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Atelocollagen-mediated siRNA delivery: future promise for therapeutic application

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Drug-delivery systems for nucleic acid-based medicine

In recent years, nucleic acid-based medicine has attracted attention for its therapeutic applications to cancers and infectious, hereditary, and other diseases. Moreover, treatment by gene transfer with a viral or nonviral vector has been actively studied. In addition, owing to the development of novel gene-transfer reagents and gene systems, the administration of a gene itself has been tested for potential therapeutic efficacy in gene expression. Most recently, the discovery of RNAi has triggered a global competition among many companies and researchers to find effective delivery systems that capitalize on the clinical benefits of siRNA.

For *in vivo* delivery of nucleic acids including siRNA, research and development have focused on the improvement of delivery efficiency, resulting in a wide use of gene-delivery systems based on cationic liposomes or polymers. However, these delivery systems still entail clinically relevant problems such as cellular toxicity and the induction of non-specific immune responses, as the expression of immune-responsive genes and apoptosis-related genes is enhanced after administration. Establishment of an ideal delivery system with lower toxicity and higher stability is a big challenge [1–10].

To solve these problems, we and others have pioneered *in vivo* nucleic acid-delivery systems, such as siRNA systemic delivery mediated by highly bio-compatible and low-immunogenic atelocollagen, which many researchers have extensively employed in studies of various disease models. We also

investigated the combined use of siRNA/atelocollagen with anticancer agents, targeting the antiapoptotic factor *Bcl-xL* gene. In brief, an siRNA targeting human *Bcl-xL* complexed with atelocollagen was injected three-times weekly for a total of 12-times through the caudal vein in mice with a human prostate cancer cell line (PC-3) xenograft already grown subcutaneously. These injections significantly inhibited growth as well as the expression of both *Bcl-xL* mRNA and its protein in tumors. A combinational therapy of *Bcl-xL* siRNA/atelocollagen with a low dose of cisplatin (12 mg/kg) provided more intense growth inhibition [11].

Atelocollagen has been developed for artificial organs, its safety having been assured as a top-level bio-compatible material by the US FDA. Atelocollagen possesses basicity (positive charge) under physiological conditions, whereas siRNA and other nucleic acids have acidity (negative charge). Therefore, atelocollagen and siRNA are considered to form a complex by electrostatic binding. The nucleic acid complexed with atelocollagen can avoid degradation by nuclease(s) and can be efficiently transported in the bloodstream to tissues and cells, where the siRNA/atelocollagen complex may be incorporated through endocytosis. Furthermore, recent studies demonstrated that the complex itself can lower the cellular membrane potential enough to promote endocytosis. In animal model experiments, the siRNA/atelocollagen complex that arrived at a tumor tissue downregulated the target gene expression. Accordingly, expectations are growing for the siRNA/atelocollagen complex as a novel



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sustained release preparation for nucleic acid-based medicine [12].

Preventing off-target effects for innate immunoreactivity of siRNA

A great deal of attention was generated by a report that showed activation of the innate immune system by double-stranded RNA molecules, rather than by sequence-specific translation suppression as we expected, had a therapeutic effect on age-related macular degeneration, which is a top focus of siRNA drug discovery [13]. Soon, it was proved that once siRNA binds to the nucleic-acid recognizing sensor receptor TLR3 on the surface of vascular endothelial cells, IL-12 and IFN- γ are secreted, causing the intense inhibition of vascularization. Then Opko Health, Inc., (FL, USA) a multinational pharmaceutical and diagnostics company that had started on a Phase III clinical trial of siRNA, faced a sharp decline in its own stock price and discontinued the trial. More recently, various approaches have been found to prevent the activation of innate immunity by devising a nucleic acid sequence and/or a chemical modification process, although experiments toward this end must be designed carefully.

