Intra-articular injection of hyaluronate (SI-6601D) improves joint pain and synovial fluid prostaglandin E2 levels in rheumatoid arthritis: A multicenter clinical trial

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Abstract

Objective

The relationship between clinical features and biochemical parameters of synovial fluid after serial intra-articular injections of sodium hyaluronate (SI-6601D) was investigated.

Methods

SI-6601D (sodium hyaluronate with an average molecular weight of 8.4 x 10^5; 25mg/2.5ml/syringe) was injected intra-articularly into the knees of 25 patients with rheumatoid arthritis (RA) every week for 5 consecutive weeks. Clinical and biochemical parameters were monitored before and after injection. Clinical findings included pain, as a summation of 3 categories (pain at rest, pain in motion and pain in passive motion, each assessed on a 4-step rating scale), and inflammation, also as a summation of 3 categories (swelling, patellar ballotement and local warmth, each assessed on a 4-step rating scale). Pain on walking of patient was qualitatively assessed by visual analogue scale (VAS). The aspirated volume of synovial fluid (SFV) was recorded and levels of prostaglandin (PG) E2, transforming growth factor beta-1, tumor necrosis factor alpha, interleukin 1 receptor antagonist, chondroitin 4-sulfate (C4S) and chondroitin 6-sulfate were measured.

Results

Significant improvement in pain symptoms ($p < 0.0001$), inflammation ($p < 0.0001$), VAS pain ($p < 0.001$) and SFV ($p < 0.05$) were observed after the 5 injections. Levels of PGE2 ($p < 0.05$) and C4S ($p < 0.05$) in the synovial fluid were significantly decreased.

Discussion

SI-6601D improved local clinical symptoms in RA patients by suppressing PGE2 and, therefore, may be a useful treatment for local inflammation in RA.

Key words
Hyaluronate, PGE2, rheumatoid arthritis, synovial fluid.

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Introduction

Intra-articular injection of high molecular weight (HMW) sodium hyaluronate (HA) has been used as a supplementary therapy for osteoarthritis (OA) (1, 2), to improve articular functions. Its mechanisms of action include suppression of proteoglycan release (3), enhancement of articular lubrication and fluid viscosity (4) with analgesic (5) and anti-inflammatory activities (6), as well as inhibition of leukocyte functions (7) and suppression of phagocytotic activity (8).

Rheumatoid arthritis (RA) is a systemic and chronic inflammatory joint disease associated with synovitis, characterized by synovial cell proliferation, joint hydrops, and cartilage destruction. Accumulation of synovial fluid is noted as a result of local inflammation, and the subsequent pain and joint destruction usually impairs the quality of life. The severity and progression of synovitis depends on the levels of cytokines and prostaglandin (PG) E2 released from infiltrating cells such as lymphocytes and macrophages in inflamed joints (9, 10). Excessive proliferation of synovial cells is often associated with pannus formation, a vascular and fibrous extension of the perichondral portion of the synovial membrane, which grows over the articular cartilage and destroys the cartilage matrix.

Goldberg and Toole (11) have documented inhibition of cultured synovial cell proliferation by HMW-HA, and Peluso *et al.* (12) have also demonstrated suppression of mitogen-induced lymphocyte cell proliferation. Furthermore, Inhibition of PGE2 release from RA synovial cells by HMW-HA has been reported (13). These data suggest a therapeutic possibility of HMW-HA for RA synovitis and recently, intra-articular injection of HMW-HA has been found to be clinically useful (14) with biochemical normalization of RA synovial fluid (15). The aim of the present study was to investigate the relationship between the levels of PGE2 and cytokines in the synovial fluids and the local articular symptoms after intra-articular injections of sodium hyaluronate (SI-6601D).

We monitored the major inflammatory parameters constituting the pathological conditions of RA; the levels of PGE2, transforming growth factor beta-1 (TGF 1), tumor necrosis factor alpha (TNF), and interleukin 1 receptor antagonist (IL-1RA) in both synovial fluid and blood obtained simultaneously. We examined the blood as well as synovial fluid levels of cytokines to determine if the cytokine levels in the synovial fluid were influenced by the intra-articular injections of SI-6601D. We also measured the levels of chondroitin sulfate (CS) isomers, chondroitin 4-sulfate (C4S) and chondroitin 6-sulfate (C6S), in the synovial fluid. It is known that CS is normally a component whose level in synovial fluid increases with the progression of cartilage destruction in RA and OA (16, 17).

