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Formulation of Powder Dosage Form of Insulin for Intranasal Delivery

Matthew Ikhuoria Arhewoh[1], Omonkhelin Josephine Owolabi[2], Sylvester Okhuelegbe Eraga[1*], Ovbokhan Henry Iyamu[1], Osarugue Uyi[1] and Augustine O. Okhamafe[1]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author MIA conceived and designed the study. Author OJO supervision of laboratory works; author SOE analysis of data and manuscript write-up. Authors OHI and OU carried out the laboratory work; author AOO co-supervision of laboratory works and review of manuscript. We declare that this manuscript has been read and approved by all the authors.

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ABSTRACT

Aims: To develop a powder dosage form of insulin for intranasal administration and investigate its glucose-lowering effect in albino rabbits.
Methods: Nine different powder formulations, each containing 500 mg powder equivalent to 50 IU of insulin were prepared by adsorbing soluble insulin solution mixed with varying amounts of polysorbate 80, guar gum and porcine mucin on to microcrystalline cellulose (MCC) powder by solvent evaporation. The formulations were subjected to *in vitro* release studies, from which two optimized formulations (E and H) were further evaluated *in vivo* for their glucose lowering effect in albino rabbits via the intranasal route.
Results: All the formulations released > 90% of insulin within 30 min in the *in vitro* release studies.

**Corresponding author: Email: eragaso@uniben.edu;*

Formulations E and H containing 0.1% Tween 80 and either guar gum or mucin gave the highest drug release of 85% each in 15 min and were selected for the *in vivo* studies. *In vivo* results obtained after 30 and 60 min of intranasal administration showed a reduction of 30 and 15% in blood glucose level for formulation E and 40 and 30% for formulation H as against the subcutaneously administered insulin (control) with 51 and 46% reduction respectively. There was no significant difference ($p > 0.05$) between the control and the formulations with regard to their glucose-lowering effect.

Conclusion: The results of the study showed that the insulin powder dosage form prepared with MCC for intranasal delivery achieved a moderate reduction in blood glucose level in rats via the intranasal route, indicating the formulations can be further developed to achieve enhanced insulin administration.

Keywords: Insulin; intranasal; powder; glucose lowering; tween 80; microcrystalline cellulose.

1. INTRODUCTION

Nasal drug delivery has been practiced for thousands of years. It is a useful delivery method for drugs that are active in low doses and show minimal oral bioavailability such as proteins and peptides. One of the reasons for the low degree of absorption of peptides and proteins via the nasal route is rapid movement away from the absorption site in the nasal cavity due to the mucociliary clearance mechanism [1]. The nasal route circumvents hepatic first pass elimination associated with oral delivery. It is also easily accessible and suitable for self-medication. During the past decades, the feasibility of drug delivery via the nasal route is receiving increased attention from pharmaceutical scientists and clinicians. Drug candidates ranging from small metal ions to large macromolecular proteins have been tested in various animal models [2]. It has been documented that nasal administration of certain hormones and steroids has resulted in a more complete absorption [3]. This indicates the potential value of the nasal route for administration of systemic medications as well as utilizing this route for local effects.

The only effective route of insulin administration is by injection which could be subcutaneous, intramuscular or intravenous depending on the severity of the disease. Though parenteral insulin is very effective, it is not well accepted by most patients because of the pain, cumbersomeness and patient inconvenience associated with using a needle daily.

Over the last 40 years, innumerable attempt have been made to find a satisfactory means of managing and treating diabetes mellitus without the trauma of injection. Delivery routes so far explored include; ocular [4], transdermal [5], rectal [6], buccal route [7] and oral route [8]. The oral insulin delivery is often limited by poor bioavailability due to the degradation by acid and enzyme in the gut. Furthermore, insulin molecule is too large to be absorbed from the gastrointestinal tract. Transdermal and rectal routes have absorption barriers to be overcomed.

