Relationships between pain-related mediators and both synovitis and joint pain in patients with internal derangements and osteoarthritis of the temporomandibular joint

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Objective. The purpose of this study was to investigate the correlations between the concentrations of pain-related mediators in synovial fluid and the degree of synovitis and between the concentrations of pain-related mediators and the degree of joint pain in patients with internal derangement and osteoarthritis of the temporomandibular joint.
Study design. The concentrations of substance P, serotonin, bradykinin, leukotriene B_4 (LTB_4), and prostaglandin E_2 in SF and the degree of arthroscopic synovitis of 32 joints with internal derangement and osteoarthritis were assessed. The correlations between the concentration of each mediator and the score of arthroscopic synovitis and between the concentration of each mediator and the score of joint pain were analyzed statistically.
Results. The detection rates of substance P, serotonin, bradykinin, LTB_4, and prostaglandin E_2 were 25%, 25%, 91%, 53%, and 16%, respectively. Positive correlations were found between the concentrations of bradykinin and LTB_4 and the score of synovitis.
Conclusion. Bradykinin in SF might be useful as an index of the degree of synovitis.
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Temporomandibular joints (TMJs) with internal derangement (ID) and osteoarthritis tend to be painful with restricted mandibular mobility. These joints usually have synovitis,[1-5] which is thought to be a cause of the TMJ pain.[4] The pain is brought about by chemical substances, produced in inflammatory lesions that act on the pain receptors. These are generally termed pain-related mediators and include substance P (SP), serotonin, bradykinin, leukotriene B_4 (LTB_4), and prostaglandin E_2 (PGE_2). Pain-related mediators are thought to be involved in the pathogenesis of TMJ disorders (TMDs).[3,6,7] However, whether these mediators are involved in the pathogenesis of the synovitis is still unclear. In this study, we analyzed the relationships between SP, serotonin, bradykinin, LTB_4, and PGE_2 concentrations in the synovial fluid (SF) and, on scales of 0 to 4 assessed with arthroscopic observation, the degree of synovitis and joint pain.

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MATERIAL AND METHODS

Subjects

This study involved 32 TMJs with ID (14 joints) and osteoarthritis (18 joints) in 28 patients (25 female and 3 male) who underwent arthroscopic lysis and lavage or open TMJ surgery. Of the 14 joints with ID, 10 had anterior disk displacement without reduction, 2 had anterior disk displacement with reduction, and 2 had normal disk positions, according to magnetic resonance imaging (MRI). All 18 joints with osteoarthritis had anterior disk displacement without reduction and bony changes, such as osteophyte, erosion, and flattening, according to MRI. One surgeon (NS) read the MRI findings of all patients. The patients ranged in age from 17 to 74 years (median, 40 years), and the duration of morbidity ranged from 1 month to 5 years (median, 7 months). Before surgery, the patients indicated on a visual analog scale of 0 to 10 the degree of joint pain during mouth opening. The pain scores ranged from 1.5 to 9.5 (median, 5.7), with the higher scores indicating greater pain.

Synovial fluid sample preparation

In all patients, the same surgeon (NS) injected 2.0 mL saline solution into the superior joint space with a 21-gauge needle and aspirated the diluted SF and reinjected it a total of 10 times before drawing off the final sample.[8,9] The withdrawal volume of the SF samples was 2.0 mL ± 5%. The SF samples were centrifuged

(×3000 rpm for 20 minutes at 4°C) and were stored at −80°C until assay.

Measurement of pain-related mediators and total protein concentrations

One surgeon (MN) determined the concentrations of the pain-related mediators with an enzyme-linked immunosorbent assay (ELISA) kit (Assay Designs, Ann Arbor, Mich, for SP, LTB_4, and PGE_2; ICN Pharmaceuticals, Costa Mesa, Calif, for serotonin; and Dainippon, Osaka, Japan, for bradykinin) according to manufacturer instructions. Protein was assayed with a Bio RAD protein assay kit (Bio-RAD, Hercules, Calif). All samples and standards were assayed in duplicate. The total protein concentration was used to compare the concentrations of the pain-related mediators in the samples despite variance in the volume of the SF aspirated from the TMJs.[5,10-12] The concentration of each pain-related mediator was calculated per 100 μg of SF total protein. The specificity of each mediator ELISA kit was as follows.

