induced TMJ pain model was examined in freely moving rats.

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ABBREVIATIONS: TMJ, temporomandibular joint; IL-1 β, interleukin-1 β; CFA, complete freund's adjuvant; IL-1ra, interleukin-1 receptor antagonist.

MEHTODS

Animals

Experiments were carried out on 63 Sprague-Dawley male rats, weighing 220~280 g. Rats were maintained in a temperature-controlled room (23±1°C) with a 12/12 h light-dark cycle. All procedures involving the use of animals were approved by the Institutional Care and Use Committee of the School of Dentistry, Kyungpook National University and carried out in accordance with the ethical guidelines for the investigation of experimental pain in conscious animals of the International Association for the Study of Pain.

TMJ-formalin test in freely moving rats

Each animal was first placed in a test chamber (30×30×30 cm) for a 30 min habituation period to minimize stress, as described previously (Abbott et al, 1986). Rats did not have access to food or water during the test. A formalin test was applied to the TMJ region, as previously described with modification (Roberoni et al, 2001). After the period of adaptation, the animal was removed from the test chamber and lightly anesthetized by inhalation of 5% halothane to allow the TMJ injection. Under anesthesia, a 30-gauge needle was introduced into the right TMJ region for injection of formalin. Fifty μl of 5% formalin was injected into the TMJ region. Following the TMJ injection, the rats were given 2 min to recover from the anesthesia before behavioral observations began and were returned to the test chamber for 45 min observation period. For each animal, the number of noxious behavioral responses, such as grooming, rubbing and/or scratching the facial region (Roberoni et al, 2001), was recorded for 9 successive 5-min intervals. To minimize the possibility that the behaviors induced by formalin might have resulted from its effect on regions outside the TMJ region, off site injections were performed: same volume of formalin previously used was injected into the right masseter muscle, and saline was also injected into the TMJ region as a control for formalin injection. Behavioral responses were measured by an investigator blinded to the animal's group assignment.

Effect of intra-articular injection of IL-1β on formalin induced TMJ pain

IL-1β (10 pg, 100 pg or 1 ng/ 10 μl) was administered into the TMJ region 1 hour prior to injection of formalin. After the administration of IL-1β, we examined changes in the number of behavioral responses produced by injection of formalin into the TMJ region. To investigate whether IL-1 receptor was involved in the IL-1β-induced hyperalgesic response, interleukin-1 receptor antagonist (IL-1ra, 50 ng/ 10 μl) was administered together with IL-1β. IL-1β and interleukin-1 receptor antagonist were dissolved in sterile normal saline.

Verification of inflammation

In order to confirm the TMJ injection site at post-mortem, formalin induced plasma extravasation of Evans' blue dye bound to plasma protein was measured, as described previously (Harada et al, 1971; Haas et al, 1992). This procedure also confirms that the plasma extravasation induced by the TMJ injection was restricted to the TMJ region. After the termination of each experiment, the animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.), and Evans' blue dye (0.1%, 5 mg/kg) was injected into the right femoral vein. Ten minutes after the injection, each rat was perfused through the heart with normal saline. Joint tissues were dissected from right-hand side, weighed and stored at −20°C until analysis. The tissues were incubated overnight in a 7 : 3 mixture of acetone and 5% sodium sulphate solution at room temperature with intermittent shaking. After incubation, samples were centrifuged at 300 rpm for 10 minutes and the supernatant was separated. The samples were analyzed for the amount of the dye present by spectrophotometrically measuring absorbance at 620 nm. The recovery of the extravasated dye per gram weight of tissue (μg/g) was calculated by comparing the absorbency of the supernatant with a standard curve. The standard curve was generated from a series of the same extraction solution mixed with known amount of Evans' blue dye.