Powder dosage forms of insulin for intranasal administration with specialized polymers that can confer good properties seem promising. Biodegradable polymers are preferred because they are broken down into biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathway. The objectives of this study were to formulate microcrystalline cellulose (MCC) based powder of insulin and investigate the effect of surfactant (polysorbate 80), guar gum and mucin concentrations on the *in vitro* release properties. Blood glucose lowering capacity on the formulated powder dosage form will also be investigated *in vivo.*

2. MATERIALS AND METHODS

2.1 Materials

Human insulin (100 IU/ml) was from Novo Nordisk A/S, Denmark. Microcrystalline cellulose (Pharmacel 101) was a gift sample from DFE Pharma, DMV International, Veghel Netherlands. Guar gum was obtained from Hindustan Gum & Chemicals Co. Ltd., India. Polysorbate 80 (Tween 80) was supplied by Carbowax Industrial Company, USA. Alloxan produced by Sigma-Aldrich Chemical Company, Germany. All other chemicals and reagents used were of analytical grades.

2.2 Methods

2.2.1 Formulation of insulin powders

The various batches of the insulin powders were prepared using the formula in Table 1. Two (2) milliliters of the soluble insulin was made into a solution and adsorbed on to microcrystalline cellulose (MCC) in a petri dish. For batches A, B, and C, the soluble insulin was first mixed with the required quantities of Tween 80 solutions or water before adsorption while for the other batches, the insulin-Tween 80 or insulin-water solutions were mixed with guar gum or porcine mucin before adsorption. The admixture was then dried in a vacuum evaporator at a pressure of 27 mmHg at 25℃ before packaging in airtight containers in readiness for administration.

2.2.2 Powder particle size

The powders was thinly spread over a glass slide and viewed under a light microscope (Labo Microsystems GmbH, Germany) via a calibrated eyepiece and the sizes and shape of the particles were recorded at a magnification of 10 (MICAM 1.4, ScopeImage 9.0).

2.2.3 Preparation of standard calibration curve

Standard solutions of insulin were prepared by serially diluting 1 ml stock solution of insulin equivalent to 100 IU/ml with distilled water using the double dilution method to obtain ten different concentrations. The absorbance of the standard solutions was measured at a wavelength of 275 nm using UV/Visible spectrophotometer (T70 PG Instrument Ltd, USA). The plot of the absorbance versus concentration was drawn.

2.2.4 *In vitro* release studies

In vitro release of insulin from the powder dosage form was determined in a pH 6.8 phosphate buffer solution spanning the physiological pH of the nasal cavity. Dried powder formulations (500 mg) of the various batches, each equivalent to 50 IU insulin were weighed into diffusion membrane tied at both ends with the aid of a thread and thereafter placed in a flask of 500 ml dissolution medium maintained at 37±0.5℃. The stirrer was operated at 50 rpm and the insulin release was monitored spectrophotometrically at 275 nm. Samples (5 ml) for spectrophotometric analysis were withdrawn at 5 min intervals for 30 min. In order to create sink conditions, the withdrawn samples were replenished on each occasion with an equal volume of fresh dissolution medium.

2.2.5 *In vivo* release studies

Healthy albino rabbits of either sex, with mean weight of 1.2 kg were purchased from the Department of Biochemistry, University of Benin, kept and allowed to acclimatize for two weeks in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. Ethical approval for the study was obtained from the Ethical Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The animals were treated according to the principle established for the care and use of laboratory animals [9]. The rabbits were induced with diabetes using Alloxan at a dose of 150 mg/kg body weight [10]. The drug was administered intravenously through their external marginal ear vein and they were left for 72 h.

Table 1. Formula for preparation of insulin powder

Batch	Insulin (100 IU/ml)	MCC (g)	Tween 80		Water (ml)	1% Guar Gum (mg)	Porcine Mucin (mg)
			1% (ml)	0.1% (ml)			
A	2	2	-	-	3	-	-
B	2	2	3	-	-	-	-
C	2	2	-	3	-	-	-
D	2	2	3	-	-	45	-
E	2	2	-	3	-	45	-
F	2	2	-	-	3	45	
G	2	2	3	-	-	-	45
H	2	2	-	3	-	-	45
I	2	2	-	-	3	-	45

In order to prevent hypoglycemic shock within the first 24 hours, the animals were administered with 10% glucose solution orally. After 72 hours, experimental diabetes was confirmed in the rabbits using the Accucheck glucometer and fasting blood glucose level greater than 200 mg/dl (11.1 mmol/l) was taken as being diabetic [11].

