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Original Article

Transdermal Delivery of Insulin by Biodegradable Chitosan Nanoparticles: Exvivo and *In vivo* Studies

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Abstract

Insulin-loaded biodegradable chitosan nanoparticle was prepared by the polyelectrolyte complex formation method. The prepared nanoparticles were in the size of 110 nm and had high entrapment (91.0%) capacity. The transdermal nanoinsulin was characterized by *in vivo* hypoglycemic effects. Plasma glucose was decreased to the range of 80.34 to 96.74 mg/dl, and insulin levels were increased to the range of 21.62 to 45.80 µIU/ml for up to 60 h. The pharmacokinetic and pharmacodynamic parameters like AUC, C_{max}, T_{max} and relative bioavailability of transdermal patch loaded insulin-chitosan nanoparticles were 3153.36 µIU/ml/h, 45.80 µIU/ml, 8 h and 20.02%, respectively.

Keywords: Bioavailability; Diabetes; *in vivo* studies; Insulin; Nanoparticle.
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1. Introduction

Diabetes is a Greek word "a siphon" delineated as a melting down of flesh and limbs into urine [1]. Diabetes is a disorder of metabolism in human being, the way that the body used digested food for growth and accustomed activities. Insulin is a hormone produced by the pancreas, a large gland present in abaft the stomach. When a person develops diabetes, his/her pancreas either fails to produce sufficient insulin or produce insulin that is ineffective in carrying its functions. Due to bereft or ineffectiveness of insulin, glucose builds up in the blood stream and is unable to enter the cells, which bivouac starved of energy [2]. Today, every third person we meet seems to be languishing from diabetes. According to world health organization (WHO) about 285 millions peoples are corresponding to 6.4% of the world population had languish with diabetes in 2010, and the number is expected to grow to 438 millions by 2030 [3].

Biodegradable nanoparticles, with a particle size of 1 µm or less, have certain unique advantages in drug delivery. These nanoparticles can penetrate small epidermis layer of skin allowing enhanced accumulation of nanoparticles into blood stream [4]. The skin penetration of the polymeric biodegradable nanoparticles is restricted to the stratum corneum, whereas the follicular penetration appears to be the major transport pathway for

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these particles, and it can penetrate the inner layer of the skin. Only few studies have investigated size-depended penetration into the skin [5]. Some of the studies showed the permeation and distribution of fluorescent labeled 20-200 nm size polystyrene nanoparticles through the porcine skin. No nanoparticle was found in the corneocyte or inner corneocyte spaces [6]. The objective of the present study is to enhance the penetration of nanoparticles into skin and maintain sustained delivery of insulin by transdermal patch containing insulin loaded biodegradable chitosan nanoparticles.

2. Materials and methods

2.1. Materials

Insulin sample was purchased from Bioton, Poland. Chitosan were from SD Fine Chemicals, Mumbai, India. Methocel K100M and DMSO (dimethyl sulphoxide) was obtained from Merk Chemicals, Mumbai, India. Streptozocin was purchased from Calbiochem, USA. ELISA kit were obtained from Bhabha Atomic Research Centre, Mumbai, India. All pther reagents were of analytical grades.

2.2. Preparation of insulin nanoparticle

About 100 mg of chitosan was dissolved in 100 ml of 6% aqueous acetic acid solution and the pH 5.5 was adjusted with 1 N NaOH. Insulin (100 IU) was dissolved in 10 mM Tris buffer and the pH of the solution was adjusted to 8.2 by using 1 N NaOH. Equal volumes of insulin solution and chitosan solution were mixed in a beaker under gentle magnetic stirring at room temperature for 30 min. The mixture was further homogenized for 4 min with an ultra- probe sonicator. The nanoparticles were precipitated and separated by refrigerated centrifuge with 20000 rpm for 10 min. The sediment was washed twice with double distilled water. The separated nanoparticles were lyophilized to get dried power. The dried nanoparticles were used for further studies.

2.3. Characterization of the insulin nanoparticle

The particle size was measured by Mastersizer 2000 (Malvern instruments, Ltd, UK). The entrapment efficient of insulin was calculated by measuring the amount of unencapsulated insulin from nanoparticles. The nanoparticle suspension was centrifuged for 10 min at 15000 rpm, and the supernated liquid containing insulin was determined by RP-HPLC method (Shimadzu scientific instruments, MD, USA) using C18 column with UV detector at 214 nm. The mixture of water and acetonitrile with trifluroacetic acid was used as solvent for quantification of insulin from major peak was found in the chromatogram.

Zeta potential was measured by Zetasizer nano ZS (Malvern Instrument Ltd, UK). The dried nanoparticles were homogenized with 5 ml distilled water and the sample was analyzed by zeta potential.

