Transduction of siRNA Targeting TNF-α to Rat Knee Joint via Sonoporation

INTRODUCTION:
Biological agents which hinder tumor necrosis factor-α (TNF-α) have been clinically developed to treat rheumatoid arthritis (RA), and studies have demonstrated that such agents are more effective than conventional antirheumatic agents in suppressing inflammation and joint destruction. These agents are generally administered systemically. However, development of new local administration therapies for patients with single-joint or a few joints RA are necessary in terms of safety and efficacy.

We previously reported that targeted suppression of the tumor necrosis factor-α gene through electroporation significantly ameliorated collagen-induced arthritis in rats. However, adverse effects of electroporation, such as muscle damage, have been reported, and less invasive methods are preferred for clinical use. At this time, the inhibitory effects of small interfering RNA targeting TNF-α (siTNF) via sonoporation on the synovium were analyzed.

METHODS:

Cell preparation
Synovial fibroblast-like cells (FLSs) were isolated from the knee joints of 8-week-old male Sprague Dawley (SD) rats.

siRNA duplexes
Two small interfering RNA (siRNA) duplexes targeting the rat TNF-α gene (siRNA-A and siRNA-B) were synthesized. Experiments were conducted in vitro on the most effective siRNA sequences for suppression of TNF expression in rats. Each siRNA was transfected into cultured FLSs from rats to compare silencing competence. To transduct siRNA, the FLSs were seeded. After 24 hours, the medium was changed, and the cells were transfected with 50 nM siRNA.

RNA extraction and real time RT-PCR in vitro
After 24 hours, total RNA was extracted and then the extracted RNA was reverse transcribed. Next, gene expression of TNF was measured by quantitative real-time PCR.

Transduction of fluorescence-labeled siRNA or siTNF into the synovium
Rats were anesthetized and hair around the left knee was shaved off. 10 μl of microbubbles was added to 40 μl of siRNA (siTNF or fluorescence-labeled siRNA), and the mixture was injected into the left knee joint of the rats. Immediately after injection, the skin around the knee was coated with ultrasound conduction gel, and ultrasound sonication was performed with an 8 mm diameter probe.

Fluorescence microscopic observation
After 24 hours, the rats were sacrificed. The knee joint of the fluorescence-labeled siRNA group was dissected and examined under fluorescence stereomicroscope.

RNA extraction and real time RT-PCR in vivo
The synovia of the knee joints of the siTNF group were excised, and samples were immersed in Sepasol and snap frozen in liquid nitrogen. The extracted RNA was reverse transcribed, and gene expression of TNF was measured by quantitative real-time PCR.

RESULTS SECTION:
Although significant silencing of TNF mRNA was induced by both siRNAs (p<0.05) (n=3 in each group), the most potent effect was observed with TNF-specific siRNA-B. TNF-specific siRNA-B was then used for the following examinations.

The fluorescence-labeled siRNA was seen in the synovium around the patella, femur, and tibia.

Gene expressions of TNF in the synovial tissue of the siRNA-B injected knee joints followed by sonoporation significantly decreased 48.3%, in comparison with that of the control group (p<0.05) (n=3 in each group).

One-way ANOVA followed by Tukey-Kramer multiple comparison were used to evaluate differences in statistical significance.

DISCUSSION:
We previously reported that transduction of various siRNA suppressed arthritis via electroporation in the rat knee. Agents such as TNF-α, IL-1β, IL-6, and RANKL are involved in the development of arthritis. For these, anti-TNF-α therapy by the in vivo electroporation method significantly ameliorated arthritis model rats. Therefore, preventing the function of TNF-α in joints is a promising strategy in the treatment of joint diseases.

However, adverse effects of electroporation, such as muscle damage, have been reported, and there is some difficulty in its clinical use. Therefore sonoporation was used as a less invasive method.

In this experiment, fluorescently labeled siRNA could be transducted with microbubbles via sonoporation, and the samples were observed under fluorescence microscopy 24 hours after transduction. We could also show that gene expression of TNF in the synovium could be suppressed in rat knees administered siTNF by sonoporation. Inhibiting arthritis by local administration may be useful for patients with a single-joint or a few joints RA.

SIGNIFICANCE:
We analyzed transduction of siTNF to rat knee joints via sonoporation. Reduction of gene expression of TNF via sonoporation may ameliorate synovitis.

ACKNOWLEDGEMENTS:
This work was supported by a grant from the Research Unit for Development of Livelihood Support Medical Equipment, Kyoto Prefectural University of Medicine, for their technical assistance.

REFERENCES:

Fig.1 Suppression of TNF mRNA in vitro

Fig.2 Suppression of TNF mRNA in vivo