The diabetic animals fasted overnight were divided into four groups of five animals each but had access to water prior to the experiment. The control group was orally given 10 ml of distilled water using an orogastric syringe. The second group was given subcutaneous injection of insulin (equivalent to 10 IU per kg body weight). Two optimum preparations (batches) from the in vitro studies were used for the third and fourth groups of the animals. The animals were anesthetized 15 min prior to drug administration by intravenous injection of Ketamine (20 mg/kg body weight). Powder preparations equivalent to 10 IU per kg body weight were carefully placed on one of the nostril of the animal with the aid of patch while breathing was allowed through the other nostril. Insulin absorption was monitored based on the effects on blood glucose level. Blood samples were obtained from the external marginal ear vein over a period of 4 h following insulin administration for all groups of animals. Blood glucose level was determined immediately after sampling using Accuchek Active glucometer (Roche, USA) and expressed at a percentage of the initial level, prior to drug administration.

2.2.6 Statistical analysis

Statistical analysis was performed utilizing Student's t-test (GraphPad InStat software version 3.10). All the results obtained were analyzed using one-way analysis of variance (ANOVA) at a confidence interval of 95%.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Particle size

Microscopic evaluation shows that the powdered formulations of insulin of the various samples are crystalline and spherical in shape with a mean size of 5.95±1.36 µm.

3.1.2 *In vitro* release

The *in vitro* insulin release from the powder formulations is shown in Fig. 1. Drug release pattern of insulin from the powder varied according to the inclusion of the surfactant polysorbate 80 (Tween 80) which was the most important factor affecting the drug release. Formulations E and H had the highest drug release in 15 min with 85% each. They were followed by batches A, C and F. All the batches released above 90% of their drug within 30 min. The highest release within 30 min was from batch E with 97% and followed by batches A and B with 94% each while other batches had 91% each.

3.1.3 *In vivo* release

Changes in blood glucose level after administration to the nasal mucosa at a dose of 10 IU/kg of the powder insulin preparation and of the liquid insulin preparation are shown in Fig. 2. In the case of the liquid insulin, the plasma glucose level decreased down to 51±3.57% after 30 min of administration, 46±3.71% after 1 h and then, 30.83±2.78% after 2 h. For the powder preparations, the blood glucose level decreased down to 29.86±2.73% for batch E and 40.37±1.03% for batch H after 30 min of administration, 15.39±1.96% and 30.75±3.35 after 1 h and rose slightly to 19.01±2.18% and 32.5±6.92 after 2h for the batches, respectively. There was no significant difference (P = .05) in the mean percentage glucose level between the standard insulin injection administered subcutaneously and powder insulin preparations.

3.2 Discussion

This investigation attempts to deliver insulin intranasally using an insoluble crystalline base (MCC). This is a practical deviation from previous major efforts that focus mainly on the use of mucoadhesisve polymers, which equally introduced its unique delivery challenges [12-14].

The particle sizes results obtained represent majorly that of MCC which are crystalline and cylindrical. Guar gum and mucin were included to facilitate adhesion to the nasal mucosa while the insulin is expected to bind reversibly to the MCC surface. The relatively small particle size ensured larger surface area of exposure to the absorption site and subsequent clearance from the nostrils after the insulin has been absorbed [15].

In vitro release study showed variable release profile from 5 to 20 min which eventually converged after 30 min. there were however clear indication that formulations A, F and I which

did not contain Tween 80 were not efficient partly because of poor wetting of the MCC surface and secondly because Tween 80 is also required to inhibit enzyme activity at the nasal mucosa [16].

Furthermore, formulations B, D and G containing high proportions of Tween 80 did not appear to present with any significantly additional benefits over C, E and H with low amounts of Tween 80. There was no significant difference in the insulin release profiles hence under such circumstances lower concentration would be considered optimal [17].

