EFFICIENT NON-VIRAL GENE DELIVERY IN VIVO INTO SYNOVUM

*Ohashi, S; **Kubo, T; *Takahashi, k; *Arai, Y; *Ikeda, T; *Terauchi, R; **Satoh, E; **Imanishi, J; **Mazda, O; *Hirasawa, Y

**Department of Orthopaedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan. Kamigy-ku, Kyoto, 602-8566, Japan, +81-75-251-5549, Fax: +81-75-251-5841, tkubo@koto.kpu-m.ac.jp

INTRODUCTION:

Gene delivery into joints is considered to become a useful strategy for the treatment of joint diseases. Viral vectors are commonly used because of their high transduction efficiencies. Despite of recent improvement of the vectors, the viral vectors are associated with some unsolved problems such as induction of inflammatory responses and undesired generation of replication-competent viruses. In treatment for joint diseases that are problematic but not lethal, the safety issue is quite important. In order to meet this point, the non-viral vectors has attracted attention of researchers. However, the gene delivery by non-viral vectors has given only transient, low levels of gene expression after the intra-articular injection. To improve the transduction efficiency in vivo, we employed the Epstein-Barr virus (EBV)-based episomal vector combined with a cationic polymer, i.e., degraded polyamidoamine (PAMAM) dendrimer (the EBV/polyplex).

METHODS:

The EBV-based plasmid vector, pGEG.ß, is composed of the E.coli ß-gal gene (LacZ) located downstream of the CAG promoter, EBV oriP and EBV nuclear antigen (EBNA) 1 gene under the control of another CAG promoter (2). Ten microgram of pGEG.ß was conjugated with 105µg of PAMAM dendrimer, and injected into the left knee joints of female Wister rats (B.W. 250g). The right knee joints of the rats were injected with the same amount of the PAMAM dendrimer alone as control. Rats were injected on day 0 and then divided into three groups each n=3. The first group (Group 1) received no additional injection. The second group (Group 2) received second injection of the same amount of DNA/dendrimer on day 3 (2 injections in total), while the third group (Group 3) was given two more injections on days 3 and 6 (3 injections in total). Animals were sacrificed 3 days after the last injection and the joints were harvested. The tissue block was fixed in 2% glutaraldehyde followed by staining with X-gal. The blue staining level was evaluated and classified into 4 grades, i.e., Grade 1 (no staining), Grade 2 (weak staining), Grade 3 (strong staining). Histological examination was performed with frozen sections of the specimens.

RESULTS:

Grossly, the synovial tissues of all three groups stained blue with X-gal, while articular cartilage did not significantly stain. Average score of the Group 1 was 1.0 while those of Groups 2 and 3 were 2.0 and 1.7, respectively. Histologically, LacZ expression was found in lining cells of the synovium but not in chondrocytes in articular cartilage. There was no apparent infiltration of inflammatory cells in all tissues. The synovial tissues from animals of the Groups 2 and 3 showed higher intensity of X-gal staining as well as higher proportion of staining cells in comparison with the same tissues from the Group 1 animals. No remarkable difference was observed in the staining levels between Groups 2 and 3.

DISCUSSION:

In the previous study, we demonstrated high transfection efficiency into chondrocytes via the EBV/polyplex in vitro (1). EBV-based episomal vector is a non-viral plasmid vector carrying the EBNA-1 gene and EBV oriP element. Any infectious viral particle can not be produced by using this vector. We have already reported that extremely efficient transfection can be succeeded in various cells by this vector system (2). In the present study, high transfection efficiency into synovium was achieved by repetitive intra-articular injections. The EBNA-1 expression resulted from the first transfection may augment expression of genes delivered by the following injections. No apparent inflammatory reaction was observed with the multiple injections. The results suggest that the repetitive transduction with EBV/polyplex may provide useful and safe methods for direct intra-articular gene delivery in vivo.

ACKNOWLEDGMENT:

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES:


Fig. 1. LacZ gene expression in the knee joints after pGEG.ß/PAMAM dendrimer injection. (A): Control knees showed no blue staining. (B): Highly efficient gene transfer was observed in the joint of Group 2.