GENE THERAPY USING IN VIVO ELECTROPORATION TO TREAT RAT OSTEONECROSIS

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Introduction: Electroporation is one of the alternative tools of gene transfer for clinical use. Whereas this procedure is easy, costless and rarely induces immuno-response, the efficacy of gene transfer is lower than that of gene therapies using viral vectors. The pCAGGS vector, recently we developed, improved this problem. This vector demonstrated acceptable efficacy of gene transfer when it was injected into muscles by in vivo electroporation. Bone marrow is rich with progenitor cells for tissue regeneration and will be reasonable target for gene therapy. The aims of this study were two folds as follows; 1) to establish a gene transfer into bone marrow by in vivo electroporation, and 2) to transfer the hepatocyte growth factor (HGF), a potent angiogenetic factor, into the rat osteonecrosis model to analyze the histological changes.

Materials and Methods: To determine the optimum condition for gene transfer, we transferred the pCAGGS vector, harboring luciferase gene, into bone marrow in the proximal femora of Wistar rats (11wks old, female). The DNA solution was injected into the intertrochanteric region of the proximal femur. The electrodes were placed on the femoral head and in the intramedullary space. Square pulses were produced between the electrodes by using of a pulse generator (CUY21EDIT, Neppagene, Japan) (Figure 1). The efficacy of gene transfer depended on the volt and current applied to the DNA injected site. Next, we transferred pCAGGS-HGF to the Wistar rat osteonecrosis model (10 months old, female) and the proximal femora were analyzed histologically on day 7, 21, and 42 after electroporation. The osteonecrosis of the rat femoral head was created surgically as previously described by Norman et al. with some modifications. These procedures were reviewed and approved by the animal care and use committee of Niigata University Graduate School of Medical and Dental Sciences.

Results: The optimal gene transfer could be achieved when 12 square pulses of 100V each were delivered to the injection site. The pulse rate was 1 Hz and each pulse lasted for 50 milliseconds. The luciferase activity was detectable for 6 weeks after gene transfer with its peak on day 2. Histological analysis of the femur never showed irreversible damages in the bone marrow around the electroporation site. We observed the fibroblast-like cells at the injection site in the HGF group on day 7. On day 21, vascular-like invasion was detected between necrotic bone and normal bone marrow. Additional bone formation, which was usually observed in the late phase of bone regeneration after osteonecrosis, was observed much more in the HGF-treated rats than that of the control rats on day 42.

Discussion: This is the first report to transfer a foreign gene into rat bone marrow by in vivo electroporation. The optimal conditions for gene transfer were almost same as those of other reports in which target genes were transferred into other tissues by in vivo electroporation. The luciferase was detected only for 6 weeks, suggesting the duration of gene expression was short with this method. Our results of HGF gene transfer in the rat osteonecrosis suggests that in vivo electroporation is an optimal tool to accerate the healing process of osteonecrosis of the femoral head.

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Figure 1: The picture of electroporation to the rat proximal femur.