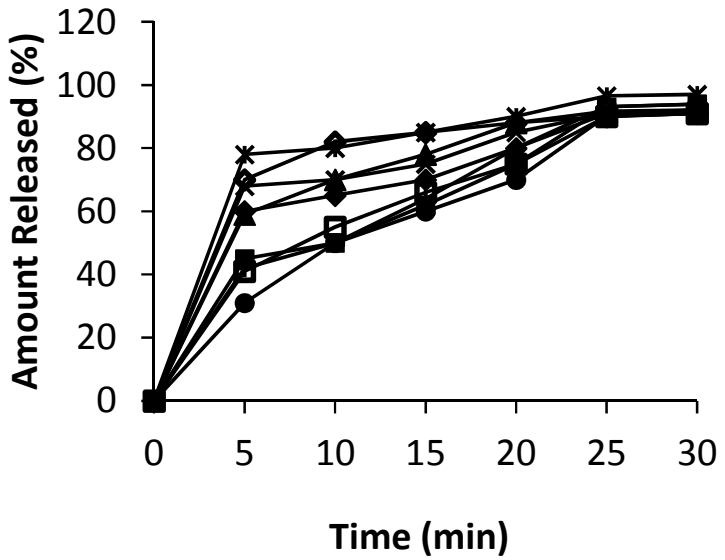

Fig. 1. *In vitro* insulin release profile of the powder formulations A(◆), B (■), C (▲), D (●), E (✱), F (×), G (+), H (◈) and I (□)

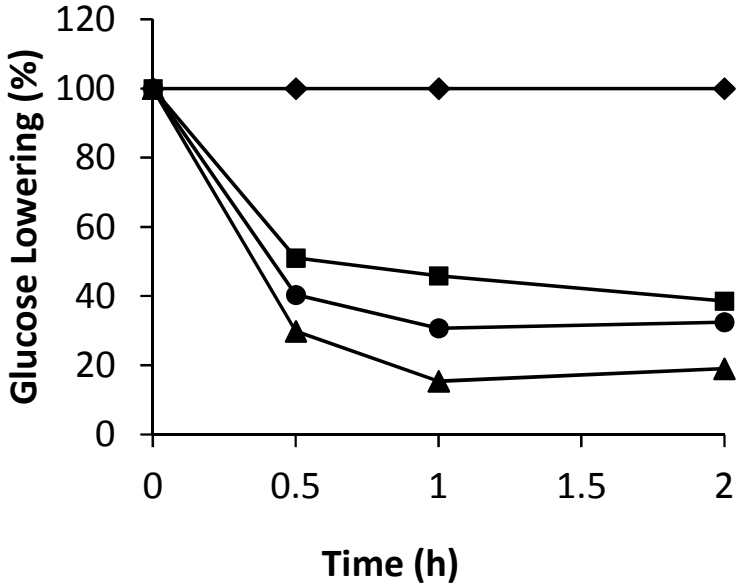

Fig. 2. Effect of treatment on glucose level after intranasal administration of liquid insulin (Control (■), Subcutaneous (◆)) and powder insulin preparation (Batch E (▲) and Batch H (●)). Data are expressed as the mean ± S.E. (n= 2)

Guar gum and mucin were incorporated in fixed quantities and would be useful to confer mucoadhesion properties on the powder. They did not appear to have imparted negatively on insulin release from the dosage form because the amounts used were rather low. There is the possibility that higher amounts could confer a sustained release property on the powdered formulations or alter the physical state of the powders by changing them to patches.

The fact that formulations C did not yield all the insulin contained in it when compared to E and H suggest that the gums in E and H may have also facilitated the release of insulin from the dosage form by competitive binding on MCC surface hence making more insulin available for absorption. Therefore, formulations E and H were used for *in vivo* blood glucose lowering studies.

Interesting result obtained from monitoring blood glucose level after intranasal administration showed similar glucose lowering ability for both test formulations and standard subcutaneously administered insulin. This is similar to previously reported results by Sintov et al. [18] and Forst et al. [19]. This shows that this formulation can release therapeutically sufficient quantities of insulin to achieve normoglycemia. In all cases, hyperglycemia started returning after 2 h of administration due to the effect of streptozotocin and also coupled with the fact that the insulin was being metabolized. There would therefore be the need to consider formulating a sustained release dosage form of this device or administering a high dose. In any case, the distress experience in frequent administration of injections is completely overcome.

4. CONCLUSION

Powder insulin formulated with microcrystalline cellulose, guar gum or mucin and polysorbate 80 achieved above 90% release of insulin within 30 min in vitro and a similar blood glucose lowering activity with standard subcutaneously administered insulin in vivo. Powder insulin for intranasal delivery would be a potential alternative for insulin administration.

CONSENT

It is not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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