Patients and methods

Patients

25 patients with active RA, defined by the 1987 ACR criteria and giving informed consent, were selected. Patients with the following characteristics were excluded: 1) individuals with a history of any other severe or chronic disease, and 2) those who had started therapy with DMARDs (disease-modifying anti-rheumatic drugs: methotrexate, D-penicillamine, bucillamine, sulfasalazine, auranofin and injectable gold) or immunosuppressants within the preceding 3 months, or oral or injectable corticosteroids (prednisolone equivalent ≥ 5 mg/day), or NSAIDs (nonsteroidal anti-inflammatory drugs), or physical therapy within 4 weeks prior to the study. Rheumatologists in ten institutions in Japan participated.

Study drug and dose

SI-6601D, ultra-purified sodium hyaluronate derived from rooster combs with an average molecular weight of 8.4×10^5, was manufactured by Seikagaku Corporation (Tokyo, Japan). Individual syringe-type ampules (2.5 ml) containing 25 mg hyaluronate (1% SI-6601D in phosphate buffered saline) were used.

Study design

The study design was based on the

method of sodium hyaluronate therapy for OA. The same knee joint received one ampule of SI-6601D once every week for five consecutive weeks. Even if both knees were affected, only one knee for each patient was selected for injection during the study. Injection of SI-6601D into the joint cavity was performed under aseptic conditions without any local anesthesia, following the aspiration of the entire fluid whenever possible. Blood was sampled before and after the SI-6601D clinical trial. For PGE2 analysis, the synovial fluid and plasma were sampled with the addition of EDTA 2Na and indomethacin in the syringe. For the cytokine analysis, the synovial fluid and serum were sampled without an additive in the syringe. The samples were centrifuged at 4°C at 2,000 x g for 10 minutes, and the supernatants were collected and stored at -20°C until analyzed.

Concurrent medications and therapy
Patients were allowed to take prednisolone (< 5 mg) or its equivalent per day. The dosage and type of drugs taken were constant for more than 4 weeks (for NSAIDs and prednisolone) or 3 months (for DMARDs) prior to the study, and could not be changed during the study. Other concurrent medications that were allowed included antibiotics for infection. The patients were instructed to continue their programs of physical and occupational therapy during the study if they had started at least 4 weeks before.

Efficacy on local clinical symptoms
The clinical efficacy of SI-6601D in the injected knee joints was studied by summing the total scores for parameters (I) and (II) at the start of the study (baseline score) and one week after the final injection (endpoint score). (I) The degree of joint pain was based on three different categories:pain at rest, pain in motion and pain in passive motion, using a 4-step rating scale of 0 = none, 1 = mild, 2 = moderate, and 3 = severe. (II) The degree of joint inflammation was based on three different categories: swelling, floating patella and local warmth, using a 4-step rating scale of 0=none, 1=mild, 2=moderate, and 3 = severe. Improvement in both degrees was evaluated by comparing the total scores at the baseline and endpoint. The final global improvement rate (FGIR) was expressed as the % change from the baseline score with the following formula:
FGIR = 100% x (baseline score - endpoint score) / baseline score
Both the baseline and endpoint scores were obtained by summing the scores for the parameters I and II as described above. Patients who showed ≥ 50% were categorized as responders and those with FGIR < 50% as non-responders.
The patient's self-assessment for pain on walking was studied by a visual analogue scale (VAS; 0-100 mm).

Measurement of biochemical parameters
PGE2 and cytokines: Levels of PGE2 in the synovial fluid and plasma were assessed by RIA (PGE2 [^{125}I] assay system, Amersham). The following cytokines in the synovial fluid and the serum were measured: TGF 1 (Human TGF 1, R&D Systems) and TNF (TNF EASIA, Medgenix-Diagnostics) analyzed by EIA, and IL-1RA (IL-1ra Human ELISA system,Amersham) analyzed by ELISA.
Glycosaminoglycans (GAGs): Levels of C4S and C6S in the synovial fluid were analyzed by high performance liquid chromatography (HPLC). HPLC analysis of the unsaturated disaccharides: di-4S and di-6S, derived from CS was performed according to the method of Shinmei *et al*. (18).