Substance P. The ELISA kit (Assay Designs) used a goat antibody specific to rabbit immunoglobulin G (IgG) and an alkaline phosphatase (AP)–labeled polyclonal rabbit antibody to SP. There was no cross-reactivity with other mediators. The sensitivity was 8.04 pg/mL, the intraassay variance was less than 6.7%, and the interassay variance was less than 7.3%, with a recovery rate of 81% to 116%.

Leukotriene B_4. The ELISA kit (Assay Designs, Inc) used a goat antibody specific to rabbit IgG and an AP–labeled polyclonal rabbit antibody to LTB_4. There was no cross reactivity with other mediators. The sensitivity was 47.9 pg/mL, the intraassay variance was less than 7.9%, and the interassay variance was less than 9.7%, with a recovery rate of 95% to 108.8%.

Prostaglandin E_2. The ELISA kit (Assay Designs) used a goat antibody specific to mouse IgG and an AP–labeled monoclonal antibody to PGE_2. There was no cross-reactivity with other mediators. The sensitivity was 36.2 pg/mL, the intra-assay variance was less than 17.5%, and the interassay variance was less than 5.1%, with a recovery rate of 101.2% to 126.1%.

Serotonin. The ELISA kit (ICN Pharmaceuticals) used a goat antibody specific to rabbit IgG and an AP–labeled antibody to serotonin. There was no cross-reactivity with other mediators. The sensitivity was 0.03 ng/mL, the intra-assay variance was less than 10.9%, and the interassay variance was less than 7.4%, with a recovery rate of 92.0% to 97.6%.

Bradykinin. The ELISA kit (Dai-nippon Pharmaceutical Corp) used a goat antibody specific to rabbit IgG and a horseradish peroxidase–labeled antibody to bradykinin. The sensitivity was 4.9 pg/mL, and there was no cross-reactivity with other mediators.[13]

Table I. Arthroscopic intensity of synovitis

Grade	*Findings*
0	Normal pale, almost translucent, synovial lining with fine network of anastomosing small blood vessels
1	Increased vascularity and capillary hyperemia
2	Capillary dilatation and increasing network
3	Contact bleeding on probe palpation
4	Microbleeding and effusion; granulative change, effusion, and debris

Evaluation of synovitis

Immediately after the collection of the SF samples, conventional diagnostic arthroscopy of the whole area of the superior joint compartment was performed and videotaped for approximately 10 minutes. This was followed by sweepage with lysis and lavage. The degree of synovitis was statistically evaluated according to the criteria of Murakami et al.[4] Namely, the most pronounced degree of synovitis in the superior compartment was given a score on a scale of 0 to 4 (Table I). Two oral surgeons (NS and MM) gave the scores, blinded to the patient names and conditions, while retrospectively viewing the videotapes. The synovitis score was reproducible because a surgeon (NS), one of the scorers, examined the criteria of Murakami et al.[4]

Statistical analysis of correlations

The correlations between the concentrations of each pain-related mediator and the scores of synovitis and pain were analyzed statistically with the Spearman rank correlation coefficient.

RESULTS

The results of the concentrations of the pain-related mediators and the scores of synovitis and pain of each TMJ are shown in Table II. The detection rates of the pain-related mediators ranged from 15.6% to 90.6% (Table III). Bradykinin was detected with the highest rate (90.6%). On comparison of joints with ID and osteoarthritis, no differences were seen in the concentrations of the pain-related mediators, the score of synovitis, and the pain score (data not shown). Significantly positive correlations were found between bradykinin and LTB_4 concentrations and the synovitis score ($P < .05$; Table IV; Figure). No correlation was found between each mediator concentration and the pain score (Table IV).

Table II. Concentrations of pain-related mediators and scores of synovitis and pain of each TMJ

