Safety of Intra-articular Methotrexate Injection With and Without Electroporation for Inflammatory Small Joints in Patients With Rheumatoid Arthritis

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ABSTRACT: The general disease activity of patients with rheumatoid arthritis (RA) is well controlled by disease-modifying antirheumatic drugs, but local inflammation often remains in a few small joints. Electroperation, making small pores in cell membranes, has proven useful for drug delivery. The safety of a combination therapy of methotrexate (MTX) and electroporation for local joint inflammation in RA was investigated in a prospective, randomized, double-blind, placebo-controlled, exploratory study (UMIN000016606). The patients were randomly allocated to groups receiving a combination of MTX and electroporation (True-EP) and MTX alone (False-EP) groups. The MTX solution was injected into finger joints under ultrasound guidance. The True-EP group underwent electroporation with MTX, and the False-EP group was given MTX but only pinched using the electrode. The ultrasound grade, disease activity, and safety were evaluated from baseline to 26 weeks. Five patients (3 True-EP and 2 False-EP) with a mean age of 57.4 years and disease duration of 10.2 years were enrolled. The grey-scale grade was unchanged in 3 cases (2 True-EP and 1 False-EP) and increased in 2 cases (1 True-EP and 1 False-EP). Disease activity was alleviated in 3 cases (2 True-EP and 1 False-EP). No patients experienced burned skin or electroshock. The combination therapy of electroporation and MTX was safe for RA patients.

KEYWORDS: rheumatoid arthritis, electroporation, methotrexate, local inflammation, ultrasound imaging

Introduction

Biological disease-modifying antirheumatic drugs (DMARDs) have brought about dramatic changes in disease activity and have made it possible to achieve and maintain remission in patients with rheumatoid arthritis (RA). For patients with RA who respond insufficiently to conventional synthetic DMARDs, it is recommended that biological DMARDs be used in combination with methotrexate (MTX). Although general disease activity is well controlled by biological and conventional synthetic DMARDs, local inflammation is still observed in a few small joints of the fingers and toes, such as the proximal interphalangeal (PIP) joints, metacarpophalangeal (MCP) joints, and metatarsophalangeal joints. Imaging-detected subclinical joint inflammation indicates structural deterioration in RA patients who are in clinical remission. Such cases mandate the need to develop a new treatment that suppresses local residual inflammation.

Electroporation was developed to transport genetic material into cells. It is based on electric stimulation producing small pores in the cell membrane, thereby allowing nonpermeable molecules, including not only genetic material, but all kinds of hydrophilic molecules, to enter the cell. We previously reported that electroporation is useful not only for moving genetic material but also drugs and that it is directly applicable to drug-delivery systems in vivo. Electroporation provides increased efficacy at lower doses because it increases the rate of drug penetration into cells. Electroporation systems are available clinically to deliver anticancer drugs into malignant solid tumour cells, a technique known as electrochemotherapy. Good clinical results have been reported for its use in battling malignancies in terms of efficacy, safety, and cost. There were early reports of MTX intra-articular injections at the knee for patients with RA to suppress local inflammation, but this technique is not widely used because its efficacy is limited, and the cell permeability of MTX is weak. Thus, electroporation may answer the need for an effective way to increase intracellular MTX levels and attenuate the inflammatory response. This study is the first trial and exploratory study of locally applied electroporation combined with intra-articular MTX injection. The safety of combined MTX and electroporation to relieve local joint inflammation in patients with RA was investigated.
Material and Methods

Trial design

A double-blind, placebo-controlled, exploratory pilot study was performed to investigate the safety and effects of the combined intra-articular injection of MTX solution and electroporation to relieve local joint inflammation in patients with RA. This study was registered with the UMIN Clinical Trials Registry [http://www.umin.ac.jp/ctr/] (UMIN000016606, April 1, 2015). It was conducted at a single hospital between November 2015 and September 2017.