Systemic clinical assessment
Systemic clinical assessment included the examination of C-reactive protein (CRP; mg/dl), Westergren's erythrocyte sedimentation rate (ESR; mm/h) and duration of early morning stiffness (EMS; min) before and after the SI-6601D clinical trial.

Safety assessment
Drug safety was monitored at all visits and at the completion of the study, or upon withdrawal from the trial,through laboratory tests that included a complete blood cell count, urinalysis, and blood chemistry. The overall safety rate was evaluated on the basis of the combination of the treatment-related clinical adverse reactions and abnormal laboratory data observed during the entire study.

Follow-up monitoring of improvement
As symptoms of pain in motion and swelling were recognized in all the patients at the beginning of the present study, we investigated a follow-up monitoring of the improvements in pain in motion and swelling, and radiographical changes of the knee at arbitrary points (approximately 1-2 years) after the end of the study.

Statistical analysis
Quantitative variables were expressed as mean ± SEM. The statistical significance of changes from baseline was tested by Wilcoxon's signed rank test. The reason for using a non-parametric method was that these variables were not normally distributed.

Results
Patients
The demographic background of the 25 patients is presented in Table I. The patients are divided into two groups (13 responders and 12 non-responders) according to the efficacy of SI-6601D. No statistically significant differences were observed between the responder and the non-responder groups in terms of age, sex, body weight, disease duration, diagnosis, and concurrent medication. The ACR criteria at baseline of the 25 patients is presented in Table II. No differences were observed in the criteria between the responder and non-responder groups.

Local clinical symptoms
Both pain scores (from 4.48 ± 0.44 to 2.60 ± 0.52, $p < 0.0001$) (Fig. 1A) and inflammatory symptom scores (from 4.48 ± 0.04 to 2.28 ± 0.56, $p < 0.0001$) (Fig.1B) were significantly decreased, and so was the volume of synovial fluid (SFV) (from 18.7 ± 3.20 ml to 13.8 ± 3.23 ml, $p < 0.05$) (Fig. 1C) after the SI-6601D treatment. Also, VAS pain by patient's self|-assessment (from 56.2 ± 3.31 mm to 38.0 ± 3.71 mm, $p < 0.001$)

Table I. Demographic profile of the patients.

Demographic characteristics	Total patients (n=25)	Responders (n=13)	Non-responders (n=12)
Age (year)			
Mean ± SEM	60.6 ± 2.20	59.5 ± 3.66	61.8 ± 2.86
Range	(30-76)	(30-74)	(42-76)
Sex			
Male : Female	3 : 22	2 : 11	1 : 11
Weight(kg)			
Mean ± SEM	50.8 ± 1.62	48.8 ± 2.18	53.0 ± 2.35
Range	(38-70)	(38-70)	(42-70)
Disease duration (year)			
Mean ± SEM	8.97 ± 1.09	7.00 ± 1.20	11.1 ± 1.69
Range	(0.83-24)	(1-16)	(0.83-24)
Diagnosis			
Stage I	0	0	0
II	8 (32%)	4 (31%)	4 (33%)
III	5 (20%)	3 (23%)	2 (17%)
IV	12 (48%)	6 (46%)	6 (50%)
Class 1	1 (4%)	1 (8%)	0
2	17 (68%)	10 (77%)	7 (58%)
3	7 (28%)	2 (15%)	5 (42%)
4	0	0	0
Concurrent medication			
Corticosteroid (≤ 5 mg/day)	17 (68%)	11 (85%)	6 (50%)
NSAIDs	22 (88%)	13 (100%)	9 (75%)
DMARDs	23 (92%)	12 (92%)	11 (92%)
Detail:			
Methotrexate	4	1	3
D-penicillamine	6	2	4
Bucillamine	3	2	1
Sulfasalazine	3	2	1
Injectable gold	7	5	2

Table II. The ACR criteria at baseline.