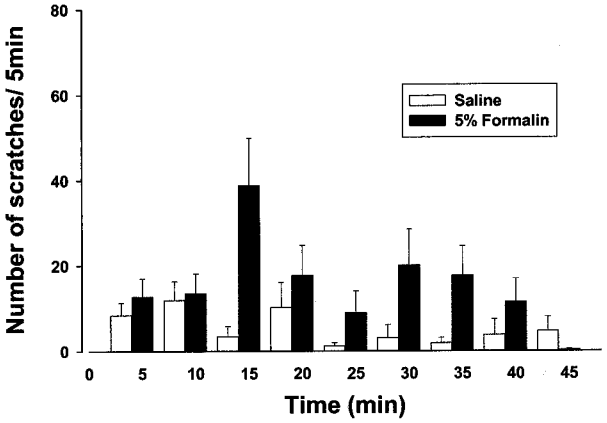

Fig. 1. Time course of formalin induced behavioral responses in TMJ. Animals were intra-articularly injected into the TMJ region with a 50 μl of 5% formalin solution. The number of behavioral responses was measured for 9 successive 5-min intervals. Nine animals were in each group.

Statistical analysis

Statistical analysis of post injection means behavioral data was carried out with one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc analysis. Comparisons between two means were performed by Student's T-test. In all statistical comparisons, $p<0.05$ was used as the criterion for statistical significance. All data are represented as mean standard error.

RESULTS

In the present study, formalin-induced TMJ pain model was used as a pain assessment method in freely moving rats. Time course of formalin induced behavioral responses are illustrated in Fig. 1. Rats received 50 μl intra-articular injection of 5% formalin solution into the TMJ region. Microinjection of 50 μl of 5% formalin significantly produced

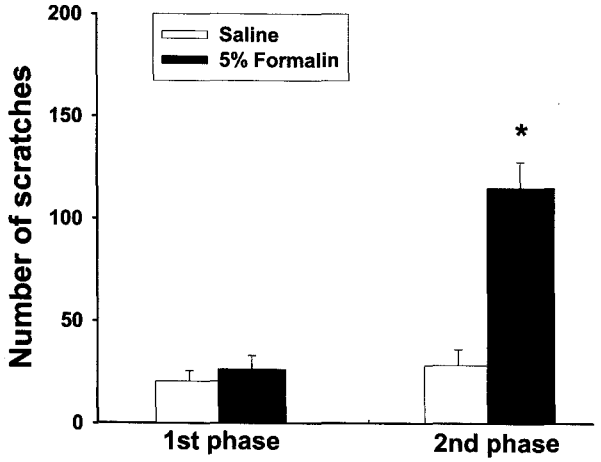

Fig. 2. The TMJ formalin responses were divided into two phases separated by a time of relative inactivity with an early short lasting response (1st phase; 0~10 min) and a continuous prolonged response (2nd phase; 10~45 min). Nine animals were in each group. *$p<0.05$, saline- vs. formalin-injected group.

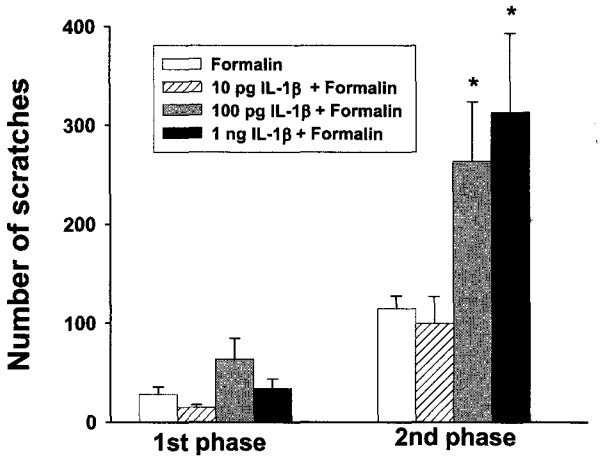

Fig. 4. Effects of intra-articular injection of IL-1β on the formalin induced behavioral responses in TMJ. Intra-articular injection of IL-1β increased the number of scratches of the 2nd phase. Nine animals were in each group. *$p<0.05$, formalin- vs. IL-1β+ formalin-injected group.

