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A high affinity kidney targeting by chitobionic acid-conjugated polysorbitol gene transporter alleviates unilateral ureteral obstruction in rats

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ABSTRACT

Aside from kidney transplantation – a procedure which is exceedingly dependent on donor-match and availability leading to excessive costs – there are currently no permanent treatments available which reverse kidney injury and failure. However, kidney-specific targeted gene therapy has outstanding potential to treat kidney-related dysfunction. Herein we report a novel kidney-specific targeted gene delivery system developed through the conjugation of chitobionic acid (CBA) to a polysorbitol gene transporter (PSGT) synthesized from sorbitol diacrylate and low molecular weight polyethylenimine (PEI) carrying hepatocyte growth factor (HGF) gene to alleviate unilateral ureteral obstruction (UUO) in rats. CBA-PSGT performed exceptionally well for targeted delivery of HGF to kidney tissues compared to its non-targeted counterparts ($P < 0.001$) after systemic tail-vein injection and significantly reduced the UUO symptoms, returning the UUO rats to a normal health status. The kidney-targeted CBA-PSGT-delivered HGF also strikingly reduced various pathologic and molecular markers *in vivo* such as the level of collagens (type I and II), blood urea nitrogen (BUN), creatinine, and the expressions of ICAM-1, TIMP-1 and α-SMA which play a critical role in obstructive kidney functions. Therefore, CBA-PSGT should be further investigated because of its potential to alleviate UUO and kidney-related diseases using high affinity kidney targeting.

1. Introduction

Chronic kidney disease (CKD), a leading cause of kidney failure, is still considered as one of the major health problems, causes millions of deaths worldwide. In the USA alone, one in three adults are at high risk of kidney diseases and 82,000 people die from kidney failure each year. The current treatments have a poor ability to reduce the progression of the disease; in addition there is no permanent treatment or way to reverse the damage of kidney failure, except kidney transplantation, which is highly expensive and extremely reliant on donor match and availability [1]. Considerable researches have been conducted recently to find out the key regulators (such as HIPK2 [2], epithelial to mesenchymal transition [3], defective fatty acid oxidation in renal epithelial cells

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[4], mitochondrial abnormality [5], fibroblasts and myofibroblasts [6,7]) of kidney fibrosis and the underlying mechanisms causing CKD to kidney failure, suggesting that a clinically-relevant treatment strategy is a prior demand to prevent this one of the most devastating human health problems.

For the past several decades, gene therapy has been considered as one of the promising strategies to treat inherited or acquired diseases and several clinical trials of gene therapy products have been showing its brilliant future to translate this field into noticeable bed-side applications. In this regard, a targeted gene therapy specific to kidneys could be an ideal strategy to improve the therapeutic efficacy of the functional gene and find out effective measures to treat kidney-related diseases. Kidney is a well-organized and differentiated organ having specialized compartments, consisting of tubules, vasculatures, glomerulus and interstitium, all of which make the gene delivery system to travel through several hurdles and these anatomic barriers encounter gene carriers before transfection at specific sites [8]. Thus, the success of kidney targeted gene delivery systems is considered highly challenging.

As targeted gene carriers, the non-viral polymeric nanoparticles have been investigated with immense potentials because of their diversity in modification, less toxicity, minimal immunogenicity and ability to encapsulate a bulk amount of genetic molecules, all of which, show promise to overcome the drawbacks of viral-mediated gene delivery systems. For efficient and specific delivery of genes to kidney, targeting vimentin receptors can be a vital strategy because it was previously found that vimentin, which is a type of intermediate filament protein, expresses in developing and adult human kidneys [9]. Several other reports also described the distribution of vimentin in both normal and carcinoma kidney tissues [10–12]. Moreover, Scanziani et al. reported that vimentin not only expresses in human kidneys (normal or damaged) tissues, but also expresses in bovine kidneys with nephritis at interstitial regions [13]. In normal kidneys, vimentin can be found in arterioles, interstitial fibroblasts, glomeruli, but not in tubules. Interestingly, however, vimentin expression can be seen abundantly in tubular injury (atrophy, inflammation and/or fibrosis) in experimental models such as urethral obstruction [14], kidney aging [15], ischemic injury [16,17], proteinuria [18], nephropathy by adriamycin [19], suggesting that overexpression of vimentin can be considered as a marker for tubular injury [20,21]. Moreover, it was demonstrated that after kidney transplantation, vimentin expression could be investigated as an indicator of renal dysfunction [17]. All these evidences strongly suggest that vimentin can be a potent receptor to target kidneys, especially the damaged or obstructive kidney tissues.