Criterion		Total patients (n=25)	Responders (n=13)	Non-responders (n=12)
Morning stiffness	Yes	19	11	8
	No	6	2	4
Arthritis ≥ 3 joint areas	Yes	24	13	11
	No	1	0	1
Arthritis of hand joints	Yes	24	13	11
	No	1	0	1
Symmetric arthritis	Yes	24	13	11
	No	1	0	1
Rheumatoid nodules	Yes	3	1	2
	No	22	12	10
Rheumatoid factor	Yes	24	13	11
	No	1	0	1
Radiographic change	Yes	24	13	11
	No	1	0	1

(Fig.1D) was significantly decreased after the SI-6601D treatment.

Local biochemical parameters

PGE2 and cytokines. Level of PGE2 in the synovial fluid was significantly decreased (from 330 ± 67.5 pg/ml to 236 ± 52.0 pg/ml, $p < 0.05$) after the treatment with SI-6601D (Fig 2). However, the levels of TGF 1 (from 2401 ± 320 pg/ml to 2953 ± 453 pg/ml), TNF (from 57.8 ± 7.29 pg/ml to 59.6 ± 10.8 pg/ml) and IL-1RA (from 37821 ± 10833 pg/ml to 44651 ± 15371 pg/ml) in the synovial fluid were statistically unchanged.

GAGs. C4S level in the synovial fluid was significantly decreased (from 21.0 ± 2.24 nmol/ml to 17.4 ± 1.66 nmol/ml, $p < 0.05$) (Fig. 3). While that of C6S (from 31.0 ± 5.95 nmol/ml to 25.6 ± 4.35 nmol/ml) was insignificantly decreased by SI-6601D.

Comparison of biochemical parameters between responders and non-responders

In the synovial fluid, significant decreases in the responders were observed for PGE2 (from 305 ± 70.8 pg/ml to 194 ± 63.5 pg/ml, $p < 0.05$) (Fig. 4A), C4S (from 25.8 ± 3.08 nmol/ml to 19.4 ± 2.23 nmol/ml, $p < 0.01$) (Fig. 4B) and C6S (from 42.2 ± 8.86 nmol/ ml to 30.5 ± 6.13 nmol/ml, $p < 0.01$) (Fig. 4C) after treatment with SI-6601D. The levels of TGF 1(from 3119 ± 523 pg/ml to 3549 ± 735 pg/ml), TNF (from 70.9 ± 12.0 pg/ml to 62.8 ± 16.8 pg/ml) and IL-1RA (from 56619 ± 19535 pg/ml to 49033 ± 22891 pg/ml) in the responder group were significantly unchanged. All the biochemical parameters studied in the synovial fluid were insignificantly changed in the non-responders.

Systemic change of PGE2 and cytokines

Plasma level of PGE2 (from 1854 ± 454 pg/ml to 1421 ± 361 pg/ml) was insignificantly changed (Fig. 2). Similarly, the serum levels of TGF 1(from 49972 ± 3656 pg/ml to 51124 ± 3911 pg/ml), TNF (from 25.0 ± 2.55 pg/ml to 26.7 ± 2.69 pg/ml) and IL-1RA (from 425 ± 82.0 pg/ml to 417 ± 70.5 pg/ml)

