Effect of arthrocentesis and sodium hyaluronate injection on nitrite, nitrate, and thiobarbituric acid–reactive substance levels in the synovial fluid

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Objective. To evaluate the effect of arthrocentesis and sodium hyaluronate (SH) injections on nitrite, nitrate, and thiobarbituric acid–reactive substance (TBA-RS) levels in temporomandibular joint internal derangements.
Study design. Arthrocentesis was performed on 10 patients, and 15 patients received a supplemental injection of SH after arthrocentesis. All these patients received an SH injection 15 days after the first intervention. The synovial fluid samples were obtained before arthrocentesis on the first appointment and before the SH injection 15 days later. Nitrite and nitrate levels were measured with a highly sensitive and specific chemiluminescence detection method, and the concentration of lipid peroxidation products was assessed by means of the thiobarbituric acid reaction.
Results. Symptomatic improvement was seen in both groups. Nitrite, nitrate, and TBA-RS levels only decreased significantly ($P < .05$) with a supplemental SH injection after arthrocentesis.
Conclusions. Intra-articular injections of SH may reduce nitrite, nitrate, and TBA-RS levels that play a role in the pathogenesis of various temporomandibular disorders.
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In recent years, synovial fluid of the temporomandibular joint (TMJ), obtained either by direct aspiration or by lavage, has been analyzed extensively for the presence of various mediators and free radicals that may be used as markers of joint disease.[1-9] If one could demonstrate that certain mediators are specific to a condition, it would contribute toward accurate diagnoses in cases of joint disease and might allow for treatments to be directed toward the elimination or reduction of these mediators.

Nitric oxide (NO) is a free radical synthesized from L-arginine by the NO synthases. NO is considered an important endogenous mediator for neurotransmission, vasodilatation, nerve function, and immune defense mechanisms. Physiologic functions involving a change in flow or shear stress, a change in oxygen tension, and a variety of vasoactive substances cause the release of NO from endothelial cells.[10,11] Overproduction of NO has been shown in certain temporomandibular disorders; whereas a detectable nitrite concentration was found, in one study, in only 1 of 10 normal (control) joints.[7] Resting cultures of rabbit synoviocytes normally produce no, or very little, NO.[12]

Lipid peroxidation and disruption of lysosomal integrity are also implicated in the pathogenesis of inflammatory processes. Lipid peroxides are oxidation products of the unsaturated fatty acids that may serve as indicators of oxidative stress. Lipid radicals increase edema and produce prostaglandins and various endoperoxides by increasing vascular permeability, inflammation, and chemotaxis. An increased lipid peroxidation in rheumatoid arthritis and increased levels of thiobarbituric acid–reactive material in the synovial fluid were reported in previous studies.[13-16]

The presence of elevated levels of certain mediators in certain conditions raises the possibility that a selective inhibition of their synthesis or a lowering of their levels may be of therapeutic value.

TMJ arthrocentesis is widely used in the treatment of TMJ internal derangements with a high success rate,

Table I. Patient characteristics

	Arthrocentesis	*Arthrocentesis + SH*
Patients/joints	10/10	15/15
Men/women	1/9	4/11
Age (y) mean/range	28.6/18-44	28.5/17-50

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Table II. Schematic representation of treatment and synovial fluid aspiration sequences

	Day 1	*Day 15*
Group I	1. Obtaining first synovial fluid sample	1. Obtaining second synovial fluid sample
	2. Arthrocentesis and lavage of the upper joint space	2. 1 mL SH injection
Group II	1. Obtaining first synovial fluid sample	1. Obtaining second synovial fluid sample
	2. Arthrocentesis and lavage of the upper joint space	2. 1 mL SH injection
	3. 1 mL SH injection	

significantly improving maximum mouth opening and jaw function and reducing pain, particularly in cases of acute closed lock.[17] Intra-articular injection of a high molecular weight of sodium hyaluronate (SH) has also been reported to have some beneficial effects in the treatment of TMJ internal derangements, with a significant long-term therapeutic effect similar to that of corticosteroids.[18,19]

This study was undertaken to evaluate the effect of arthrocentesis and the therapeutic use of SH on nitrite, nitrate, and thiobarbituric acid–reactive substance (TBA-RS) levels in the synovial fluid of patients with TMJ internal derangements.

