Cell-Penetrating Peptides in Oral Delivery Systems for Insulin
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ABSTRACT SUMMARY
Oral delivery of insulin was achieved using cell-penetrating peptides (CPPs) resulting in reduced blood glucose levels (BGLs) in mice. L- and D-form of penetratin was used to investigate the importance of CPP resistance to enzymatic degradation.

Results demonstrated that a prolonged hypoglycemic effect was achieved using D-penetratin compared to the L-form.

INTRODUCTION
Subcutaneous injections (SC) of insulin are currently an integral part in the daily treatment of diabetes, but several side effects such as hyperinsulinemia, pain and allergic reactions result in patient discomfort and low compliance. An alternative delivery route for insulin is therefore of great importance in the future development of diabetes treatments.

Oral delivery of insulin is of great interest as not only does insulin absorbed from the intestines mimic the dynamics of endogenous insulin release, but oral delivery in general is typically associated with high patient compliance. Permeation of insulin across the mucosa of the intestines, however, is restricted by the hydrophilic nature and size of insulin as well as enzymatic degradation which poses a severe barrier for oral insulin delivery.

CPPs belong to a class of peptides which promotes cellular uptake of biomolecules such as proteins and oligonucleotides by facilitating uptake across cellular membranes [1]. Penetratin is one such CPP and is characterized by its amphipathic alpha-helical structure as well as a number of cationic residues. The cationic amino acids enable interaction with insulin as well as the glycocalyx while the hydrophobic tryptophan and phenylalanine residues enable penetratin to insert itself into the cell membrane as part of the uptake process.

In this study, we investigated the effect of using the D-form of penetratin compared to the L-form and a penetratin analogue (L-Sample 6) [2] in order to address the enzymatic degradation of the CPPs in the gastrointestinal system. Physical mixtures of insulin and penetratin was dosed by oral gavage to mice and followed by measurement of insulin plasma concentrations and the reduction of BGLs.

EXPERIMENTAL METHODS
Specific amounts of recombinant human insulin were dissolved in 100 μl 0.1N HCl in polypropylene tubes, then the insulin solution was diluted with 2.8ml phosphate buffered saline (PBS), pH 6, containing 0.001% methylcellulose, which prevents the adsorption of the CPP on the tube surface, and normalized with 100 μl 0.1N NaOH. Specific amounts of L-penetratin and D-penetratin were measured in polypropylene tubes and an aliquot of insulin solution was added to the tubes and mixed gently.

For measurement of the hyperglycemic effect, animals were fasted for 24h prior to dosing. 100μl of the insulin-CPP (10IU/kg) solution was administered by oral gavage and BGL was measured at specific time points up to 6 hours after dosing. The pharmacological availability (compared to SC injection) was calculated from the area above the curve (AAC). Insulin plasma concentrations was measured in separate experiment to avoid influence of anesthesia on BGLs. Animals were dosed (10IU/kg) as described above. 10 minutes after dosing, anesthesia (50mg/kg) was administered by i.p. injection and blood samples were collected from the jugular vein at time points up
RESULTS AND DISCUSSION

BGLs decreased in dose-dependent manner after oral administration of the insulin (10IU/kg)-penetratin (5mM) physical mixture. In the case of D-penetratin reduction of BGL lasted up to 6h reaching 40% after 4h compared to an insulin control group (Fig. 1).

Previous studies have shown L-Penetratin to enhance the uptake of insulin more efficiently than D-penetratin in in situ loop models [3]. We speculate that the high reduction of BGLs for the insulin-D-penetratin observed in this study is a result of the increased enzymatic stability of the D-form passing through the gastrointestinal system. Significant increase in the AAC was seen for both L (~10 times), D-penetratin (~30 times) and L-Sample 6 (~20 times) compared to the insulin control (Fig. 2). A pharmacological availability of 21% was obtained compared to a subcutaneous administration (1IU/kg) of insulin highlighting the promise of the use of enzymatically stable CPPs in oral delivery of insulin.

Insulin plasma concentrations were measured to confirm the effect of insulin on BGLs after administration of the insulin-penetratin solution. In line with the BGLs, a significant increase in insulin plasma concentrations was observed in animals receiving D-penetratin and insulin compared to the insulin control group.

CONCLUSION

In conclusion, CPPs stand out as promising delivery agents in meeting the needs of an oral insulin formulation. As demonstrated in this study, increased enzymatic stability of penetratin aids in overcoming parts of the barriers associated with oral protein delivery.

REFERENCES


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