2.4. Preparation of the nanoparticulated transdermal patch

About 100 mg of Methocel K100 M was soaked in craved quantity of water overnight. In these polymeric solutions, amalgamated 2 IU equivalent of insulin containing nanoparticles were mixed with 1% DMSO

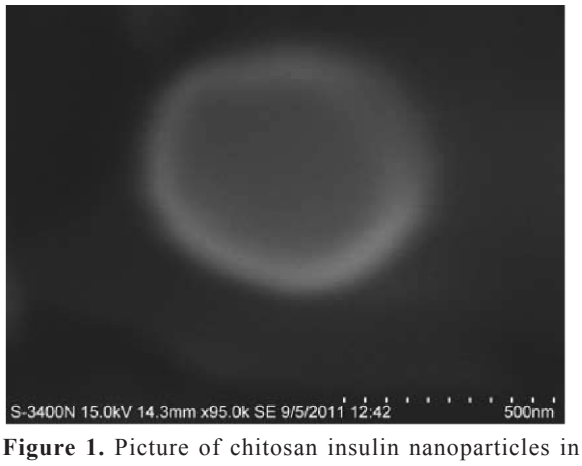

Figure 1. Picture of chitosan insulin nanoparticles in transdermal patch.

Table 1. Pharmacokinetic AND pharmacokinetic parameters of transdermal nanoinsulin after *in vivo* administration in diabetes rats.

Treatment	AUC_0^{60} (µIU/ml/h)	C_{max} (µIU/ml)	T_{max} (h)
Subcutaneous injection	2614.08*	46.68	2
Transdermal nanoinsulin	3153.36*	45.80	8

*Statistically significant (p<0.01); AUC060 -Area under curve of plasma insulin versus time plot and was calculated by trapezoidal method; C_{max} & T_{max} - calculated from insulin concentration versus time plot; all the values are mean (n=3) with SEM.

and homogenized by mechanical stirrer. The obtained clear suspension was casted on prelubricated glass mould (5 X 5 cm) and dried at room temperature for two days.

2.5. Ex-vivo permeation studies

An Ex-vivo permeation study was done on the rat skin. After accessing approval from institutional animal ethical permission, the experiment was carried out. Wister rats (200-250 g) were sacrificed by excessive ether anesthesia and the hair was removed from the dorsal portion using mechanical hair clipper, and the hairless portion of the skin was removed and the fat adhering to the dermis side was removed using a scalpel with magnifying lens. Finally, the excised skin was washed twice in double distilled water and stored at -20 ºC in Aluminum foils. The skin should be used within 7 days after treatment. Phosphate buffer (pH 7.4) was prepared and sonicated for 30 min and was filled in the receptor and donor chamber divided by prepared animal skin (area of 0.8 cm2). The diffusion cell was equilibration overnight at 37±0.2 ºC with a constant stirring speed of 100 rpm in a magnetic stirring module. A 2 cm2 of transdermal patch containing chitosan insulin nanoparticles (equivalent of 2 IU insulin) was placed on the surface of the skin (the patch exposed to epidermis layer of the skin). At predetermined time intervals, 3 ml samples were collected from the receptor compartment and replaced immediately with fresh buffer solution. Samples were collected upto 60 h and the samples were filtered through membrane filter. The filtered solution was analyzed by HPLC at 214 nm.

2.6. In vivo experiments

2.6.1. Preparation of rats

Wister rats (250-300 g) were housed in the institutional central animal laboratory and diabetes was induced by injection of streptozotocin dissolved in sodium citrate buffer (pH 4.5; i.p.; 65 mg/kg body weight). The rats were allowed to bolt for 3 days. Only rats whose blood glucose levels were more than 250 mg/dl were considered as a diabetic. One day before the experiment, hair in the upper back region of rats was removed by mechanical hair remover. The prepared animals were divided into the following

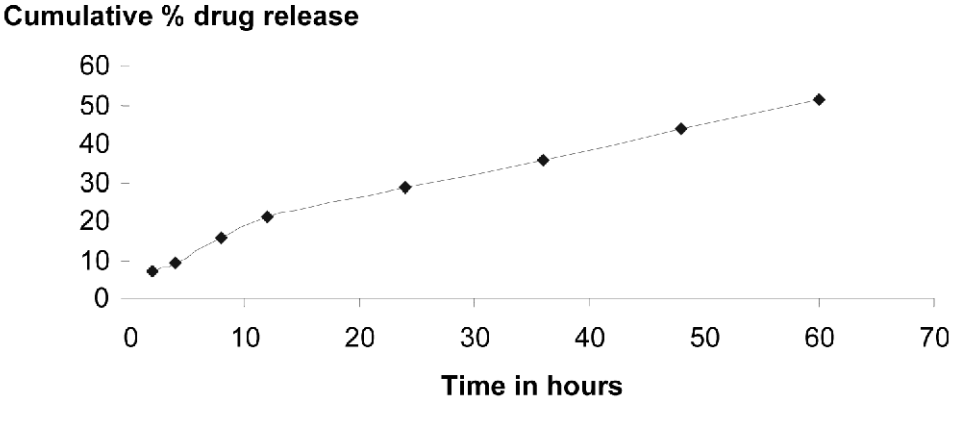

Figure 2. Ex- vivo permeation studies of transdermal patch contain nanoinsulin.

groups. Each group containing 4 rats (n=4) and individually labeled by color pen in the tip of the tail. Blood samples were arranged in a similar manner.