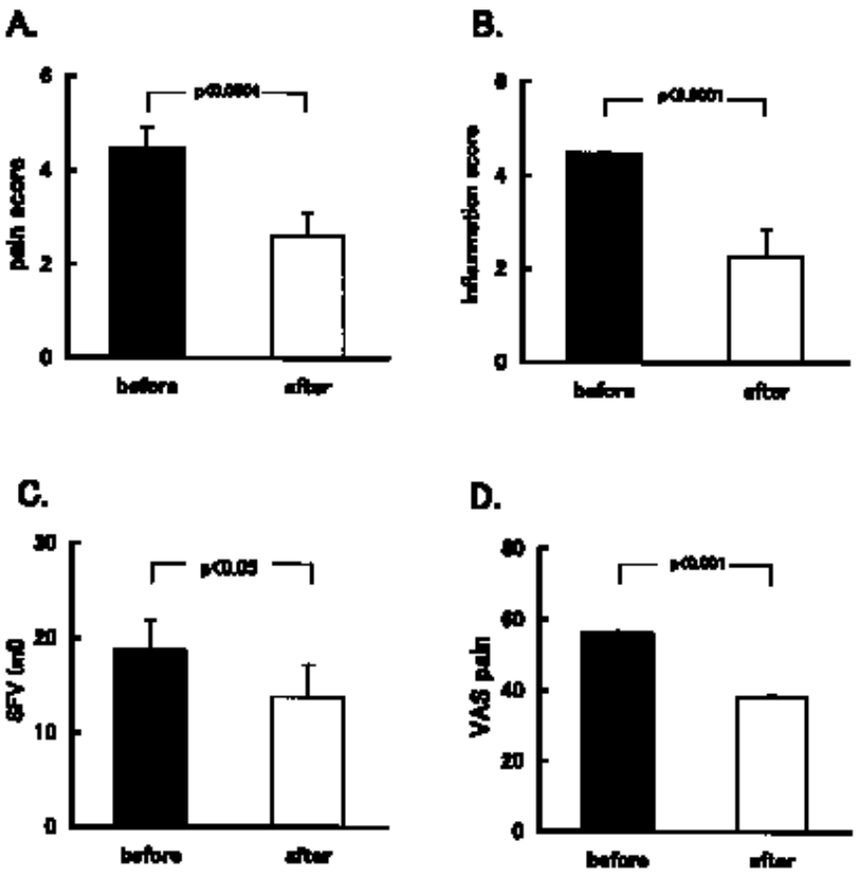

Fig. 1. Clinical improvement of injected knee joints: (**A**) pain score; (**B**) inflammation score; (**C**) synovial fluid volume (SFV); (**D**) VAS pain on walking. Pain and inflammation were evaluated using 4-step rating scales of 0-3, and the total scores (mean ± SEM) are shown for before and after the treatment.

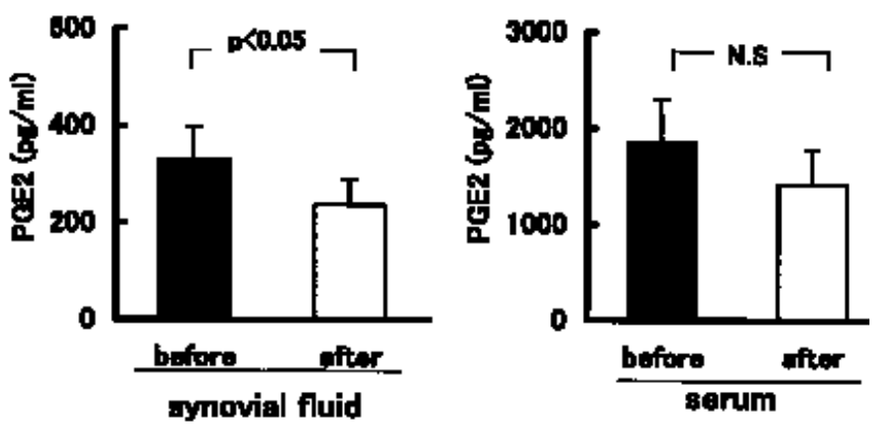

Fig. 2. PGE2 levels in the synovial fluid and serum. The values represent the means ± SEM. N.S: not significant.

were basically unchanged during the study.

Comparison of local and systemic levels of PGE2 and cytokines

Both the plasma level of PGE2 (Fig. 2) and serum level of TGF 1 were significantly higher ($p < 0.0001$) (1854 ± 454 pg/ml and 49972 ± 3656 pg/ml, respectively) than their levels in the synovial fluid (330 ± 67.5 pg/ml and 2401 ± 320 pg/ml, respectively). Both TNF and IL-1RA were significantly higher ($p < 0.001$) in the synovial fluid (57.8 ± 7.29 pg/ml and 37821 ± 10833 pg/ml, respectively) than in the serum (25.0 ± 2.55 pg/ml and 425 ± 82.0 pg/ml, respectively).

Systemic clinical assessment

The mean CRP values were 4.14 ± 0.76 mg/dl before the treatment and 3.47 ± 0.63 mg/ml after the treatment, the mean ESR values were 76.3± 7.76 mm/h before the treatment and 74.4 ± 7.90 mm/h after the treatment, and the mean EMS values were 52.4 ± 18.8 min before the treatment and 48.5 ± 15.7 min after the treatment. These inflammatory measurements did not change significantly before and after the treatment.