Recently, experiments in which age-related macular degeneration model mice were similarly prepared via laser irradiation showed that the atelocollagen drug delivery system (DDS) does not cause the off-target effect [14]. Among the atelocollagen DDSs, the control siRNA did not suppress vascularization; VEGF siRNA offered sequence-specific therapeutic effects that are confirmable along with the siRNA incorporated into the retinal pigment epithelium.

Furthermore, we used siRNAs carrying type I IFN inducible sequences to evaluate IFN and inflammatory cytokine responses to various nucleic acid DDSs, including an atelocollagen DDS. The atelocollagen DDS exhibited no immune activation when administered to Balb/c mice or added to human peripheral blood monocyte cultures, whereas a cationic lipid DDS enhanced inflammatory cytokine responses (IFN- α , TNF- α , IL-1 β , IL-6 and IL-12) [15]. We also demonstrated the therapeutic effectiveness of systemic atelocollagen-mediated delivery of a siRNA targeting MCP-1, a leukocyte chemotactic factor, in the treatment of contact hypersensitivity of the skin [7]. In contact hypersensitivity model mice, intravenous administration of MCP-1 siRNA/atelocollagen significantly reduced the expression of MCP-1 protein at the inflammation-induced site and effectively inhibited the infiltration of monocytes, macrophages, and T cells, leading to a favorable outcome for inflammation-induced auricular swelling [7]. These results indicate that atelocollagen is an excellent vehicle for nucleic acid DDSs.

Atelocollagen is safe in living bodies

To assess the biotoxicity and related risk of a drug for systemic administration, various studies including acute toxicity testing, antigenicity testing, and carcinogenicity testing have been conducted. However, every test requires extraordinary time and labor. In recent years, DNA microarray procedures have been preferred as simpler and more efficient alternatives to other procedures for analyzing the toxicity and efficacy of a drug [16]. A DNA microarray can analyze tens of thousands to hundreds of thousands of DNA spots simultaneously to measure the transcription amount of each gene. Therefore, the microarray technique enables efficient and comprehensive analyses of gene behaviors. These characteristics of this technology promise broad utilization in clinical settings and testing of drugs and food additives [17].

Indeed, using DNA microarray technology, we compared gene expression in the liver of mice 24 h after administration of atelocollagen or a cationic polymer (both commercially available agents to introduce nucleic acids *in vivo*) through the caudal vein. The toxicity of the cationic polymer versus a lower toxicity of atelocollagen was quantitatively verified. In the cationic polymer group, apoptosis- or inflammatory-responsive, cell-toxic genes have increased in number to more than 100-times or at least to 2.04-times; in contrast, such genes no more than doubled in the atelocollagen group. These findings indicate that atelocollagen is biologically safe even at a very high level [18].

Application of atelocollagen as a vehicle for sustained release of drugs

For DDS preparations, it is important to deliver a drug to the target tissue(s) at an effective dose over many hours.

Studies are now focusing on administration methods and dosage forms that minimize adverse drug reactions and enhance drug efficacy as much as possible. Successful DDS preparations will be obtained after these questions are answered: what materials should be used and how should a drug be delivered and slowly released?

Atelocollagen can be shaped into any form and, after the drug is released, atelocollagen is absorbed, metabolized and finally degraded *in vivo*. These properties are favorable for a base material of DDS. Therefore, atelocollagen can be used not only in solution, but also in sheet, sponge, powder, and other forms, providing a broad range of clinical applications.

Administration through the caudal vein has delivered many nucleic acids/drugs to tumors, owing to an enhanced permeability and retention effect, although poor delivery to many other nontarget

normal tissues and organs has also been simultaneously observed. One of the delivery related problems in the treatment of a disease is the need to select a method of delivering only to the target organ while lessening effects on normal organs and tissues as much as possible. In this regard, there is still a high hurdle to be jumped for the systemic administration of not only atelocollagen but also many other DDS vehicles in clinical application. The development of atelocollagen with organ-specific modifications and other devices will lead to a bright future of clinical application to humans.

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