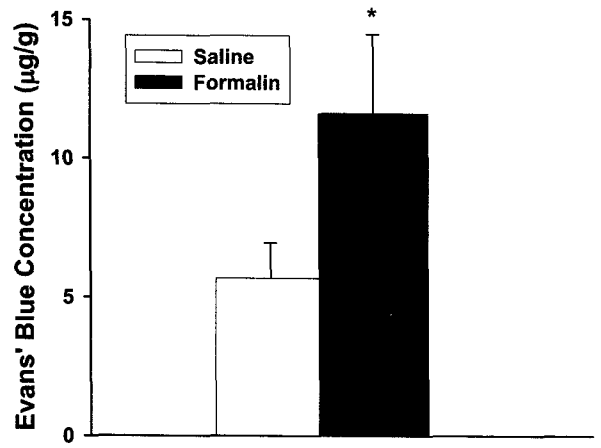

Fig. 3. Formalin-induced TMJ inflammation as indicated by plasma extravasation. Evans' blue dye was extracted from the inflamed tissue and spectrophotometically measured at 620 nm *$p<0.05$, saline- vs. formalin-injected group.

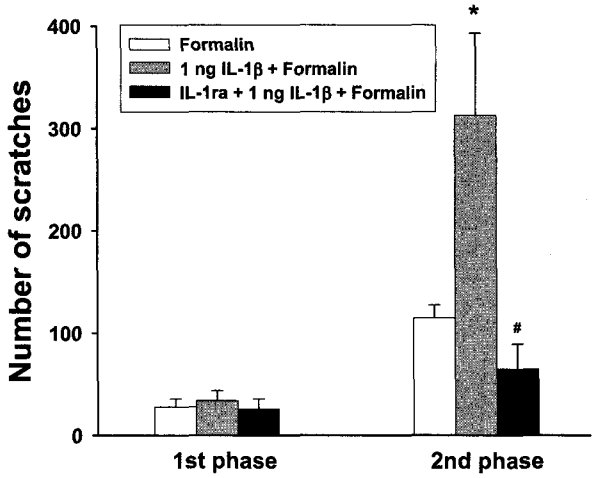

Fig. 5. Effect of co-administration of interleukin-1 receptor antagonist (IL-1ra, 50 ng) on the hyperalgesic response produced by IL-1β injected into the TMJ. Co-administration of interleukin-1 receptor antagonist abolished the increases in the number of scratching responses of the 2nd phase. Nine animals were in each group. *$p<0.05$, formalin- vs. 1 ng of IL-1β-injected group. #$p<0.05$, 1 ng of IL-1β- vs. IL-1 ra+1 ng of IL-1β-injected group.

noxious scratching behavioral response and the scratching behavior lasted for 40 min. The microinjection produced 114 ±52 in number of scratches ($p<0.05$) of the 2nd phase, while it did not change the scratching behavior compared with the saline-treated group of the 1st phase (Fig. 2). Generally, behavioral responses produced by formalin-induced pain model are composed of two distinct phases (Choi et al, 2002 & 2003a, b, c; Clavelou et al, 1989 & 1995): Two phases are separated by a time of relative inactivity with an early short lasting response (0~10 min, 1st phase) and a continuous prolonged response (10~45 min, 2nd phase). The present study demonstrated that an injection of formalin into the TMJ region did not show two distinct phases, because the early phase was masked by the anesthesia for the TMJ injections. However, it is well known that the early short lasting response is possibly caused by the direct effect of formalin on the sensory receptors, and that continuous prolonged responses are due to the inflammatory components with the release of different pain mediating substances and central sensitization. The later response characterizes clinical pain better than the early phase provoked by a transient stimulus.

After the conclusion of each experiment, Evans' blue dye

(0.1%, 5 mg/kg) was injected to confirm the TMJ injection site at post-mortem as described above. The changes in the plasma extravasation of Evans' Blue after injection of formalin into the TMJ region are illustrated in Fig. 3. Compared with the animals receiving saline, administration of formalin into the TMJ region significantly increased the extravasated Evans' Blue dye concentration ($p<0.05$).

