

A Trial of Shockwave-mediated plasmid DNA transfection

Authors:

Ryo Murata*, Seiji Ohtori*, Nobuyasu Ochiai*, Keiji Takahashi*, Takashi Saisu**, Yuichi Wada***, Hideshige Moriya*

*: Department of Orthopaedic Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan

** : Division of Orthopaedic Surgery, Chiba Children's Hospital, Chiba, Japan

***: Department of Orthopaedic Surgery, Teikyo University Ichihara Hospital, Ichihara, Chiba, Japan

Introduction:

Viral vectors have been shown to be effective for gene transfer with high efficacy rate. However, for the therapeutic application it requires a number of improvements including the issues of safety, immunogenicity. Non-viral gene transfer systems are safe and easy to apply, but low efficacy rate remains unsolved. Among these methods ultrasound-mediated plasmid DNA transfection with microbubble agent is reported to be useful and reveal a certain level of efficiency. In considering an ideal gene therapy for orthopaedic disorders, to modify non-viral plasmid DNA transfection would be desirable. Thus we tried to develop shockwave-mediated plasmid DNA transfection.

Methods:

Male rats (200-300g) were used for the experiment. Previously conditioned luciferase plasmid DNA solution and pEGFP plasmid DNA solution were injected directly into the pretibial muscle of the rat hindlimb under general anaesthesia. After that low-energy extracorporeal shock waves were exposed to the identical area. Two days later they were sacrificed and the muscles were harvested. GFP expression was observed by fluorescence microscope and luciferase activity was quantitatively measured by a luciferase assay system to evaluate efficacy rate of gene transfection. The advantageous effect of a microbubble agent (Optison) was also evaluated.

Results:

GFP expression and luciferase activity were significantly enhanced when the specimens were exposed to shock waves with Optison. Plasmid DNA injection without Optison or shock wave application was failed to increase transfection efficiency. Complication was not occurred except for minor subcutaneous hemorrhage of the exposed site.

Discussion:

We showed that the shockwave-mediated plasmid DNA transfection was achieved with a microbubble agent. The cavitation effect, which is augmented by this agent, may cause transient hole formation on the neighbour cell surface. Plasmid DNA may be transferred into the target cell through this hole. This phenomenon called as "Sonoporation" may explain the mechanism of our method. The efficacy rate of gene transfection is not as high as other methods such as adenoviral vectors. Although it needs so many improvements before application for the clinical use, this safe and easy method may provide a possibility of gene therapy for various orthopaedic disorders.