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Polymeric gene carrier for insulin secreting cells: Poly(L-lysine)-*g*-sulfonylurea for receptor mediated transfection

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Abstract

Ex vivo transfer of therapeutic genes to cells is one of the potential strategies to prolong the life span of cell transplants. However, relatively safe non-viral carriers have not been extensively investigated due to their lower transfection efficiency. In this study, poly(L-lysine)-*g*-sulfonylurea varying SU content (PLL-SU) was synthesized to promote gene delivery efficacy to an insulin secreting cell line, RINm5F, which is known to express sulfonylurea receptor (SUR). The polymer formed complexes with a model reporter gene of pCMV-Luc (DNA) and the size of resulting particles was around 100 nm. The transfection efficiency of a polymer synthesized with 5 mol% of SU in the reaction feed (PLL-SU5%) to RINm5F cell was at least 5 times higher than that of PLL. The cytotoxicity of PLL-SU5%/DNA complex was equivalent to that of PLL/DNA complex. PLL-SU5% showed less transfection efficiency than PLL to NIH3T3 and HepG2 cells which are SUR negative. In RINm5F cells, the addition of free SU decreased the transfection efficiency of PLL-SU5%/DNA complex, suggesting that the complex shares the same receptors for SU. The PLL-SU5%/DNA complex seems to be internalized via SUR-mediated endocytosis pathway as suggested by vacuolar ATPases inhibition by Bafilomycin A_1. It is noted that RINm5F cells treated with PLL-SU5%/DNA complex secreted more insulin than control, untreated cells, suggesting the insulinotropic effect of SU in PLL-SU5%. In conclusion, PLL-SU may be useful for transfer of therapeutic genes into insulin secreting cells.

Keywords: Gene delivery; Receptor-mediated endocytosis; Polymeric non-viral vector; Sulfonylurea; Insulin-secreting cells

1. Introduction

Cell transplantation has potential for repair, replacement, or enhancement of biological functions within the body which utilizes autologous, allogeneic, or xenogeneic cell sources. Transplanted

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allogeneic or xenogeneic cells are commonly exposed to and attacked by the host immune system causing graft failure [1–3]. In order to prevent graft rejection, immunomodulatory genes have been introduced via various routes such as in vivo intravenous or intramuscular gene delivery and ex vivo gene modification. Ex vivo gene transfer makes it possible to have local transgene delivery with improved transfection efficiency and less cytotoxicity compared to in vivo gene therapy [4]. The transplanted cells with secretory functions such as neuroendocrinal or pancreatic islet cells, which are typically quiescent in culture, also face inappropriate environmental conditions of hypoxia and/or apoptosis [5–7], losing their biochemical modulating functions. To increase the viability and long-term biochemical functionality of transplanted functional cells, the introduction of immunomodulatory (e.g., IL10, CTLA-4Ig) [4,8], anti-apoptotic (e.g., Bcl-2, A20) [8,9], and/or neovascularizing (e.g., VEGF) [7,10] genes has been proposed. Ex vivo gene transfer offers further merits such as the ability to replicate and reduced apoptosis during preservation [11], hence overcoming immense supply shortages [12,13].

To date, almost all gene delivery systems for secreting cells have used viral vectors [5,7,8,14,15] (e.g., adenovirus, adeno-associated virus (AAV), retrovirus, and herpes simplex virus). A few non-viral systems have been tried using either commercial cationic liposomes [10,15–21] (e.g., Lipofectin™, Lipofectamine™, and DOTAP) or cationic polymers [22–24] (e.g., poly(L-lysine) and poly(ethyleneimine) (PEI)). Viral vectors show undesirable characteristics such as immunogenicity, cytotoxicity, integration into host genome, and limited loading capacity of DNA, although they presented higher transfection efficiency compared to non-viral gene carriers [8]. Also, when applying adenovirus and AAV, their high multiplicity of infection (MOI) induced fibrosis and malignant matrix formation. Since non-viral systems have low cytotoxicity and immunogenicity, they could be an alternative for transfecting secreting cells.

Many non-viral systems have been developed for high transfection efficiency. Receptor-mediated internalization [25] is one of the efficient strategies, which can be coupled with fusogenic material-containing systems for disruption of endosomal membrane [26,27] and with nuclear localization signal for facilitated nuclear import [28]. Receptor-mediated gene carriers not only showed improved transfection due to facilitative internalization but also showed the ability to target cells with specific receptors [25]. Until now various receptors have been used for receptor-mediated gene delivery such as asialoglycoprotein receptor [29], folate receptor [30], integrin receptor [31], transferrin receptor [32], and nerve growth factor receptor [33]. However, the application of sulfonylurea receptor for receptor-mediated gene delivery has not been studied. This receptor exists in the cellular membrane of endocrine and neuroendocrine cells such as RINm5F, HIT-T15, AtT-20, GH3, α-TC6, and PC12 cells [34,35].

Sulfonylurea receptor (SUR) and sulfonylureas (SU) have been studied for their biological roles in affecting the secretion of various bioactive molecules such as insulin from insulinoma cells [36,37], catecholamine from PC12 and adrenal chromaffin cells [38,39], and atrial natriuretic factor from atrial myocytes [40]. For insulin-secreting cells, in particular, SU–SUR interactions have been utilized to reduce hyperglycemia by stimulating insulin release. SU-labeled radioisotopes [41] and fluorescent moieties [42] have been investigated for islet imaging. Furthermore, Bae's group has shown that water-soluble and non-cationic polymers conjugated with sulfonylurea were internalized into pancreatic rat islets [42,43] and improved insulin secretion [44].

In this study, as a first step toward developing ex vivo non-viral gene carriers for secreting cells, sulfonylurea-conjugated poly(L-lysine) (PLL-SU) was synthesized to test SUR-mediated transfection. An insulin-secreting cell line, RINm5F cell, which expresses SUR on plasma and intracellular membranes [34] was selected.

2. Materials and methods

2.1. Materials

Poly(L-lysine) hydrobromide (PLL·HBr; M_w (viscosity)=27,400, M_w (MALLS)=30,200), Dulbecco's phosphate buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, glibenclamide, trinitrobenzene sulfonate (TNBS), borax decahydrate, bovine serum albumin (BSA),