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Oral Insulin Delivery in Diabetic Rats by PLGA Nanoparticles Combined with a Protease Inhibitor (N-Ethylmaleimide)

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SUMMARY

Nanoparticles (NP) have shown a certain potential to overcome the drawbacks of oral peptide delivery in the gastrointestinal tract such as low peptide stability and permeability. Insulin PLGA NP were prepared using a modified double emulsion solvent evaporation technique. Insulin PLGA NP were composed from human insulin (5 mg) encapsulated in PLGA 2.5% w/v mixed with PEG (2 kDa, 5% w/w) and the external aqueous phase contained 1.25% of PVA. The resulting nanoparticles of 202.6 nm diameter and loaded with 33.86 µg insulin per mg of polymer were utilised in this study to examine the hypoglycaemic effect after combination with a protease inhibitor, N-Ethylmaleimide.

INTRODUCTION

Insulin is sensitively degraded by proteolytic enzymes in the gastrointestinal tract. In addition, this peptide is very poorly absorbed after oral administration. To protect it from biodegradation and to improve its intestinal absorption, insulin has been associated with protease inhibitors, hydrogels, or combined with absorption enhancers and surfactants.

N-Ethylmaleimide (NEM) is a thiol blocker (irreversible inhibitor of all cysteine peptidases). Intragastrical administration of NEM produced a dose-dependent reduction of the transmucosal potential difference and the mucosal non-protein SH levels. NEM could inhibit insulin degradation and protect insulin throughout different gastrointestinal segments (Bai et al., 1996). Insulin was also encapsulated in polymeric biodegradable systems such as nanocapsules, or micro/nanoparticles that might be associated to surfactants to improve its oral bioavailability (Damgé et al., 2007).

MATERIALS AND METHODS

Insulin PLGA NP were prepared using a modified, double-emulsion, solvent technique. In vitro characterisation consisted of particle size determination, zeta potential, percent entrapment efficiency and in vitro release profile (Abdelkader et al., 2018a)(Abdelkader et al., 2018b).

Male Sprague–Dawley rats (12-week-old) weighing 250-300 g, were fasted for 6 hours prior to the

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induction of type I diabetes via i.p injection of streptozotocin (50-60 mg.kg-1, pH=4.5). Three percent sodium bicarbonate solution (500µL) in phosphate buffered saline (PBS) was administered through an oral gavage to neutralize gastric acid (Hyuk Sang Yoo, 2004), a protease inhibitor -PI- (N-Ethylmaleimide, 2 mM) was physically mixed with free insulin or insulin PLGA-NP before oral administration. To investigate the effect of protease inhibitor (PI), two other animal groups of rats were utilised for free insulin and insulin PLGA-NP without PI.

RESULTS AND DISCUSSION

As shown in Figure 1, a non-significant difference was observed between control, free insulin and free insulin with PI. Free insulin could be rapidly broken-down due to the enzyme activity of GIT, so the blood glucose level did not significantly decrease. The pH value of the stomach, higher than expected due to sodium bicarbonate administrated prior to study, could affect negatively on free insulin absorption from stomach which demonstrated the non-significant difference between free insulin with PI and free insulin without PI or control. Although insulin NP had a significant hypoglycaemic effect compared to free insulin, control and free insulin with PI. It was found that addition of PI exaggerated the hypoglycaemic effect with a significant difference ($p < 0.05$) comparing to insulin NP.

CONCLUSIONS

The addition of protease inhibitor combined with nano delivery system might have a potential strategy that increases the possibility to administer human insulin orally with better patient convenience.

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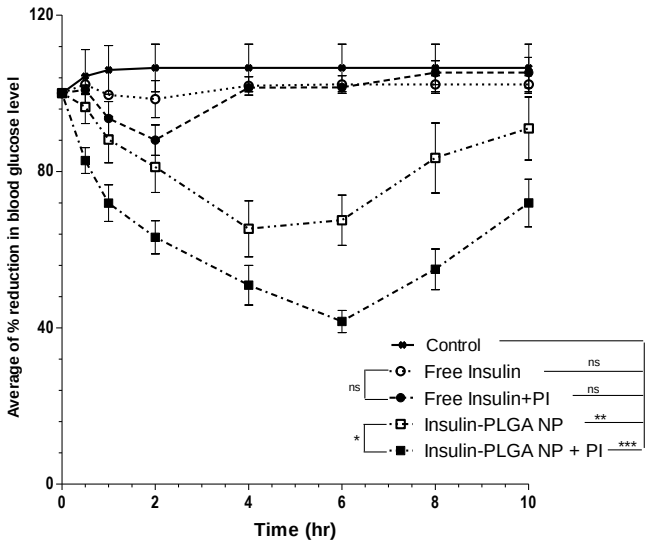

Fig. 1. *Effect of protease inhibitor-PI- (N-Ethylmaleimide) on the hypoglycaemic action of free insulin and insulin-PLGA NP after oral administration in streptozotocin-induced diabetic rats. Results are expressed as means ± SE. N= 6 per groups of control (x), free insulin (○) and free insulin with PI (●), N= 12 per groups of insulin NP (□) and insulin NP with PI (■). Insulin was orally administered at the concentration of 100 IU/kg. The average blood glucose level was 532.28±71.42 mg/Dl at time zero. One-way ANOVA statistical analysis was shown to be (*p < 0.05), (**p < 0.01), (***p < 0.001) and ns for non-significant difference.*

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