ADVANCES IN ORAL INSULIN DELIVERY

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Background

Insulin Oral Delivery Challenge

Experimental Work

Content

Glucosamine Effect

Glutathione Effect

Results and Conclusion
Insulin is the most used medicine for diabetes mellitus.
Insulin can be delivered by injection or by other delivery systems, among these is the oral route.
In order to deliver insulin orally it must be modified by:

Chemical Methods : New Moieties
Physical Methods   : Formulations
Various attempts to deliver insulin orally are at different stages. The main focus is to deliver oral insulin using solid and liquid dosage forms.
Among these is an internationally patented technology where a polyelectrolyte insulin-chitosan complex is formed and solubilized in a reverse micelle.
This patented system was conceptually proved and tested on 50 human healthy volunteers.
The challenge is to improve the bioactivity of the tested formulations
Strategy followed is to minimize metabolism of insulin in liver
Glutathione dehydrogenase is responsible for \( \frac{1}{3} \) of insulin metabolism.

Glucosamine HCL was found to reduce or inhibit metabolism of many small molecules.
Delivered insulin concentration

Glucosamine effect on delivered insulin

Glutathione oxidized and reduced effect on delivered insulin
**Solubilized Insulin in Reverse Micelle Preparation**

- Dissolve 100mg of insulin 1ml of 0.1M HCl
- Dilute in 3ml of 1M TRIS buffer pH 7.0
- In a separate vial, dissolve 125mg of 13KDa Chitosan (99% DDA) in 5ml of dH₂O
- Adjust the pH of chitosan solution to 5.5 using 0.2N NaOH
- Equal volumes of 25mg/ml insulin solution and 25mg/ml chitosan solution are mixed gently with frequent inversions
- Glutathione or Glucosamine complexes are added to aqueous phase

**Oily Base**

- Weigh the following into a beaker: oleic acid (16g), labrasol (2g), and plurol (2g), and stir for 10 minutes
- Add 400μl of the PEC dropwise in 20g of the oily base (2%, v/v) with vortexing for 30 seconds.
Changes in % glucose levels (n=10) versus time profiles of STZ diabetic rats injected initially with multiple doses of insulin (0.5, 0.75 and 1 IU/kg) every two hours followed by oral glucose administration (50 mg/rat) post 6 hours of blood glucose measurements. % Glucose levels were significantly different post oral glucose administration (p < 0.05).
Cumulative release of insulin from the IC-RMs using LMWC (13 kDa).
Effect of GlcN·HCl SC administration on insulin bioactivity.
Notes: GlcN·HCl solution was SC injected with doses of 0, 50, 100 and 200 mg/kg prior to SC insulin (1 IU/kg) administration. The reduction in blood glucose levels was confirmed to be significant as P values were < 0.01.
Abbreviations: GlcN, glucosamine; SC, subcutaneous; IU, insulin unit.
Effect of simultaneous SC insulin-GlcN-HCl administration on insulin bioactivity.

Notes: Insulin-GlcN-HCl mixture solutions of mass ratio 1:0, 1:1, 1:4, 1:10 and 1:20 were SC injected (1 IU/kg) to fasted rats. The mixtures prepared at mass ratios of 1:10 and 1:20 (indicated by *) induced significant reductions in the blood glucose levels of the tested rats compared to the free insulin group at 0.5 and 4 h time intervals (P < 0.05).

Abbreviations: GlcN, glucosamine; SC, subcutaneous; IU, insulin unit
Effect of continuous oral GlcN·HCl administration on insulin bioactivity.

Notes: Rats were fed with GlcN·HCl (25 mg GlcN·HCl/mL) for 5 days. Blood glucose levels post SC insulin (1 IU/kg) administration was significantly reduced when compared to rats offered water (P = 0.028).

Abbreviations: GlcN, glucosamine; SC, subcutaneous; IU, insulin unit.
Effect of GlcN·HCl on IC-RM bioactivity in diabetic rats.
Notes: The hypoglycemic activity of IC-RM containing GlcN·HCl was higher compared with IC-RM containing no GlcN·HCl and the difference is significant (P<0.05).
Abbreviations: GlcN, glucosamine; SC, subcutaneous; IU, insulin unit; IC, insulin-LMWC; PEC, polyelectrolyte complex; RM, reverse micelle; LMWC, Low molecular weight chitosan
It has been reported that GSH has an important role in the degradation and regulation of insulin
Percentage blood glucose levels in non-diabetic rats after subcutaneous (SC) administration of different doses of reduced glutathione (GSH) prior to SC insulin injection (1 IU/kg). Glucose measurements were performed at different time intervals (0 – 4 h). Each data point represents the mean ± SEM (n = 10). GSH significantly reduced the activity of insulin in a dose-dependent manner (p < 0.05).
Percentage blood glucose levels in non-diabetic rats after subcutaneous (SC) administration of 50 mg/kg of reduced (GSH) or oxidized glutathione (GSSG) prior to SC insulin injection (1 IU/kg). Glucose measurements were performed at different time intervals (0 – 4 h). Each data point represents the mean±SEM (n = 10). GSSG administration significantly enhanced hypoglycemia (p <0.05).
Percentage blood glucose levels in non-diabetic rats after subcutaneous (SC) administration of 50 mg/kg of reduced glutathione (GSH) at different time intervals prior to SC insulin injection (1 IU/kg). Glucose measurements were performed at different time intervals (0 – 4 h). Each data point represents the mean ± SEM (n = 10).
Percentage blood glucose levels in diabetic rats after oral administration of insulin-chitosan reverse micelle (IC-RM) containing 50 IU insulin/mL and different concentrations of reduced glutathione (GSH) in comparison with SC insulin injections (1 IU/kg). Glucose measurements were performed at different time intervals (0 – 10 h). Each data point represents the mean±SEM (n = 10). The reduction in insulin activity was insignificant with GSH containing preparations (p > 0.05).
Percentage blood glucose levels in diabetic rats after oral administration of insulin-chitosan reverse micelles (IC-RM) containing 50 IU insulin/mL and 2.1 mg/kg of reduced (GSH) or oxidized glutathione (GSSG). Glucose measurements were performed at different time intervals (0 – 10 h). Each data point represents the mean ± SEM (n = 10). GSH (p > 0.05), GSSG (p < 0.05) compared to glutathione free preparations.
Conclusion

Glucosamine and oxidized Glutathione can enhance the bioactivity of insulin when incorporated into S.C and oral formulations
Thank You