Patients

To be eligible for this study, patients had to meet the following criteria: (1) age > 20 years; (2) fulfilled the American College of Rheumatology 1987 classification criteria14 or the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria15; (3) had been treated using a DMARD with or without biologic agents for at least 3 months; (4) had less than 3 residual swollen, tender MCP, or finger PIP joints. Patients were excluded if they had a history of implantation of an MCP or finger PIP joint, hypersensitivity to MTX, current infection, and/or were pregnant or breastfeeding.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee (No. 3094; March 31, 2015) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients provided written, informed consent prior to participation and submission for publication as a case series.

Intervention and randomization

The patients were allocated, randomly and equally, to groups receiving a combination of MTX and electroporation (True-EP group) or MTX only (False-EP group). Methotrexate solution was injected into the MCP or finger PIP joints. The True-EP group underwent electroporation from 4 directions (Figure 1), whereas the False-EP group was given MTX, but they were only pinched using an electrode without electrification (sham procedure). The treating doctors were blinded to the group with or without electroporation, and the clinical parameters and outcomes were assessed during the treatment term by blinded evaluators. Only the doctor who injected the MTX and performed the electroporation was aware of the group allocation. The procedure was performed once. Clinical assessments and ultrasound examinations were performed over a 26-week period. The primary endpoint was the safety profile of combination therapy of MTX and electroporation. Secondary endpoints were changes in synovitis, which was assessed using an ultrasound examination, and in the disease activity score 28-erythrocyte sedimentation rate (DAS28-ESR).16

Ultrasound-guided intra-articular injection and electroporation

Injectable MTX was purchased from Pfizer Japan Inc (Tokyo, Japan). The MTX dosage was set at 5 mg/mL. It was administered as a 0.5 mL (2.5 mg) injection into the MCP or finger PIP joint via a horizontal approach under ultrasound guidance. The dosage was determined using a previous report that the minimum and maximum effective dosages were 2.5 and 10.0 mg, respectively.12

An electroporation apparatus (CUY-21; Bex Co, Ltd, Tokyo, Japan) was used to generate transient pores in cell membranes of inflamed soft tissues in the joint. Direct-current electrical pulses (4 Hz, 100 ms pulse duration, 30 V electrode distance) of 1-s duration were delivered from 4 directions during a single procedure. Two parallel stainless-steel electrodes were made for MCP and finger PIP joints, as indicated in Figure 1. This specific delivery of electrical pulses was selected for the safety of the patient based on a preliminary test. Electrode paste (Cardio cream; Nihon Kohden, Tokyo, Japan) was used to prevent skin burns and enable better energy delivery.
Ultrasound examination

Ultrasound was performed on all patients by a single rheumatologist (T.O.), who was experienced in musculoskeletal ultrasonography and was blinded to the treatment/control groups and the clinical response of each patient. Ultrasound scans were performed using the HI VISION Avisus device (Hitachi Aloka Medical, Ltd, Tokyo, Japan). Local inflammation of the synovium was evaluated via a systematic multiplanar grey-scale (GS) and power Doppler (PD) ultrasound examination at the PIP or MCP joint. Grey-scale and PD were scored on a scale of 0-3 using a semi-quantitative method and the European League Against Rheumatism-OMERACT scoring system.17,18 Longitudinal dorsal midline scans were used to score each joint. The intraclass correlation coefficient of intraobserver agreement was good (0.983, 95% confidence interval=0.971-0.992).

Radiographic scoring

Radiographs, which were obtained at baseline, were scored independently (using the Larsen grade)19,20 by 2 trained readers (Y.S. and K.M.) blinded to the treatment/control groups and the clinical response of each patient. Altogether, 10% of the films were re-read to analyse intra-reader variability.

Clinical assessment

Disease activity was evaluated using the DAS28-ESR,16 simplified disease activity index,21 laboratory data, and patients’ local pain visual analogue scale scores at baseline and after 2, 4, 12, and 26 weeks. Safety assessments were performed throughout the study, covering pain, infection, and local burn injury.

Statistical analysis

A sample size of 40 joints (20 joints in the True-EP group, 20 joints in the False-EP group) was calculated to provide at least 80% power for detecting a significant (P<0.05) difference in the safety profile when comparing MTX monotherapy versus combined MTX and electroporation, as we previously reported.6 However, only 5 patients were finally enrolled because there was a lack of patients with only 1 or 2 swollen, tender joints, and several patients did not consent to an intra-articular MTX injection and electroporation. In addition, the risk/benefit ratio of this procedure was high. Hence, a statistical analysis could not be performed, and the study is reported as a case series.

