Delivery of GLP-1 gene and DPP-4 siRNA using chitosan-based nanoparticles for Type-2 Diabetes treatment

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ABSTRACT SUMMARY

Novel chitosan (CS)-based nanoparticles (NP) were tested for the delivery of plasmid coding for native and modified GLP-1 and for the delivery of siRNA specific to the DDP-4 gene. Nanoparticle characteristics and their therapeutic potential were tested in vitro and in vivo.

INTRODUCTION

Glucagon like peptide 1 (GLP-1), an incretin hormone that regulates blood glucose level post-prandially, is rapidly inactivated by the dipeptidyl peptidase-4 (DPP-4) enzyme, which results in a short circulating half-life of the active form of GLP-1. GLP-1 analogues and DPP-IV inhibitors are both currently used for the treatment of Type 2 Diabetes. Here we evaluate the potential of novel CS-nucleic acid nanoparticles (NP) for the delivery of plasmid DNA expressing native and modified GLP-1 and of siRNA specific for DPP-IV knockdown. CS is a promising non-viral polycationic delivery vector because of its biocompatibility, biodegradability, low immunogenicity and ease of manufacturing.

EXPERIMENTAL METHODS

NP were prepared by mixing nucleic acids and CS with specific molecular weight (Mn), degree of deacetylation (DDA) and ratio of chitosan amine to nucleic acid phosphate (N:P ratio), named 92-10-5, 80-10-5, 80-80-5 [DDA-MW-N:P ratio]. Environmental scanning electron microscopy (ESEM) and dynamic light scattering (DLS) were used to characterize nanoparticles shape, size and surface charge. To assess CS protection against nuclease attack, nanoparticles were incubated with DNase I. Nanoparticle internalization was studied using confocal microscopy and FACS. Recombinant GLP-1 expression level and DPP-IV gene silencing efficiency were characterized by ELISA and by qPCR respectively. NP were administrated to Diabetic ZDF rat model by intramuscular (IM), and subcutaneous (SC) injections. ELISA and intraperitoneal glucose tolerance tests were performed to evaluate the efficacy and longevity of the treatment in ZDF rats.

RESULTS AND DISCUSSION

Chitosans form spherical and positively charged NP that protect from nucleases with diameters of 68–283 nm for plasmid and ODN (mimicking siRNA) respectively. CS NP were significantly more internalized in cells with an uptake of 99% when compared to commercial phospholipid NP with an uptake of 55% (Figure 1). The weak uptake performance for phospholipid NP is probably due to the cell toxicity associated with the lipid-based NP. Quantitative real time PCR (qRT-PCR) showed NP-mediated inhibition of DPP-IV coding mRNA at levels similar to that of the positive control DharmaFECT (72% DPP-IV gene silencing) but without toxicity seen with liposomal systems. DPP-IV enzymatic activity was reduced to 56% in HepG2 and Caco-2 cell lines by CS-siRNA NP. Cells transfected with DPP-IV resistant analogues showed GLP-1 levels of about 5 fold higher than those transfected with native GLP-1 (up to 115 ng/L). Diabetic ZDF rats injected subcutaneously and intramuscularly with CS-based nanoparticles showed GLP-1 plasma levels of about 5 fold
higher versus non-treated animals (Figure 2). The insulinotropic effect of recombinant GLP-1 in treated animals was reflected by an increase in plasma insulin levels compared to controls. Intraperitoneal glucose tolerance tests revealed efficacious decrease of blood glucose to near-normal levels in treated groups versus controls for up to 24 days following treatment.

CONCLUSION
The versatility, safety and efficiency of CS-based delivery of pDNA and siRNA indicates a strong potential of this delivery platform in the treatment of Type 2 Diabetes.

REFERENCES

ACKNOWLEDGMENTS
Financial support was provided by The Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research (CIHR).