The effects of IL-1β injected intra-articularly on the number of behavioral responses are illustrated in Fig. 4. Intra-articular administration of saline or 10 pg of IL-1β did not change the number of behavioral responses produced by formalin injection both in the 1st and the 2nd phases. However, intra-articular injection of 100 pg or 1 ng of IL-1β significantly increased the number of scratches in the 2nd phase by 130% or 174% (263 ± 60 or 313 ± 80 in number of scratches, $p<0.05$), respectively.

To investigate whether IL-1 receptor was involved in the hyperalgesic response of IL-1β injected intra-articularly, IL-1 receptor antagonist (IL-1ra, 50 ng) was administrated together with IL-1β. IL-1 receptor antagonist co-administered with saline did not produce any behavioral response in the TMJ. However, IL-1 receptor antagonist administered together with IL-1β decreased the number of scratches in the 2nd phase from 313 ± 80 to 64 ± 24 ($p<0.05$).

DISCUSSION

The present study demonstrates the hyperalgesic effect of intra-articular administration of IL-1β on the formalin-induced TMJ pain in freely moving rats. We used formalin-induced TMJ pain model as a pain assessment method as described previously (Roberoni et al, 2001). Intra-articular injection of formalin into the TMJ region of rats produced nociceptive scratching behavioral responses. Generally, in the formalin test, behavioral responses of animals produced by the subcutaneous injection of formalin into the forepaw or hindpaw are made of two phases. The behavioral responses by the orofacial formalin test also show two distinct phases (Clavelou et al, 1989 & 1995; Choi et al, 2002 & 2003a, b, c). In the present study, however, the injection of formalin into the TMJ region of rats showed only one phase, because the first phase was apparently masked by the inhalation of halothane to allow the TMJ injections. It is important to point out that TMJ injections without any type of anesthetic induction would be practically impossible and ethically unacceptable because the TMJ is a deep tissue with difficult access (Roberoni et al, 2001). Although formalin-induced TMJ pain behavioral responses did not produce any significant facilitation in the first phase, it is known that the early short lasting response is possibly caused by the direct effect of formalin on the sensory receptors and continuous prolonged responses due to the inflammatory components with the release of different pain mediating substances and central sensitization (Dubuisson & Dennis, 1977; Hunskaar & Hole, 1987; Tjolsen et al, 1992). The later response, such as described in the present study, better characterizes overt pain and bears more resemblance to clinical pain than that provoked by a transient stimulus.

After the termination of each experiment, Evans' blue dye (0.1%, 5 mg/kg) was injected systemically in order to confirm the TMJ injection site at post-mortem, as previously described (Haas et al, 1992; Fukuoka et al, 1994). In the present study, administration of formalin into the TMJ region significantly increased plasma extravasation of the dye bound to plasma protein, compared with saline injection. Formalin-induced inflammation was verified and quantified by measuring tissue Evans' blue extravasation. Swelling was caused by increased capillary permeability and subsequent plasma extravasation of fluids and proteins. Evans' blue dye binds to plasma proteins normally contained within the vasculature, but released with increased vascular permeability. To eliminate the possibility that the behaviors induced by formalin might have resulted from its effect on regions outside the TMJ region, off site injections were performed: same volume of formalin previously used was injected into the right masseter muscle. Off site injection of formalin did not increase the plasma extravasation of the dye in the TMJ, indicating that TMJ inflammation was induced by formalin injection into the TMJ region.

