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The role of osmotic polysorbitol-based transporter in RNAi silencing via caveolae-mediated endocytosis and COX-2 expression

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ABSTRACT

Polymeric diversity allows us to design gene carriers as an alternative to viral vectors, control cellular uptake, target intracellular molecules, and improve transfection and silencing capacity. Recently, we developed a polysorbitol-based osmotically active transporter (PSOAT), which exhibits several interesting mechanisms to accelerate gene delivery into cells. Herein, we report the efficacy of using the PSOAT system for small interfering RNA (siRNA) delivery and its specific mechanism for cellular uptake to accelerate targeted gene silencing. We found that PSOAT functioned via a caveolae-mediated uptake mechanism due to hyperosmotic activity of the transporter. Moreover, this selective caveolae-mediated endocytosis of the polyplexes (PSOAT/siRNA) was regulated coincidently with the expression of caveolin (Cav)-1 and cyclooxygenase (COX)-2. Interestingly, COX-2 expression decreased dramatically over time due to degradation by the constant expression of Cav-1, as confirmed by high COX-2 expression after the inhibition of Cav-1, suggesting that PSOAT-mediated induction of Cav-1 directly influenced the selective caveolae-mediated endocytosis of the polyplexes. Furthermore, COX-2 expression was involved in the initial phase for rapid caveolae endocytic uptake of the particles synergistically with Cav-1, resulting in accelerated PSOAT-mediated target gene silencing.

1. Introduction

Since the discovery of RNA interference (RNAi) by Fire and Mello in 1998, for which they received the Nobel Prize, the concept and its applications for effective and targeted gene silencing have been explored considerably [1]. Small interfering RNAs (siRNAs) were introduced in early 2001 [2,3] and thereafter, the first experimental approach for a gene silencing strategy targeting hepatitis C in mice was achieved using siRNA [4]. Since the remarkable breakthrough, experts in this field have made considerable advancements in siRNA therapeutics against various diseases including viral infections and cancers [5–7]. However, this exciting and promising approach has not yet been translated into the clinical side, owing to several limitations. The most significant challenges are the relatively large size (~13 kDa) and negative charges of siRNA molecules, together with their susceptibility to degradation by endogenous enzymes [6,7]. Although naked siRNAs have been shown to be effective in a few physiological settings and applications [8,9], an improved delivery system to facilitate siRNA transfection is required in most body tissues because naked siRNAs are unable to cross cellular membranes freely due to their strong anionic charges. Thus, an effective delivery system for siRNA remains a challenge and the most critical barrier between siRNA technology and its therapeutic application. At present, numerous gene therapies in clinical trials use recombinant viral vectors due to their excellent transfection ability; however, safety issues have halted their further advancement. Therefore, to achieve therapeutic advantages using siRNA, not only clinical safety but also an effective delivery system must be in place; non-viral polymeric carriers could be the best, most effective alternative.

Cationic polyethylenimine (PEI), one of the most widely investigated polymers, has been used as a non-viral gene carrier. However, PEI-mediated gene transfer may cause severe cellular

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toxicity due to its non-degradable nature and has a low transfection capability relative to viral vectors, major drawbacks hindering its clinical application [10]. Hence, the modification of PEI using degradable cross-linkers has been investigated extensively to introduce degradable properties to improve its cellular viability [10]. Recently, we developed a class of gene transporter, the polysorbitol-based osmotically active transporter (PSOAT), prepared from sorbitol dimethacrylate (SDM) and low-molecular-weight linear polyethylenimine (LMW LPEI), which exhibits accelerated gene transfer capability. The transfection activity of PSOAT is greatly hampered by COX-2 and vacuolar-type proton ATPase-specific inhibitors but is enhanced by osmotic activity and many hydroxyl groups contained in the polysorbitol chain of the transporter [10]. Although hydroxyl groups of polymers have been reported to reduce gene transfection efficiency [11,12], interestingly, PSOAT shows an accelerated gene transfer capability despite its many hydroxyl groups [10]. The osmotic PSOAT exploits a transporter mechanism related with its polysorbitol backbone, which enhances cellular internalization. However, the precise cellular uptake mechanism, which is essential for its use as a delivery tool for therapeutic agents such as siRNA, remains unknown.

