Recombinant Protease Inhibitor Enhances Oral Insulin Pharmacodynamics in Pigs

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BACKGROUND

Oral insulin delivery is projected to provide both physiologic and technical benefits, and has been the focus of rigorous research efforts in recent years. The ORMD-0801 oral insulin formulation relies on the activities of both protease inhibitors and an absorption enhancer to ensure insulin integrity and bioavailability. The special blend of excipients both hinder proteolysis in the small intestine and enhance translocation of insulin across the gut epithelial lining. Once transported across the gut wall, the insulin is ferried to the hepatic portal vein, mimicking the natural route of endogenous pancreatic insulin, and then subjected to first-pass metabolism in the liver, before being delivered to peripheral sites of action.

OBJECTIVES

- To develop a recombinant protease inhibitor (rPI) identical to its naturally sourced (nsPI) counterpart
- To characterize the effectiveness of the rPI in vitro and in vivo

IN VITRO

rPI was cloned and expressed in the *Pichia pastoris* expression vector (pPIC9K), in-frame with the *Saccharomyces cerevisiae* α-mating factor pre-sequence and the αHC secretary signal peptide. The in silico-translated protein, which demonstrated 100% alignment with nsPI, was isolated and purified by a series of IEX chromatography steps followed by buffer-exchange with the final formulation buffer. The protein sample showed >95% purity.

Protein function was determined by incubating increasing concentrations of rPI or nsPI with a constant concentration of its substrate, followed by continuous spectroscopic rate determination (A_{256}) rPI effectively inhibited its standard substrate in a dose-dependent manner (Figure 1A) and demonstrated >2.5-fold higher activity as compared to nsPI (Figure 1B).

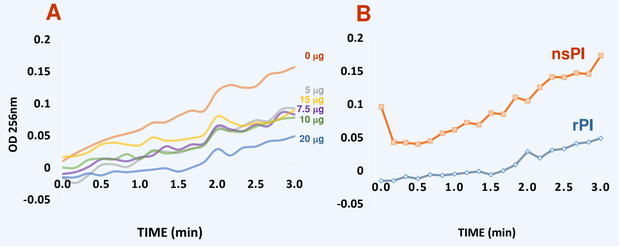
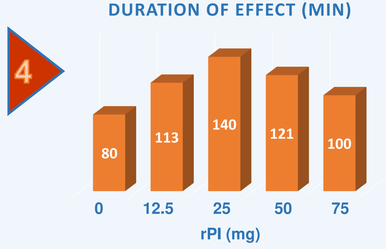


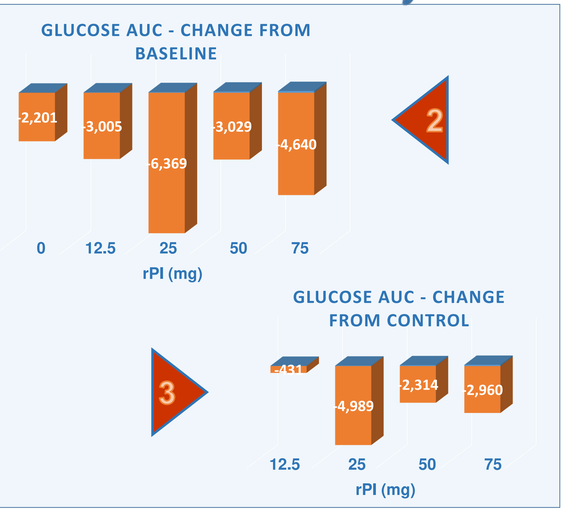
Figure 1. Enzyme inhibition by the purified rPI. A fixed concentration of the substrate and enzyme (in 1:1 ratio) was incubated with increasing concentration of the purified rPI (A) for 15 min, at room temperature (25°C). Readings were taken over 3 min. Enzyme inhibition by 20 µg rPI vs. 20 µg nsPI (B).

IN VIVO

The glucose-lowering effect of ORMD-0801 formulated with increasing doses of rPI (12.5-75 mg) was assessed in 8 healthy, fasting, anesthetized (isoflurane 2L O_2/min) commercial pigs (3-4 years old, 25-30 kg) administered the preparation directly to the duodenum through an endoscopic catheter, under endoscopic guidance. Blood samples were drawn periodically, for up to 3h, to determine glucose concentrations.

When integrated in the ORMD-0801 formulation, a rPI dose-dependent increase in the oral insulin efficacy was observed within the lower range of tested rPI concentrations (Figures 2-3). The maximal glucose-lowering effect was observed in pigs treated with the formulation containing 25 mg rPI, as manifested by a 3-fold greater change from baseline serum glucose concentrations as compared to those treated with the PI-free formulation (AUC: -6369 mg/dL vs. -2201 mg/dL, respectively). The duration of the effect (140±33 minutes) was 75% longer than the duration of the effect of the PI-free formulation (80±37 minutes) (Figure 4). Formulations containing >25 mg rPI provided a more modest glucose-lowering effect, which was still greater than that observed in animals treated with a PI-free formulation (Figures 2-3).





CONCLUSIONS

rPI was successfully cloned, expressed and purified and showed higher anti-proteolytic activity when compared to the nsPI. When formulated with insulin, rPI provided a protective environment, preserving insulin integrity and function, as observed by its glucose-lowering effect in fasting pigs. The formulation containing 25 mg rPI provided for the greatest anti-glycemic effect when compared to both control and other tested rPI doses. Taken together, rPI is likely to both enhance ORMD-0801 bioavailability and cut manufacturing costs.

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