The present results demonstrated that intra-articular injection of 100 pg or 1 ng of IL-1β increased the number of scratching behavioral responses produced by injection of formalin into the TMJ region. These results are consistent with previous reports that peripheral cytokines are involved in facilitation of pain transmission. Peripheral IL-1β plays an important role in cutaneous hyperalgesia by activating polymodal receptors to mechanical and thermal stimulation in rats (Fukuoka et al, 1994) and peripheral or subcutaneous administration of IL-1β produced thermal hyperalgesia in rats (Ferreira et al, 1988; Watkins et al, 1994; Safieh-Garabedian et al, 1995; Roberoni et al, 2001) and mechanical allodynia in the orofacial area of rats (Ahn et al, 2004). These results indicate that peripheral IL-1β is involved in hyperalgesia. Several studies have also demonstrated IL-1β in the TMJ region to be involved in inflammation or hyperalgesia. Injury to TMJ can cause inflammation of the intracapsular and surrounding tissues, and IL-1β is released in the synovium of TMJs during antigen and CFA-induced arthritis (Harper et al, 2001; Tominaga et al, 2001). Moreover, high concentrations of IL-1β in arthritic synovial fluid are associated with high disease activity as well as bone and cartilage destruction (Tjolsen et al, 1992; Maier et al, 1993; Safieh-Garabedian et al, 1995; Alstergren et al, 1998; Roberoni et al, 2001). These results indicate that IL-1β is one of key mediators in the inflammatory process and contributes to destruction of cartilage in inflammatory joint diseases such as rheumatoid arthritis (Arend & Dayer, 1990). Although these reports suggest that IL-1β is involved in nociceptive response in the TMJ region, direct behavioral evidences for involvement of peripheral IL-1β in TMJ pain has largely remained unclear. The present data demonstrated that intra-articular injection of IL-1β facilitated the formalin-induced scratching behavioral responses in the TMJ area. These results, together with previous data, suggest that peripheral IL-1β facilitates of pain transmission in the TMJ. To investigate whether IL-1 receptor is involved in the hyperalgesic response of IL-1β in the TMJ region, interleukin-1 receptor antagonist (IL-1ra, 50 ng) was co-administered with IL-1β injection. The IL-1β-induced hyperalgesia was blocked by co-application of IL-1 receptor antagonist, suggesting that peripheral IL-1β-induced hyperalgesia is mediated by the IL-1 receptor.

In conclusion, intra-articular injection of 100 pg and 1 ng of IL-1β significantly increased noxious behavioral responses. Co-administration of IL-1 receptor antagonist blocked the IL-1β-induced hyperalgesic responses in the TMJ region. These data suggest that peripheral IL-1β facilitates the

transmission of nociceptive information in the TMJ area, and that IL-1β-induced hyperalgesia seems to be mediated by the IL-1 receptor.

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REFERENCES

Abbott FV, Franklin KBJ, Conel B. The stress of a novel environment reduces formalin pain: possible role of serotonin. *Eur J Pharmacol* 126: 126-141, 1986

Ahn DK, Jung CY, Lee HJ, Choi HS, Ju JS, Bae YC. Peripheral glutamate receptors participate in interleukin-1-induced mechanical allodynia in the orofacial area of rats. *Neurosci Lett* 357: 203-206, 2004

Alstergren P, Ernberg M, Kvarnstrom M, Kopp S. Interleukin-1β synovial fluid from the arthritic temporomandibular joint and its relation to pain, mobility, and anterior open bite. *J Oral Maxillofac Surg* 56: 1059-1065, 1998

Arend WP, Dayer JM. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum* 33: 305-315, 1990

Choi HS, Ju JS, Lee HJ, Jung CY, Kim BC, Park JS, Ahn DK. Effects of TNF-alpha injected intracisternally on the nociceptive jaw opening reflex and orofacial formalin test in freely moving rats. *Prog Neuropsychopharmacol Biol Psychiatry* 27: 613-618, 2003a

Choi HS, Ju JS, Lee HJ, Kim BC, Park JS, Ahn DK. Effects of intracisternal injection of interleukin-6 on nociceptive jaw opening reflex and orofacial formalin test in freely moving rats. *Brain Res Bull* 59: 365-370, 2003b

Choi HS, Lee HJ, Jung CY, Ju JS, Kim BC, Park JS, Ahn DK. Central cyclooxygenase-2 participates in interleukin-1β-induced hyperalgesia in the orofacial formalin test of freely moving rats. *Neurosci Lett* 352: 187-190, 2003c

Choi HS, Park JS, Ahn DK. Differential responses of intracisternal injection of interleukin-1β to acute and inflammatory orofacial pain model in freely moving rats. *Neurosci Res Commun* 31: 145-154, 2002