Results

Patients’ characteristics

The baseline demographic data for the 5 RA patients are shown in Table 1. The mean age was 57.4 years, and the mean disease duration was 10.2 years. Swelling and tenderness persisted at small local joints. The procedure positions were 3 PIP joints (2 True-EP and 1 False-EP) and 2 MCP joints (1 True-EP and 1 False-EP). Disease activity was low or moderate (DAS28-ESR: 2.46-3.48 and simplified disease activity index: 4.48-13.91), and serology was positive in all patients, except Case 2. Synovitis in local joints was active (assessed using ultrasound GS and PD), and joint destruction was moderate (assessed by the Larsen grade). All patients were treated with MTX (4-12 mg/week), and 2 patients were also treated with biological DMARDs (abatacept).

Disease activity and safety

The GS did not change from Grade 3 in 3 cases and increased from Grade 2 to Grade 3 in Case 2 (False-EP) and Case 3 (True-EP). The PD grade did not change in 4 cases (Cases 1, 2, 4, and 5) and increased from Grade 2 to Grade 3 in Case 3. The results are summarized in Figure 2 and Table 2. The efficacy of the interventions was not different between the True-EP and False-EP groups.

Case Presentation

Case 1 is outlined in Table 1 and Figure 3. A 60-year-old woman with 4 swollen and 2 tender joints, including the left third PIP joint, had been treated with MTX (10 mg/week), tacrolimus (1.5 mg/day), and abatacept (500 mg by intravenous injection once every 4 weeks). In this study, she was randomly assigned to the True-EP group. Methotrexate was injected into her articular joint, and the joint underwent electroporation. After 26 weeks, the PD (Grade 2) and GS (Grade 3) were unchanged.

Discussion

This study is the first trial of the safety of the combined MTX and electroporation regimen to treat local joint inflammation in patients with RA. With ultrasound guidance, MTX solution was accurately injected intra-articularly, and synovitis of the local joint was evaluated. There was no enhancement of the effect by the electroporation-MTX combination compared with MTX alone. This finding may have resulted from the small number of cases. Even so, there were no safety issues or adverse events with the therapy, which was the only positive result in this study.
An intra-articular MTX injection for the knee joint was first reported by Hall and Head.\textsuperscript{22} Positive effects of an intra-articular MTX injection were reported previously,\textsuperscript{11,12,23,24} although others reported negative results.\textsuperscript{25,26} Consequently, this treatment remains controversial. One reason for the negative results is that the transport of MTX into cells is not efficient. We performed an exploratory study for electroporation as a drug-delivery system to improve MTX efficacy through

