Comparison of anti-rheumatic effects of local siRNA therapy using various cytokine genes as molecular targets

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Introduction: RNA interference (RNAi) provides the powerful means of sequence-specific gene silencing (1). Several studies applied RNAi to the treatment of various disorders in animal models and showed that RNAi may provide promising strategies to treat human diseases by suppressing disease-responsible genes in vivo. We previously reported that small interfering RNA (siRNA) was transfected into articular synovium by in vivo electroporation method effectively without using any viral vectors, and the progression of collagen-induced arthritis (CIA) was suppressed by transducing tumor necrosis factor-α (TNF-α) specific siRNA into synovium (2). The aim of this study was to compare the effects on CIA in rats through the suppression of cytokine gene by delivery of siRNA targeting for TNF-α, interleukin-1β (IL-1β), interleukin-6 (IL-6) or receptor activator of NF-κB ligand (RANKL).

Materials and Methods: Four rats TNF-α and IL-1β specific siRNA and two rats IL-6 and RANKL specific siRNA were synthesized. We selected the most potent siRNA, which induced silencing effect in vitro study.

The each specific siRNA was delivered into the knee joints of CIA rats through the in vivo electroporation method on 7,10,13,16 days after immunization. The control group was transduced no siRNA after immunization. To analyze the silencing effects in vivo, the expression of each cytokine in the synovium was examined using real-time PCR. To compare the therapeutic effect of each siRNA in vivo, gross morphological, radiographical and histological examinations were performed.

Results: The expressions of TNF-α, IL-1β and IL-6 in the knee joints were reduced 57.8%, 44.9% and 14.9% compared those of control joints. The expressions of each cytokines in the ipsilateral ankle joints were also reduced 52.0% 57.0% and 19.2%.

The siTNF-α and siIL-1β suppressed and delayed paw swelling of CIA rats. siIL-6 and siRANKL also induced the amelioration of arthritis, but the effect was milder than that of siTNF-α or siIL-1β. These effects were not observed in the control group (Fig.1).

Discussion: In the treatment for rheumatoid arthritis (RA), anti-cytokine agent has been attention as a novel anti-rheumatic agent. However, the side effects could not be neglected because it is administrated systemically. If inflammatory cytokine can be locally inhibited in joints, it would be a safe and effective therapy for RA. The transfection of siTNF-α into synovium induced the amelioration of CIA (2). However, the suppression of TNF-α can not always induce the effective amelioration in RA, because the various cytokines, such as TNF-α, IL-1β and IL-6, have influence on the onset and progression of joint destruction in RA(3).

We demonstrated the down-regulation of target cytokine in vivo at the RNA level. In the case of siTNF-α and siIL-1β, 57.8% and 44.9% silencing of cytokine expression in vivo induced the therapeutic effects. But the strongest silencing (14.9%) of cytokine expression in vivo did not induce the therapeutic effects in the case of siIL-6. According to these results of in vivo cytokine expressions and the therapeutical results, TNF-α is located in the up-stream of the cytokine cascade and is the targeting cytokine which we should suppress in the local arthritis.

This strategy- in vivo siRNA transfection method- was useful for the analysis of pathology of joint disease.

References: (1) Hannon GJ. Nature 2002; 418:244-51.

Fig.1. Kinetic change of paw volume of CIA rats.

. Arthritis inhibitory effects were observed in the hind limb on the delivered side, but not on the opposite side. According to the radiographical and histological examinations of the paw, the control group showed typical signs of arthritis. In contrast, the paws of siTNF-α and siIL-1β treated animals were virtually normal (Fig.2 and 3)

Fig.2. Radiographic evaluation of CIA rats.

Fig.3. Histological demonstration of therapeutic outcome of siRNA therapy.

. Radiographical and histological arthritis scoring also indicated that manifestations of arthritis were significantly milder in the siTNF-α and siIL-1β treated groups than in control groups.

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