Adverse events and laboratory abnormalities

In the 25 patients, one adverse event occurred, with one patient withdrawn after receiving 4 injections of SI-6601D due to aggravation of systemic rheumatoid inflammation. This adverse event was probably not related to SI-6601D, and no laboratory abnormalities unequivocally related to the treatment were observed during the entire study.

Discussion

In the present study, intra-articular injection of SI-6601D improved the local symptoms and reduced the synovial fluid volume with an associated suppression of the PGE2 level. PGE2 is known to enhance vascular permeability (19) to induce hydrops, potentiate the nociceptive activity of bradykinin, and amplify the pain perception (20). Punzi *et al*. (21) reported that HMW-HA suppressed synovial fluid PGE2 levels in 8 patients with various types of arthritis including 4 with RA.

We also observed a decrease in PGE2, while TNF levels remained relatively constant. Since TNF is a possible stimulator of PGE2 production in lymphocytes and macrophages (14), the results suggest that SI-6601D may directly suppress PGE2 production by the inflammatory cells.

The ratio of PGE2 reduction, which was similar to the result by Punzi *et al*. (21), was moderate. However, SI-6601D significantly improved the local pain in RA patients. The results suggest that not only a reduction of the chemi-

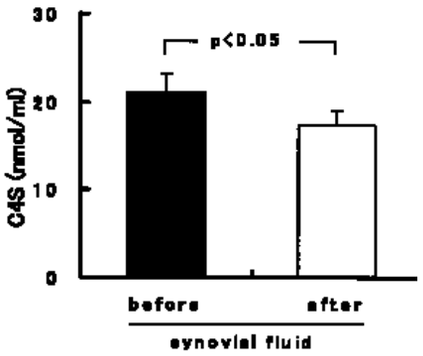

Fig. 3. C4S level in the synovial fluid. The values represent the means ± SEM.

cal mediators but also the improvement of articular lubrication with normalization of hyaluronan in RA synovial fluid (15) is important to improve the local symptoms.

We observed a reduction in C4S levels in the synovial fluid after the SI-6601D treatment. Furthermore, synovial fluid C6S and C4S levels were decreased in the responders. The synovial fluid contains CS isomers, C6S, C4S and C0S, as minor components (22). Since more than 90% of the CS isomer in human articular cartilage is C6S (23) and there is only a trace level in blood (24), it is possible that most synovial fluid C6S originates from the articular cartilage. On the other hand, C4S in synovial fluid may be derived not only from articular cartilage but also from the plasma (24), which predominantly contains C4S and C0S (25). The C4S level in the synovial fluid of RA patients was higher than that of OA, suggesting a correlation with the degree of synovitis (18). Reduction in the C4S level in the synovial fluid may thus be partly attributed to the improvement of synovitis by SI-6601D. Furthermore, the reduction in the synovial fluid C6S level in responders suggests a protective effect of SI-6601D against cartilage degradation. Therefore relief of joint symptoms may reflect not only the improvement of synovitis, but also the prevention of cartilage destruction.

The fact that both systemic biochemical and clinical parameters were basically unchanged during the study may suggest a local effect of SI-6601D in RA knees.

The synovial fluid levels of TNF and IL-1RA were found to be higher than the systemic levels, in line with an earlier report concerning interleukin-6 in RA patients (26). In contrast, the systemic levels of PGE2 and TGF 1 were higher than the synovial fluid values. This is the first report that has monitored the both local and systemic parameters obtained simultaneously.

The follow-up investigation demonstrated a long term improvement in pain on motion in 8 patients (32%) and in swelling in 11 patients (44%), lasting for at least one year, while radiographically assessed joint damage showed no further deterioration in 11 cases (44%). This follow-up monitoring suggested that the treatment with SI-6601D once every week for 5 consecutive weeks may have lasting beneficial effects in RA knee joints.