Joint no.	Sex	Age (y)	Duration of morbidity (d)	Disk position on MRI	Mediators concentration					Synovitis score	Pain score
					SP(p)	5HT(n)	BK(p)	LTB_4(p)	PGE_2(p)		
1	F	19	210	WR	2.6	*	136.0	26.0	*	4	5.1
2	M	55	30	Normal	*	*	7.0	*	*	4	†
3	F	34	90	WR	*	0.6	7.0	*	*	2	7.8
4	F	34	90	Normal	*	2.1	94.0	*	*	3	7.8
5	F	40	1233	W/OR	*	4.4	364.0	635.0	*	4	8.1
6	F	22	48	W/OR	1.8	0.7	47.0	45.0	144.7	3	3.6
7	F	41	270	W/OR	*	*	4.0	5.0	*	1	9.5
8	F	49	240	W/OR	*	*	142.0	21.0	*	4	6.1
9	F	23	448	W/OR	3.5	*	100.0	56.0	16.2	3	6
10	F	56	134	W/OR	*	*	13.0	*	*	1	2.8
11	F	73	230	W/OR	1.6	*	5.0	6.0	*	1	4.1
12	F	40	50	W/OR	*	*	3.0	*	*	2	7.5
13	M	39	133	W/OR	*	*	8.0	*	*	3	1.5
14	M	39	133	W/OR	*	*	13.0	*	*	3	1.5
15	F	59	365	W/OR	*	*	43.0	985.0	*	2	8.3
16	F	17	800	W/OR	1.0	0.5	1.0	4.0	*	2	5.7
17	F	20	1155	W/OR	0.8	*	9.0	7.0	*	2	5
18	F	56	30	W/OR	*	0.6	51.0	*	*	4	3.5
19	F	74	1825	W/OR	7.0	*	23.0	5.0	*	1	†
20	F	67	100	W/OR	*	*	32.0	*	*	4	4.8
21	F	38	210	W/OR	*	2.6	197.0	*	*	2	4.7
22	F	38	210	W/OR	*	3	258.0	2796.0	*	3	4.7
23	M	36	330	W/OR	*	*	204.0	*	504.7	3	8
24	F	27	490	W/OR	*	*	*	2006.0	174.4	4	4.9
25	F	24	400	W/OR	*	*	82.0	*	*	3	7.5
26	F	45	240	W/OR	*	*	22.0	28.0	*	1	6.5
27	F	50	400	W/OR	0.8	*	9.0	*	*	4	†
28	F	55	165	W/OR	*	*	104.0	30.0	*	2	5.7
29	F	23	448	W/OR	*	*	22.0	56.0	*	2	6
30	F	54	850	W/OR	*	0.97	102.0	465.0	117.2	1	2.3
31	F	17	152	W/OR	*	*	*	*	*	2	5.3
32	F	52	132	W/OR	*	*	*	*	*	1	8.1

Joint no. 1–14: IO no. 15–30.
P, pg/100 μg protein; *5HT*, serotonin; *n*, ng/100 μg protein; *BK*, bradykinin; *F*, female; *M*, male; *WR*, anterior disk displacement with reduction; *W/OR*, anterior disk displacement with reduction.
*Not detectable.
† No data.

Table III. Detection rates and concentrations of pain-related mediators

Pain-related mediator	Detection rate	Concentration (/100 μg protein)
PGE_2	15.6%	191.4 ± 185.0 pg
SP	25.0%	2.4 ± 2.1 pg
5HT	25.0%	1.8 ± 1.4 ng
LTB_4	53.1%	422.1 ± 806.7 pg
BK	90.6%	72.5 ± 89.1 pg

PGE_2, Prostaglandin E_2; *SP*, substance P; *5HT*, serotonin; *LTB_4*, leukotriene B_4; *BK*, bradykinin.

DISCUSSION

Pain-related mediators, including serotonin, SP, LTB_4, PGE_2, and bradykinin, are produced in inflammatory lesions and bring about pain by acting on the pain receptors. The serotonin is stored in mast cells and platelets in peripheral tissue and is released simultaneously with histamine on degranulation induced by several substances, such as SP.[13] The SP has been proposed as a mediator of pain, and its vasoactive properties are well documented. Eicosanoids, including PGE_2 and LTB_4, are derived from the oxygenation pathways of arachidonic acid, after phospholipase A2 has released fatty acids, and are involved in pain and inflammation[14] and the regulation of pain sensitivity.[15] PGE_2 is a powerful vasodilator of capillaries, increases vascular permeability, and can cause bone resorption.[16] LTB_4 triggers vascular permeability and is a powerful chemoattractant and activator of inflammatory cells. Bradykinin has been shown to have potent proinflammatory effects, and it is one of the most potent pain-

Table IV. Correlation coefficients between concentrations of pain-related mediators and scores of synovitis and pain

Pain-related mediator	*Correlation with synovitis score*	*Correlation with pain score*
PGE_2	$r = 0.34$; $P = .15$ (n = 5)	$r = 0.64$; $P = .42$ (n = 5)
SP	$r = -0.35$; $P = .59$ (n = 8)	$r = 0.31$; $P = .48$ (n = 6)
5HT	$r = 0.37$; $P = .32$ (n = 8)	$r = 0.37$; $P = .45$ (n = 8)
LTB_4	$r = 0.40$; $P = .03$ (n = 17)	$r = -0.09$; $P = .64$ (n = 16)
BK	$r = 0.35$; $P = .02$ (n = 29)	$r = 0.22$; $P = .70$ (n = 26)