Clavelou P, Pajot J, Dallel R, Raboisson P. Application of the formalin test to the study of orofacial pain in the rat. *Neurosci Lett* 103: 349-353, 1989

Clavelou P, Dallel R, Pajot J, Orliaguet T, Woda A, Raboisson P. The orofacial formalin test in rats: effects of different formalin concentrations. *Pain* 62: 295-301, 1995

Costello NL, Bragdon EE, Light KC, Sigurdsson A, Bunting S, Grewen K, Maixner W. Temporomandibular disorder and optimism: relationships to ischemic pain sensitivity and interleukin-6. *Pain* 100: 99-110, 2002

Dinarello CA. Biology of interleukin 1. *FASEB* J2: 108-115, 1998

Dubuisson D, Dennis SG. The formalin test: a quantitive study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. *Pain* 4: 161-174, 1977

Ferreira SH, Lorenzetti BB, Bristow AF, Poole S. Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. *Nature* 334: 698-700, 1988

Fukuoka H, Kawatani M, Hisamitsu T, Takeshige C. Cutaneous hyperalgesia induced by peripheral injection of interleukin-1β in the rat. *Brain Res* 657: 133-140, 1994

Haas DA, Nakanishi O, MacMillan R.E, Jordan RC, Hu JW. Development of an orofacial model of acute inflammation in the rat. *Arch Oral Biol* 37: 417-422, 1992

Harada M, Takeuchi M, Fukao T, Katagiri KA. A simple method for the quantitative extrction of dye extravasated into skin. *J Pharm Pharmc* 23: 218-219, 1971

Harper RP, Kerins CA, Mclntosh JE, Spears R, Bellinger LL. Modulation of the inflammatory response in the rat TMJ with increasing doses of complete Freund's adjuvant. *J Osteo Arthritis Res Soc Int* 9: 619-624, 2001

Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non inflammatory pain. *Pain* 30: 102-114, 1987

Kubota E, Imamura H, Kubota T, Shibata T, Murakami K. Interleukin-1β and stromelysin (MMP3) activity of synovial fluid as possible markers of osteoarthritis in the temporomandibular joint. *J Oral Maxillofac Surg* 55: 20-27, 1997

Maier SF, Wiertelak EP, Martin D, Watkins LR. Interleukin-1β mediates the behavioral hyperalgesia produced by lithium chloride and endotoxin. *Brain Res* 623: 321-324, 1993

Nordahl S, Alstergren P, Eliasson S, Kopp S. Interleukin-1β in plasma and synovial fluid in relation to radiographic changes in arthritic temporomandibular joints. *Eur J Oral Sci* 106: 559-563, 1998

Roberoni RC, Parada CA, Cecilia M, Veiga FA, Tambeli CH. Development of a behavioral model of TMJ pain in rats: the TMJ formalin test. *Pain* 94: 185-191, 2001

Rooney M, Symons JA, Duff GW. Interleukin 1-β in synovial fluid is related to local disease activity in rheumatoid arthritis. *Rheumatol Int* 10: 217-219, 1990

Safieh-Garabedian B, Poole S, Allchorne A, Winter J, Woolf CJ. Contribution of interleukin-1 beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. *Br J Pharmacol* 115: 1265-1275, 1995

Takahashi T, Kondoh T, Fukuda M, Yamazaki Y, Toyosaki T, Suzuki R. Proinflammatory cytokines detectable in synovial fluids from patients with temporomandibular disorders. *Oral Surg Oral Med Oral Path Oral Rad Endo* 85: 135-141, 1998

Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain* 51: 5-17, 1992

Tominaga K, Alstergren P, Kurita H, Matsukawa A, Fukuda J, Kopp S. Interleukin-1β in antigen induced arthritis of the rabbit temporomandibular joint. *Arch Oral Biol* 42: 539-544, 2001

Watkins LR, Wiertelak EP, Ooehler LE, Smith KP, Martin D, Maier SF. Characterization of cytokine induced hyperalgesia. *Brain Res* 654: 15-26, 1994