In conclusion, the present multicenter trial suggests the clinical usefulness of

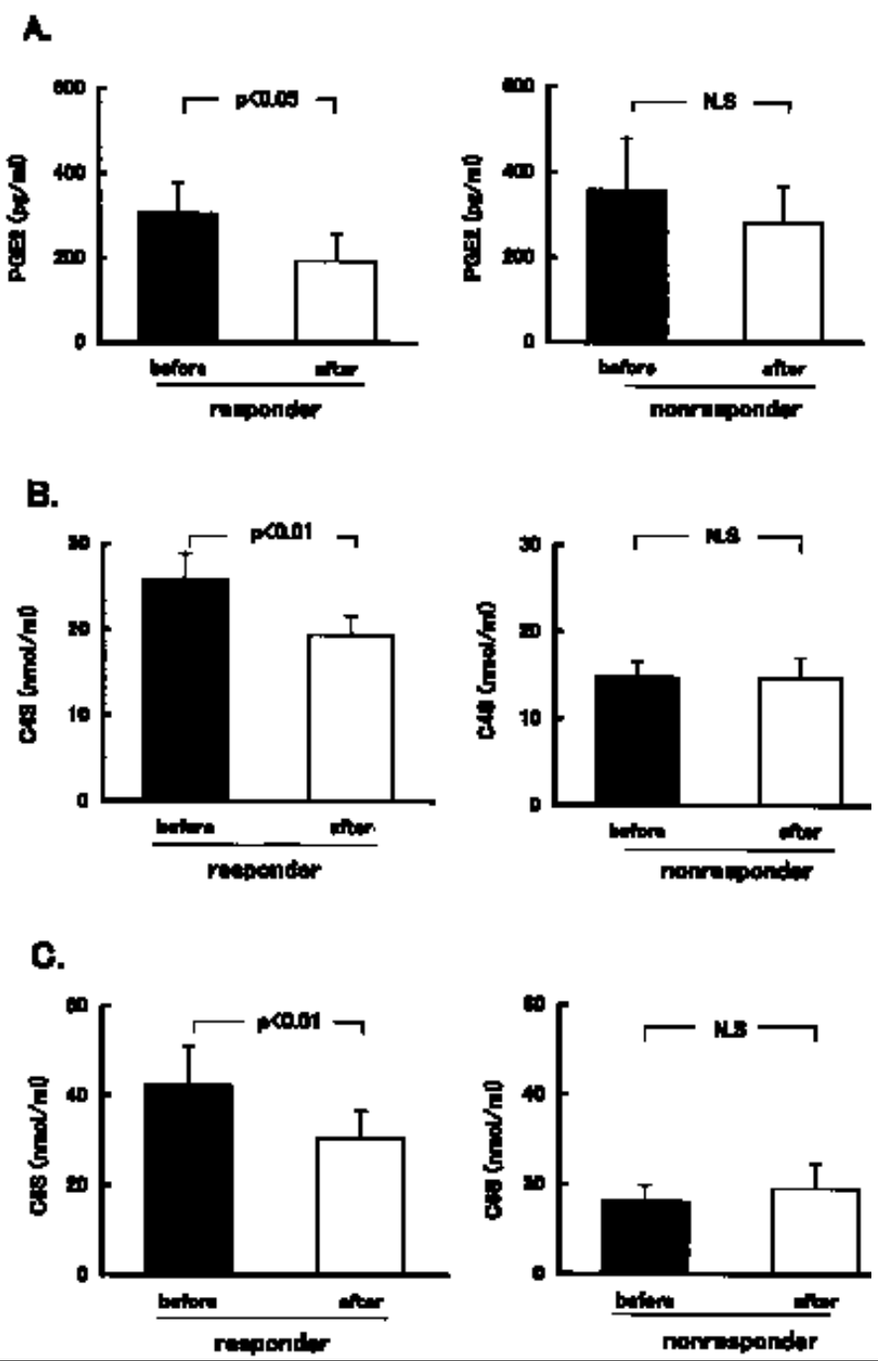

Fig. 4. PGE2 (**A**), C4S (**B**) and C6S (**C**) levels in the synovial fluid divided the responder and non-responder groups. The values represent the means ± SEM. N.S: not significant.

SI-6601D for local therapy in RA patients. SI-6601D may act in part through its mild anti-inflammatory action by suppressing PGE2 production in joints.

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