PATIENTS AND METHODS

A total of 25 patients (age range, 17-50 years; mean, 25.8 years) diagnosed as having TMJ internal derangement were included in this study (Table I). Care was taken to not include patients who had taken medication during the last 15 days. TMJ transcranial views in open and closed mouth positions were taken for radiographic evaluation. A detailed history was taken, and a thorough clinical examination was performed by the same clinicians. Patients were diagnosed as having either disk displacement with reduction or disk displacement without reduction (stage II and stage III, according to Wilkes staging) by these clinical and radiologic examinations. The scores for intensity of pain, joint noises, and joint dysfunction were measured with a 100-mm visual analog scale. Maximal mouth openings were measured by the distance between the incisal edges of the upper and lower central incisors; the range of lateral movements was measured by the distance between the upper and lower midlines on lateral movements. These scores were recorded to the files of each patient 3 times: before treatment, on the first day after surgery, and 15 days after surgery.

The treatment protocol consisted of arthrocentesis, by which lavage of the upper joint space was completed, and a supplemental injection of SH. In the first group, consisting of 10 patients, TMJ arthrocentesis was done. These patients received an intra-articular injection of 1 mL of SH 15 days after arthrocentesis. The first synovial fluid was obtained before arthrocentesis, and the second synovial fluid was obtained before an SH injection 15 days after arthrocentesis. In the second group, consisting of 15 patients, an intra-articular injection of 1 mL of SH was administered immediately after TMJ arthrocentesis and again 15 days after the first injection. The first synovial fluid in this group of patients was obtained before arthrocentesis, and the second synovial fluid was obtained before the second SH injection 15 days after the first injection (Table II).

For collection of the synovial fluid, 2 mL of saline solution was injected into the upper joint space after local anesthesia was administered with a solution containing prilocaine hydrochloride (HCl), 30 mg/mL, and Citanest Octapressin, 0.58 μg/mL. To allow the saline solution to mix with synovial fluid, the solution was aspirated and then reinjected 3 times, and finally the mixture of synovial fluid and saline solution was withdrawn into a syringe.

The obtained sample was centrifuged 5 minutes at 2000 rpm to remove cells. The upper portion of the centrifuged synovial fluid was then transferred to another test tube and stored at –20°C until the performance of the assay.

Nitrite measurement

The amount of total nitrite in the test samples was determined by a modification of the procedure described by Braman and Hendrix[20] with the purge system of a nitric oxide analyzer (Model 280 A; Sievers Instruments, Boulder, Colo). Glacial acetic acid (4 mL) and 1 mL of sodium iodide (NaI) (69 mg in 1 mL of deionized water) were added to the purge vessel and purged with nitrogen gas for 10 minutes before use. Samples were injected into the purge vessel to react with the glacial acetic acid and NaI, which converted nitrite to NO. The NO produced was stripped from the reaction chamber and detected by ozone-induced chemiluminescence in the chemiluminescence detector; parts per billion values were detected, and NO levels of the samples were calculated from the peak values. A standard curve was constructed with various concentrations of sodium nitrite (10 μm to 100 μm). Nitrite levels of the samples were calculated by using the standard curve. Results were expressed as the mean ± SD.