\begin{table}
\centering
\caption{Patient demographics and clinical characteristics at baseline.}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{CASES} & 1 & 2 & 3 & 4 & 5 \\
\hline
\textbf{Group} & True-EP & False-EP & True-EP & True-EP & False-EP \\
\hline
\textbf{Position and joint} & Left third PIP & Left fourth MCP & Left fifth PIP & Right second MCP & Left third PIP \\
\hline
\textbf{Age, y} & 60 & 75 & 57 & 49 & 46 \\
\hline
\textbf{Sex} & Female & Male & Female & Female & Female \\
\hline
\textbf{Disease duration, y} & 3.7 & 14.4 & 9 & 15.6 & 8.2 \\
\hline
\textbf{Swollen joint counts} & 4 & 2 & 3 & 1 & 2 \\
\hline
\textbf{Tender joint counts} & 2 & 2 & 2 & 3 & 1 \\
\hline
\textbf{DAS28-ESR} & 3.32 & 3.33 & 3.28 & 3.48 & 2.46 \\
\hline
\textbf{SDAI} & 13.91 & 10.31 & 8.55 & 11.51 & 4.48 \\
\hline
\textbf{CRP, mg/dL} & 0.01 & 0.31 & 0.05 & 0.01 & 0.08 \\
\hline
\textbf{MMP-3, ng/dL} & 41.8 & 96.1 & 46.7 & 66.3 & 38.6 \\
\hline
\textbf{Anti CCP antibody, U/mL} & 85.7 & 0.6 & 100 & 500 & 6.2 \\
\hline
\textbf{Rheumatoid factor, IU/mL} & 9 & 5 & 7 & 110 & 105 \\
\hline
\textbf{mHAQ} & 0.25 & 0.25 & 0 & 1.5 & 0 \\
\hline
\textbf{Local pain VAS, mm} & 43 & 72 & 30 & 62 & 45 \\
\hline
\textbf{US grey-scale} & 3 & 2 & 2 & 3 & 3 \\
\hline
\textbf{US power Doppler} & 2 & 3 & 2 & 2 & 3 \\
\hline
\textbf{Larsen grade} & III & I & I & III & III \\
\hline
\textbf{Medication, biologics} & MTX 10 mg/week ABT & MTX 8 mg/week SASP 1 g/day IGU 50 mg/day & MTX 4 mg/week ABT & MTX 12 mg/week SASP 1 g/day IGU 50 mg/day PSL 5 mg/day & MTX 12 mg/week BUC 200 mg/day IGU 50 mg/day \\
\hline
\end{tabular}
\end{table}

Abbreviations: ABT, abatacept; BUC, bucillamine; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; DAS, disease activity score; EP, electroporation; ESR, erythrocyte sedimentation rate; IGU, iguratimod; MCP, metacarpophalangeal joint; mHAQ, modified health assessment questionnaire; MMP-3, matrix metalloprotease 3; MTX, methotrexate; PIP, proximal interphalangeal joint; PSL, prednisolone; SASP, salazosulfapyridine; SDAI, simplified disease activity index; TAC, tacrolimus; US, ultrasound; VAS, visual analog scale.

Figure 2. The efficacy of MTX and electroporation is evaluated using ultrasound. The changes over time in grey-scale (A) and power Doppler (B) of each case are indicated. EP indicates electroporation; MTX, methotrexate.
enhanced cell penetration. The combined MTX and electroporation therapy was well tolerated by the patients in this study. However, improved delivery of drug to the target cells was not shown, for several possible reasons. The injection and electroporation procedure was performed only once, whereas intra-articular MTX injections were performed multiple times in the previous studies that reported efficacy.\textsuperscript{11,12,23,24} The MTX dosage in this study was based on the results of those previous studies, which reported that the minimum effective dosage was 2.5 mg and the maximum was 10 mg for the knee joint. The safety and efficacy of intra-articular MTX injection for small joints were considered, expecting that electroporation would have an enhancing effect. Therefore, an MTX dosage of 2.5 mg was selected. The concentration of MTX, however, might have been insufficient. Unfortunately, data from dose-response curves for MTX injections in PIP and MCP joints were not available.

The electrical setting was an important factor for the safety and efficacy of electroporation. The electroporation setting for soft tissue tumours was high (100 V/cm).\textsuperscript{8} In our preliminary experience, we performed electroporation on ourselves, with the electrical voltage at 100 V/cm for our PIP and MCP joints. However, we experienced pain at both joints, as well as numbness along the digital nerve. Therefore, the electrical voltage was decreased gradually until there was no pain or numbness. Ultimately, it was decided to set it at 30 V/cm for safety. Pulse duration and direction were increased to strengthen the efficacy of electroporation, instead of lowering the voltage. It was

Table 2. Changes in evaluated parameters from baseline to 26 weeks.

| CASES | GROUP  | US GS | US PD | DAS28-ESR | LOCAL PAIN VAS | SAFETY          |
|-------|--------|-------|-------|-----------|----------------|-----------------|
| 1     | True-EP| 3→3   | 2→1   | 3.32→3.02 | 43 mm→31 mm    | No pain and burn|
| 2     | False-EP| 2→3   | 3→3   | 3.33→3.71 | 72 mm→88 mm    | No pain and burn|
| 3     | True-EP| 2→3   | 2→3   | 3.28→2.31 | 30 mm→35 mm    | No pain and burn|
| 4     | True-EP| 3→3   | 2→2   | 3.48→3.41 | 62 mm→45 mm    | Pain 1 time and no burn|
| 5     | False-EP| 3→3   | 3→3   | 2.46→1.88 | 45 mm→40 mm    | No pain and burn|

Abbreviations: DAS, disease activity score; EP, electroporation; ESR, erythrocyte sedimentation rate; GS, grey-scale; PD, power Doppler; US, ultrasound; VAS, visual analog scale.