Recently, we identified that vimentin can be effectively targeted with high affinity by chitobionic acid (CBA), prepared by dimer of *N*-acetylglucosamine (GlcNAc) as a specific ligand to the vimentin-expressing cells, when conjugated with cationic polymers, because we found that the CBA-conjugated polymer showed higher transfection efficacy in vimentin expressing cells (the human embryonic kidney 293T cells and epithelial HeLa cells) compared to their unconjugated counterparts [22,23]. Mechanistically we also showed the proof-of-concept that these CBA-conjugated polymers had no effect when the surface-vimentin was knocked-down by RNA interference [22]. This remarkable targeting affinity of CBA to vimentin motivated us to apply with our recently developed polysorbitol gene transporter (PSGT; which showed incredible efficacy to deliver a variety of genes such as DNA [24–27], siRNA [28–31] and microRNA [32,33]) and exhibited remarkable potentiality to treat a number of diseases such as lung carcinoma, breast cancer and restenosis.

In the present study, we prepared a unilateral ureteral obstructive (UUO) rat model, a widely used animal model to study kidney disease [34], and hypothesized to treat UUO rat model using the CBA-conjugated PSGT-mediated delivery of hepatocyte growth factor (HGF) gene utilizing the concept of kidney-specific targeting. We used HGF as a potential genetic material to ameliorate UUO in rat because several previous gene therapy studies showed that HGF blockades the progression of chronic obstructive nephropathy [35–37]. We observed that CBA-PSGT has high affinity for kidney targeting and expresses HGF specifically in kidneys significantly better than other organs *in vivo*. As expected, CBA-PSGT remarkably alleviated ureteral obstruction in UUO rat model and we investigated the underlying molecular mechanisms of this proof of ameliorative effects.

2. Materials and methods

2.1. Materials

Branched PEI (molecular weight: 12 and 25 kDa) and 3-(4, 5-dimethyl thioazol-2-yl)-2, 5-diphenyl tetrazolium bromide reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sorbitol diacrylate (SDA) (MW, 290.27 Da) was purchased from Monomer-Polymer & Dajac Labs (Trevose, PA, USA). Chitobiose was kindly provided by Yaizu Suisankagaku Industry Co. Ltd. (Shizuoka, Japan). HGF plasmid from rat origin was cultivated in *Escherichia coli* and purified with a plasmid DNA purification kit (Cosmo Genetech, Seoul, Korea).

2.2. Synthesis and characterization of the PSGT, CBA and CBA-PSGT

As we previously described [27–29], the PSGT was synthesized by reaction between SDA and PEI (12 kDa) using a Michael addition reaction and characterized by ^{1}H nuclear magnetic resonance (NMR) (Avance™ 600, Bruker, Mannheim, Germany) and gel permeation chromatography (GPC) to confirm the success of the reaction. For GPC measurement, a column coupled with multiangle laser light scattering (GPC-MALLS) and a Sodex OHpack SB-803 HQ (Phenomenox, Torrance, CA, USA) were used to measure the actual MW of the polymer. The column temperature was 25 °C at a flow rate of 0.5 mL/min and as mobile phase, 0.5 M ammonium acetate was used.

To synthesize chitobionic acid (CBA), first a water and methanol mixture (5 mL) was used to dissolve chitobiose (0.5 g). Then iodine (1.425 g) dissolved in methanol (20 mL) was added drop by drop into chitobiose solution and mixed for 1 h at 40 °C. The color of the solution became dark brown after adding iodine solution to CBA. The solution was mixed with 4% (weight percent) potassium-hydroxide solution (in methanol) until the color of iodine solution disappeared and stirred for 2 h at 40 °C. After cooling, the sample was recrystallized in ether and ether was removed carefully to get precipitated sample. The recrystallized precipitated sample was then dissolved in distilled water and chitobionic acid [$(GlcNAc)_2$-COOH] was obtained by separating the synthesized product using an amberlite IR-120 column and repeated this separating process 2 to 3 times to obtain more purified product. The purified CBA solution was then lyophilized and stored at −70 °C until use later.

To synthesize CBA-PSGT, CBA was conjugated with PSGT using DCC/NHS chemistry as coupling agents where the mole ratios of all reactants in the reaction were 1:2:2:5 for CBA:DCC:NHS:PSGT, respectively. Methanol was used as the dissolving solvent for all the reactants. Briefly, CBA was dissolved in methanol at 2 mM concentration. The DCC and NHS were added separately (each at 4 mM concentration) and stirred the reaction at room temperature for overnight (16 h) at inert condition. The PSGT (10 mM concentration in methanol) was then added and kept the reaction at RT (and inert