Table III. Pretreatment and posttreatment scores and measurements achieved in arthrocentesis and arthrocentesis + SH groups

	Arthrocentesis			*Arthrocentesis + SH*		
	Mean	*SD*	*SE mean*	*Mean*	*SD*	*SE mean*
Pain prtx	5.80	2.45	0.78	4.69	2.46	0.66
Pain potx	2.10*	1.98	0.63	1.93*	1.95	0.52
Click prtx	5.86	4.08	1.30	6.30	3.88	1.00
Click potx	1.35*	1.90	0.60	2.88*	3.13	0.84
Dysfunctional prtx	4.79	2.85	0.90	5.16	2.23	0.60
Dysfunctional potx	2.83	2.68	0.85	2.01*	1.88	0.50
MMO prtx	36.50	6.62	2.10	35.36	8.24	2.20
MMO potx	40.20	6.58	2.10	38.50	4.67	1.20
Right lateral prtx	6.70	2.26	0.72	7.21	2.39	0.64
Right lateral potx	6.70	1.70	0.54	7.79	1.76	0.47
Left lateral prtx	6.10	2.73	0.86	6.93	2.09	0.56
Left lateral potx	7.90	1.91	0.60	7.71	1.77	0.47

*$P < .05$.
MMO, Maximal mouth opening; *prtx*, pretreatment; *potx*, posttreatment.

Nitrate measurement

The amount of total nitrate in the test samples was also determined by a modification of the procedure described by Braman and Hendrix[20] with the purge system of the same nitric oxide analyzer. A saturated solution of VCl_3 in 1 mol/L HCl was prepared and filtered before use. Five milliliters of this reagent was added to the purge vessel and purged with nitrogen gas for 10 minutes before use. The purge vessel was equipped with a cold water condenser and a water jacket to permit heating of the reagent to 95°C with a circulating water bath. The HCL vapors were removed by a gas bubbler containing 15 mL of 1 mol/L of sodium hydroxide. The gas flow rate in the chemiluminescence detector was controlled with a needle valve. Samples were injected into the purge vessel to react with the VCl_3/HCl reagent, which converted the nitrate to NO. The NO produced was stripped from the reaction chamber and detected by ozone-induced chemiluminescence in the chemiluminescence detector. Parts per billion values were detected, and NO levels of the sam-ples were calculated from the peak values. A standard curve was constructed by using various concentrations of nitrous trioxide (10 μmol/L to 100 μmol/L). Nitrate levels of the samples were calculated with the standard curve. Results were expressed as the mean ± SD.

Thiobarbituric acid test

The concentration of lipid peroxidation products was assessed in samples by means of the TBA reaction. A modified method of Rowley et al[16] was used in this study; briefly, 125 μL of synovial fluid was added to 250 μL of TBA solution (1% wt/vol in 50 mmol/L sodium hydroxide), 250 μL of HCl acid (25% vol/vol) and 200 μL of water; 125 μL of water was used in place of the sample as a negative control. The tubes were tightly capped and heated at 100°C for 1 hour, after which they were allowed to cool to room temperature before they were extracted into 1.5 mL of 1-butanol and vigorously mixed for 2 minutes. The samples were then centrifuged at 1500*g* at 4°C for 15 minutes, and the absorbance of the upper organic layer was determined at 532 nm by spectrophotometry (Milton Roy Spectronic 3000-Array-USA).

Statistical analysis was done by the Student *t* test. Distribution of the sample values, whether normal or not, were tested by Kolmogorov-Smirnov method.

RESULTS

All patients showed symptomatic improvement after treatment. Maximal mouth openings and lateral jaw movements increased in both groups. In the first group, arthrocentesis significantly ($P < .05$) reduced the average pain score, from 5.80 to 2.10, and the noise score, from 5.86 to 1.35. In the second group, injection of 1 mL of SH after arthrocentesis significantly ($P < .05$) reduced the average pain score, from 4.69 to 1.93, and the noise score, from 6.30 to 2.88. The jaw functions improved significantly ($P < .05$) in the group of patients who received intra-articular injection of SH (Table III).

Nitrite, nitrate, and TBA-RS levels were significantly reduced, from 0.94 to 0.55 ($P < .05$), 0.09 to 0.05 ($P < .05$), and 0.67 to 0.42 ($P < .05$), respectively, in all patients after the therapeutic use of SH (Table IV). The decrease in these levels was not significant in patients receiving arthrocentesis only.

