Regulation of mRNA immunogenicity by nanomicelle encapsulation for in vivo mRNA delivery

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ABSTRACT SUMMARY
Messenger RNA delivery is a promising method to provide therapeutic proteins and peptides into the body without any risk of insertion mutagenesis. However, in vivo delivery of mRNA is difficult due to its instability under physiological conditions and strong immunogenicity. In this study, we solved these two problems at the same time by encapsulating mRNA into polyplex nanomicelle, and obtained prolonged protein expression from the mRNA without significant immune responses in the central nervous system (CNS) of rodents.

INTRODUCTION
mRNA has a high potential to produce proteins and peptide for therapeutic purpose without the risk of insertion mutagenesis. However, in vivo mRNA delivery has two major problems, instability under physiological environment, and immunogenicity through Toll-like receptors (TLRs). Although immunogenicity of mRNA can be reduced by nucleoside modification using pseudouridine (ΨU), 2-thio-uridine (2tU), or 5-methyl-cytidine (5mC), complete elimination of cytokine induction cannot be accomplished, which hampered clinical usage of mRNA [1, 2]. To solve these problems, we used a nonviral carrier based on self-assembly of a polyethylene glycol (PEG)-polycationic block copolymer, polyplex nanomicelle. A core-shell structure of the nanomicelle, with mRNA-containing inner core surrounded by PEG layer, provides high stability and stealth property to the nanomicelle [3]. In the cationic segment of the block copolymer, we used PAsp(DET), which has two desirable properties for the delivery of nucleic acids, biodegradability and high endosome-escaping capability [4-6]. The nanomicelle was delivered to intrathecal space, which allows efficient delivery of therapeutic proteins into CNS tissue by avoiding blood brain barriers.

EXPERIMENTAL METHODS
mRNA loaded nanomicelle was prepared at nitrogen/phosphate (N/P) ratio of 8. mRNA was modified with ΨU, 2tU, and 5mC (10%, 10%, and 20% in total U or C, respectively). The nanomicelle was administered to cisterna magna of mice and rats. The efficiency of protein expression from mRNA was evaluated using firefly luciferase, or a secreted type of luciferase (Gaussia luciferase, Gluc). The immune responses after mRNA delivery were evaluated by quantifying the transcriptional levels of inflammatory molecules. The activation of Toll-like receptor (TLR) signaling was investigated using HEK 293 cell-lines that were transformed to express a specific TLR.

RESULTS AND DISCUSSION
In the evaluation of firefly luciferase expression in the brain stem of mice, nanomicelle showed about 10 – 100 times higher expression than naked mRNA, and other carriers, such as lipoplex (lipofectamin 2000) and polyplex without PEG shielding (polyethylene imine, and PAsp(DET)) at 4 and 24 h after the administration (p < 0.001). Time dependent profile of mRNA expression was evaluated by measuring Gluc secretion into cerebrospinal fluid (CSF). mRNA introduction by nanomicelle provided sustained expression of Gluc in CSF for almost a week, whereas introduction of Gluc protein into CSF resulted in rapid decrease of its concentration in CSF within four hours. These data demonstrated high potential of nanomicelle-mediated mRNA delivery for sustained production of therapeutic proteins and peptides in the body.

Expression of proinflammatory cytokines (IL-6, TNF-α), and type 1 interferons (IFN-α4, IFN-β1) after mRNA introduction were evaluated in the brain stem of mice at 4 h after the introduction. Naked mRNA introduction induced significant inflammatory responses even when using modified mRNA, although the modification showed some effects on reducing inflammatory responses (Fig. 1). In contrast, inflammation induced by nanomicelle was almost comparable to that of buffer-treated control even when unmodified mRNA was used.

To study the mechanism of this result, we performed in vitro analyses of TLR signaling. Naked
mRNA increased the expression of IL-8, and IFN-β1 in the 293 cells stably expressing TLR7 (293-TLR7), which recognizes mRNA, but not in 293 cells expressing TLR9 (293-TLR9), which does not react with mRNA, showing that naked mRNA induced TLR specific inflammation (Fig. 2). In contrast, nanomicelle did not induce significant inflammation in both of 293-TLR7, and 293-TLR9. Thus, nanomicelle encapsulation prevents mRNA from recognition by TLRs in endosome, presumably due to the stealth property and the capacity of facile endosome-escape of the nanomicelle.

CONCLUSION
The encapsulation of mRNA into nanomicelle simultaneously solved the two major problems of in vivo mRNA delivery, instability and immunogenicity. As a result, mRNA introduction using nanomicelle allowed sustained protein expression with reduced inflammatory responses in CNS. The low immunogenicity of this system is attributed to the reduced recognition of mRNA by TLRs after nanomicelle encapsulation. This system has high potentials for clinical applications without any risk of insertion mutagenesis.

REFERENCES

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