Sonoporation-mediated Transduction of siRNA Targeting TNF Alpha Ameliorated Experimental Arthritis

Hiroaki Inoue¹, Yuji Arai¹, Ryu Terauchi¹, Shuji Nakagawa¹, Masazumi Saito¹, Shinji Tsuchida¹, Atsuo Inoue¹, Tomohiro Matsuki², Toru Morihara¹, Toshiharu Shirai¹,², Osam Mazda³, Toshikazu Kubo¹.

¹Department of Orthopaedics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan, ²Department of Orthopaedic Surgery, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan, ³Department of Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Disclosures:

Introduction: Synovial macrophages in patients with rheumatoid arthritis (RA) produce tumor necrosis factor alpha (TNF-α), a central component in the cytokine cascade. TNF-α acts on synovial fibroblasts, inducing the expression of inflammatory mediators, indicating the important role of TNF-α in the pathophysiology of RA. In addition, inflammatory cytokines including TNF-α are potent inducers of bone destruction, and cause joint dysfunction [1]. Thus, synovial tissue, which produces inflammatory cytokines, is an important target tissue in RA treatment.

Meanwhile, sonoporation is a method for gene transduction that makes use of ultrasounds [2]. Ultrasound is already widely utilized in daily clinical practice. As no adverse biological effects have been associated with these applications of ultrasound, we regarded sonoporation as a safe technique for gene transduction.

We therefore utilized sonoporation to transduce siRNA targeting TNF-α (siTNF) into the synovium of a rat arthritis model and analyzed the safety and efficacy of this method.

Methods: DA rats were anesthetized and the hair around the left knee was shaved off. Ten μl of microbubbles was added to 40μl of siRNA (siNeg, siTNF, or fluorescence-labeled negative siRNA). The mixture was injected into the left knee joint of rats. Immediately after injection, ultrasound conduction gel was painted onto the skin around the knee, and ultrasound sonication was performed for one minute through a probe. After 24 hours, the rats were sacrificed. In the fluorescence-labeled negative siRNA group, the knee joint was resected and examined under fluorescence stereomicroscope. In the siNeg and siTNF group, the synovium of the knee joints were excised. The specimens were immersed in Sepasol and snap frozen in liquid nitrogen.

Extracted RNAs were reverse transcribed. And gene expression of TNF was measured by quantitative real-time PCR. Immediately after sonoporation, temperatures at the surface of the rat knee joint were measured. Collagen induced arthritis (CIA) rats were immunized. The mixture of microbubble and siRNA (siNeg or siTNF) was sonoporated every 3 days (i.e., 7, 10, 13, 16 days after immunization). Control group received no treatment after immunization. Foot volume and arthritis score were measured every day. Twenty-eight days after immunization, radiographs were taken of the hind paws of CIA rats. Bone and cartilage destruction were classified and scored. To investigate the effects of siTNF on the ankle synovium, sections were stained with anti-TNF-α antibody.

Results: The fluorescence-labeled siRNA was seen in the synovium around the patella, femur, and tibia. The gene expressions of TNF-α in the synovial tissues of the siTNF injected knee joints followed by sonoporation were significantly reduced 48% compared those of control group (p<0.05) (n=3 in each group). Mean skin temperatures before and soon after sonoporation were 26.8ºC and 27.3ºC, respectively. However, no adverse effects, such as burns or tissue destruction, were observed.

In vivo sonoporation of siTNF into the knee joint every 3 days (i.e., 7, 10, 13, and 16 days after immunization) resulted in significant inhibition of paw swelling 20 to 23 days after immunization compared with siNeg group (Figure 1). The radiographic scores in siTNF group were significantly reduced to 61% compared with the siNeg group. Histological examination of the paws showed that the number of TNF-α positive cells was significantly lower in areas of pannus invasion into the ankle joints of the siTNF- than of the siNeg-treated rats.

Discussion: Ultrasound is widely used in clinical practice, and there have been no reports of adverse effects, suggesting that it is quite safe. The intensity of ultrasound is important for gene transduction because cell membrane permeability is dependent on cavitation resulting from ultrasonication [3]. We therefore sonoporated articular synovia at output intensities of 0.5, 1.0, and 2.0 W/cm2, finding that the efficiency of transduction was highest at 2.0 W/cm2 [2]. We found that sonoporation did not markedly elevate skin temperature and no adverse effects, such as burns or tissue destruction, were observed, suggesting that this method was safe for siRNA transduction.

Several biological agents targeting TNF-α are used clinically, and combining these biologics with methotrexate can improve the therapeutic effects of the latter, even in patients in whom methotrexate monotherapy is ineffective. Although biological agents are administered systemically, their effects on various joints may not be uniform. Moreover, systemic administration is not
suitable for patients with monoarthritis, due to adverse effects. Rather, these patients require local inhibition of arthritis. Using specific siRNA, we knocked down TNF-α gene expression specifically in the synovium. Production of TNF-α protein in the ankle joint synovium of siTNF treated CIA rats was lower than that in CIA group or siNeg treated CIA rats. The siTNF acts specifically on TNF-α and may also inhibit inflammatory factors downstream of this cytokine, as well as controlling synovitis. Moreover, we found that radiological scores in the siTNF transduction group were significantly lower than in the other groups. These findings suggest that siTNF transduced by sonoporation may have inhibited bone destruction by suppressing osteoclast differentiation.

**Significance:** Transduced siTNF into the joints of CIA rats by sonoporation inhibited synovitis.

Sonoporation is a method for gene transduction without adverse effects such as burn.

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**References:**