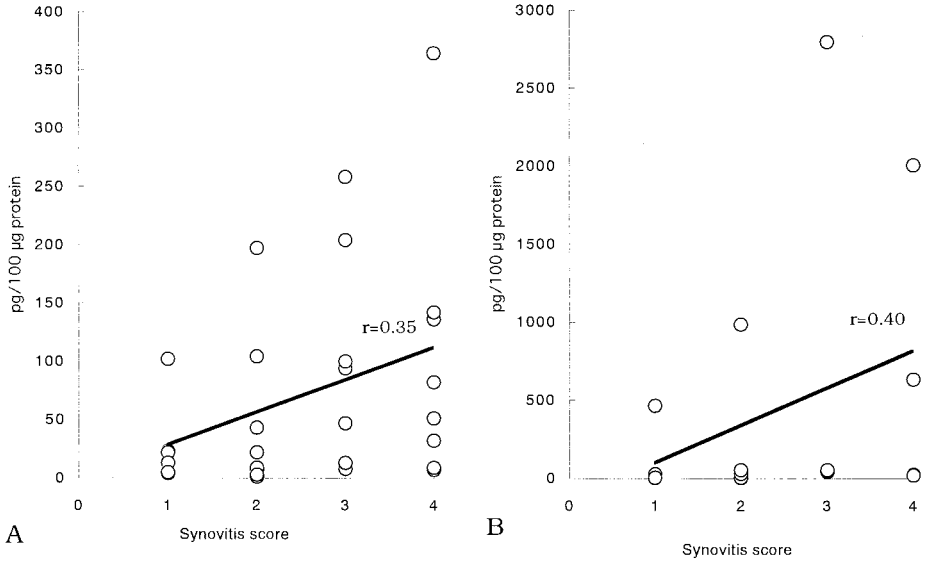

Figure. Graphs correlating individual TMJ concentrations of **(A)** bradykinin and **(B)** LTB_4 with their synovitis scores.

producing endogenous substances known. After its local release within inflamed tissues, bradykinin can initiate many important processes, including vasodilatation, plasma extravasation of blood-borne proteins, activation of immune cells, induction of leukocyte chemotaxis, and activation of nociceptive neurons.[17] The pain-related mediators have been shown to be involved in not only orthopedic inflamed joints[18-22] but also in TMD.[3,6,7] Synovitis has also been suggested to be a cause of the TMJ pain[4] and the neurogenic inflammation mechanism involved in the pathophysiology of degenerative TMD.[23] Thus, the pain-related mediators are concerned with synovitis and joint pain. As for the mechanism of synovitis by the pain-related mediators, when the synovium is stimulated physically or chemically, pain-related mediators are produced in the circulating blood, synaptic terminals, and many kinds of cells in the synovium or in the metabolism of arachidonic acid. The resulting vasodilatation and extravasation by these mediators bring about synovitis.

The detection rates of SP, serotonin, LTB_4, and PGE_2 in SF in this study were less than in past studies.[3,6,7] The differences in data may be related to differences in sensitivity and specificity among assays. The concentration of bradykinin in SF of TMJ has not been reported. In orthopedic research, the mean concentration of bradykinin in SF of patients with active rheumatoid arthritis has been reported to be 31.49 µg.[24] The differences in data may be related to differences in SF sampling procedures and disease condition; that is to say, original SF was taken in orthopedic research and rheumatoid arthritis is a systemic inflammatory disease. On the other, diluted SF was taken in TMJ because of difficulties in the collecting, and ID and osteoarthritis is a local slight inflammatory disease.

In this study, the correlations between bradykinin and LTB_4 concentrations and the synovitis score were confirmed. However, LTB_4 might not be useful as an index of synovitis because its detection rate in SF was not high (53.1%). The detection rate of bradykinin in SF was high (90.6%). Therefore, bradykinin in SF might be a useful index of synovitis in TMJs with ID and osteoarthritis. On the other hand, patients with synovitis scores of 1 have higher bradykinin than those

with synovitis scores of 4. And the SF was devoid of mediators in increased pain or in synovitis in some of the joint. This might be because of the rapid turnover or consumption of mediators within the joint cavity.[25]

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