A potential strategy for improving gene delivery by non-viral carriers is to target a particular cellular uptake process that is closely related with intracellular fate [13,14]. The mechanism for the cellular uptake of nanoparticles has been suggested to be either clathrin-dependent or clathrin-independent endocytosis [15]. Clathrin-dependent cellular uptake follows the classic endocytic pathway, which cannot avoid the fusion of endosomes carrying a target molecule(s) to a lysosome, resulting in enzymatic degradation. On the other hand, clathrin-independent, particularly caveolae-mediated endocytosis leads to the transport of endocytosed materials toward the non-acidic and non-digestive route without fusion to a lysosome [16,17]. Several reports have also demonstrated the advantages of caveolae-mediated cellular uptake [18–20]. These studies have emphasized the importance of proper design and the development of non-viral gene vectors targeting caveolae-dependent uptake to achieve effective intracellular processing for the expected gene expression profile. Moreover, other recent reports emphasized on designing nanoparticles and their translocations through cellular membrane using computer simulation strategy which may provide new ideas and concept for future experimental nanoparticle design and their potential use in drug delivery system [21–23].

It is interesting to note that hypertonic exposure of cells can be used to selectively stimulate the caveolae-mediated endocytic pathway by downregulating clathrin-dependent endocytosis and fluid-phase uptake [24]. Caveolae endocytosis involves the expression of caveolin (Cav), especially Cav-1, a major component of caveolae formation, which could stabilize caveolae receptors and the receptors at the plasma membrane outside of caveolae that affect the caveolae-mediated endocytosis mechanism [25–27]. This notion is further supported by the fact that cells under osmotic stress induce Cav-1 phosphorylation via Src-kinase activity during the process of budding and pinching off from the plasma membrane [28]. Thus, it appears that Cav-1 expression has a direct functional mechanism for caveolae endocytosis under osmotic

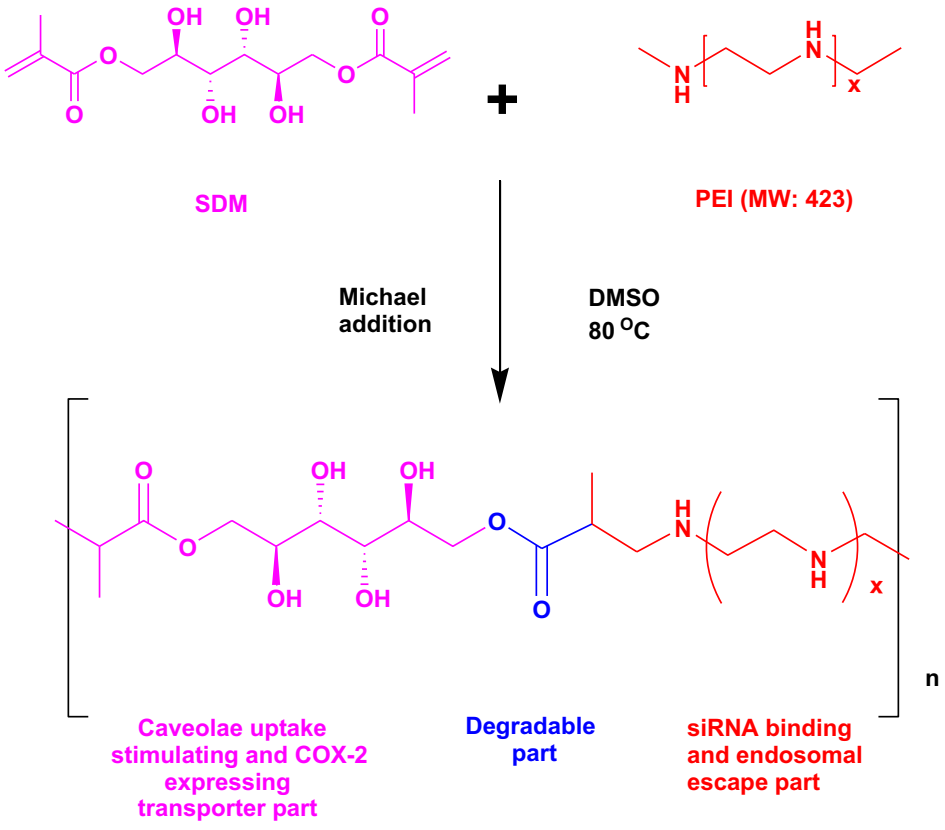

Fig. 1. Proposed reaction scheme for the synthesis of PSOAT from SDM and PEI. PSOAT was prepared through a Michael addition reaction in DMSO at 80 °C. Different colors indicate different functional parts of PSOAT. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)