Figure 3. Methotrexate is injected into the left third PIP joint (A) and the joint is electroporated using 2 parallel stainless-steel electrodes (B). The Grade 2 power Doppler value of the PIP joint at baseline improves to Grade 1 at 12 weeks. However, this has increased at 26 weeks, based on the longitudinal images (C to E). The grey-scale readings do not change, and the grade is 3 after 26 weeks.
surnised that the electrical setting for joints is different from that for soft tumours, because the PIP and MCP joints contain bone and ligament, and there is a digital (sensory) nerve along the side of the joint. Therefore, a relatively low electrical setting was used for electroporation to prevent side effects.

We previously reported on the efficacy and safety of combination therapy of electroporation and MTX when studying adjuvant arthritis in rats. Combination therapy decreased the swelling and prevented joint destruction. It also showed that microbubble-enhanced ultrasound exposure promoted MTX uptake into synovial cells, which enhanced the anti-inflammatory effects of an intra-articular MTX injection in a rabbit arthritis model. In vivo, electroporation and microbubble-enhanced ultrasound are useful as a drug-delivery system for MTX into synovial cells by making small pores in the cell membrane. However, the therapeutic effect of this combination therapy was not shown to be significant in 5 human cases.

It was reported that MTX polyglutamate is important for maintaining an effective MTX concentration inside the cell. If MTX does not contain a glutamate moiety (PG2-PG5), it cannot remain within the cell and is excreted into the extracellular environment. De Rotte et al. reported that a relatively high concentration of MTX polyglutamates in erythrocytes was associated with lower disease activity. An intra-articular MTX injection combined with electroporation could promote infiltration of MTX into the cell. However, the procedure was performed only once in this study, and the polyglutamation of MTX might not have been sufficient, causing its excretion into the extracellular environment and, thus, a lack of effect. It is unfortunate that no experiment was performed to address this possibility. We may need to change the frequency of the procedure from a single injection to multiple injections to enhance efficacy.

It has been reported that a glucocorticoid articular injection decreased swelling and relieved pain at a joint with residual local inflammation. Ultrasound guidance significantly improved the accuracy of the injection compared with clinical examination guidance. It is unknown which is more effective for inflammatory joints: glucocorticoid injection or a combination of MTX and electroporation. With the 5 patients enrolled in this study, an interim analysis was performed by an independent monitor. The combination procedure was assessed as clearly being less effective than a single intra-articular injection of glucocorticoid without electroporation. Hence, it was concluded that this study protocol provided no benefit for the participants. Therefore, it was decided to abort the study, although the safety profile of this procedure was established.

This study has some limitations. First, the number of cases enrolled was small. The efficacy of MTX and electroporation could not be shown with only 5 cases, and the study was discontinued. Second, the settings used for the electroporation procedure might not be adequate for synovitis because specific electroporation conditions were chosen based on preventing adverse events. This voltage setting was decided based solely on safety. Whether this setting was the most suitable for the efficacy of electroporation was not known. Third, MTX polyglutamate levels were not considered in this study. Methotrexate may have been excreted into the extracellular environment, despite its infiltration into the cell by electroporation.

In summary, the safety of local combination therapy of MTX and electroporation was compared with that of MTX alone. Methotrexate injection and electroporation did not result in safety concerns, causing neither burns nor pain.

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Author Contributions

Study design: M.T. and K.I.
Study implementation: M.T.
Data collection: T.O., M.T., and K.M.
Data analysis: M.T. and K.I.
Data interpretation: M.T., T.K., H.N., and K.I.
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