DISCUSSION

There is clinical evidence supporting the existence of disk displacement in TMJ internal derangement. However, recent concepts suggest that a change in the

Table IV. Results of measurements of nitrite, nitrate, and TBA-RS levels before treatment and after intra-articular injection of 1 mL of SH

	Before treatment	*X ± SD*	*After SH injection*	*X ± SD*	*P values*
Nitrite levels (μm)	0.0952	0.032	0.541	0.017	$P < .05$
Nitrate levels (μm)	0.94462	0.64	0.55	0.59	$P < .05$
TBA-RS levels (nmol/L)	0.67	0.45	0.42	0.09	$P < .05$

position of the disk is not the primary factor in dysfunction and pain of the TMJ.[21] Alterations in joint pressure, a variety of biochemical substances, and the constituents of the synovial fluid (and thereby failure of lubrication) may lead to clicking and derangement of the TMJ.[21,22]

The presence of inflammatory cells and inflammatory mediators, including arachidonic acid metabolites and cytokines, were demonstrated in symptomatic TMJs correlating with an index of TMJ disease.[1,2,5,6,8,23] Most of these inflammatory molecules are associated with NO and exert their effects through NO.[24] For this reason, blood serum and synovial fluid from patients with inflammatory joint disease and various temporomandibular disorders also contain markers of NO-dependent oxidative damage, whereas these markers were not detectable in body fluids of healthy subjects.[7,25] In this study, synovial fluid could not be obtained, for ethical reasons, from the patients with normal joints who had no symptoms. Also, it was beyond the scope of this study to compare the levels of certain mediators in the synovial fluid of normal joints and joints with temporomandibular disorders.

It has been shown in animal models of arthritis that high amounts of NO synthesized systemically and intra-articularly play an important role in inflammatory joint diseases and that administration of NO synthase inhibitors profoundly reduces this activity.[26] Lipid peroxidation and the disruption of lysosomal integrity are implicated in the pathogenesis of inflammatory processes as well.

Pain associated with temporomandibular disorders may be associated with vasodilation.[24] The release of NO and TBA-RS may result from mechanical stress and high pressures directed to the upper compartment during clenching and jaw movements, which also play a role in the cause of internal derangements. Both NO and TBA-RS cause vasodilatation and vascular permeability. It has been shown clinically that painful temporomandibular disorders, including disk derangements, are associated with a highly significant increase in the level of NO in the TMJ synovial fluid.[7] Therefore, selective inhibition of the pathologically enhanced NO synthesis may emerge as a new experimental therapeutic approach in the treatment of temporomandibular disorders that exhibit increased levels of NO and TBA-RS.

TMJ arthrocentesis is a procedure by which inflammatory mediators causing pain are lavaged from the upper joint space. It has been shown in an experimental model that lavage of the superior joint space of the TMJ removes inflammatory mediators like prostaglandins and bradykinin.[27] Lavage of the superior joint space has reduced the protein concentration in a volume-dependent manner, with a reported therapeutic volume of 100 mL.[28] In our study, the fluid for lavage of the superior joint space was also 150 to 200 mL.

It has been proposed that intra-articular injection of SH may decrease the inflammatory mediators and may stimulate increased natural hyaluronic acid production by the synovial cells.[29] Intra-articular injection of SH has been shown to significantly reduce several inflammatory mediators in patients with TMJ internal derangements.[30] These anti-inflammatory actions include the scavenging of free radicals, the inhibition of migration, and the reduction of vascular permeability.

In this study, all patients treated either by arthrocentesis or by intra-articular injection of SH after arthrocentesis showed clinical improvement. However, intra-articular injection of SH after arthrocentesis significantly reduced the NO and TBA-RS levels in the synovial fluid, whereas no significant decrease of these levels was found in patients receiving arthrocentesis only.

A significant decrease in NO and TBA-RS levels in the synovial fluid of patients receiving an intra-articular injection of SH may have long-term effects in the pathogenesis of the disease. Further studies and long-term evaluation of these patients are needed.

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