Group I: control (untreated); Group II: control (diabetic, untreated); Group III: standard (diabetic, subcutaneous injection; 2 IU insulin); Group IV: transdermal nanoinsulin (1.79 IU) treated (diabetic treated).

2.6.2. Experiment

Insulin nanoparticles containing transdermal patch applied on the back of hair removed rats, to baffle the removal of the patch, which protected by polyvinyl tape. During these experiments, 1 ml blood samples were abjured from the retro-orbital plexus at different time intervals.

2.7. Separation of plasma

Plasma separated from the blood by cooling centrifugation. Blood samples (0.5 ml) were subjected to cooling centrifuge at 10000 rpm for 5 min. After separation, plasma was stored immediately at 5 ºC until used.

2.8. Determination of plasma glucose and insulin concentration

The prepared plasma was used for calculation of glucose and insulin. The glucose concentration was measured by ONE TOUCH glucometer (Life scan Ltd. Brazil) and insulin concentration was clinched by ELISA tests.

2.9. Pharmacokinetic and pharmacodynamic parameters

From the plasma concentration and time profile curve of these experiments, the pharmacokinetic and pharmacodynamic specifications like AUC, C_{max}, T_{max}, and Relative Bioavailability were assessed using the Trapezoidal method. The relative bioavailability of transdermal nanoinsulin patch was calculated by collated with subcutaneous insulin. All the data were bully to one- way ANOVA, a significance level of $p< 0.05$.

Relative bioavailability=$[AUC]_{transdermal}$ X $dose_{s.c}$ /$[AUC]_{s.c}$ X $dose_{transdermal}$

3. Results

3.1. Preparation and characterization of insulin nanoparticles

The insulin nanoparticles were prepared by polyelectrolyte complex formation method. The prepared nanoparticles had good cellular guff, colloidal stability, favorable diameter,

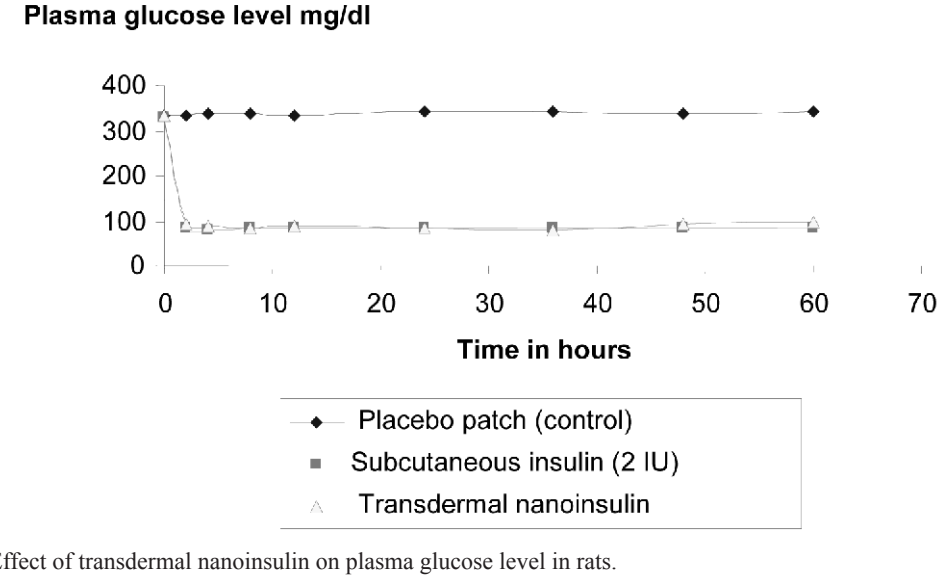

Figure 3. Effect of transdermal nanoinsulin on plasma glucose level in rats.

Table 2. Relative bioavailability of transdermal nanoinsulin.

Bioavailability calculations	Relative bioavailability (%) Mean±SEM
Plasma Insulin	20.02±6.1
Plasma Glucose	16.87±1.2

Transdermal patch contain nanoinsulin contain 2 IU of insulin/ dose/ patch, and AUC was calculated at the end of the treatment compared with AUC of subcutaneous insulin injection.

surface charge, morphology and low polydispersibity index [7]. Too boot, these method used for debarring the damage of protein and peptides. The prepared nanoparticles bear a spherical shaped size of 110 nm (Figure 1) and low polydispersibity (0.08). The insulin nanoparticles bear high entrapment efficient of 91.02±1.2 % and the zeta potential of +21.63±0.16 mV.

3.2. Ex-vivo release of insulin from transdermal patches

The nano-particels of insulin released the insulin on the rat skin during six h of experiment (Figure 2). At 2 h only about 8% of the content of patch was released and permeated from the skin, but at 60 h about 50% of the noano-insulin content was permeated through the skin (Figure 2).

3.3. In vivo hypoglycemic effects

Wister rats were bare to streptozocin and the plasma glucose level was increased from 84.17±0.12 to 331.67±0.02 mg/dl. In this experiment, the streptozocin induced diabetic condition was bolt over three days. During this period, the plasma glucose sensitive subcutaneous insulin diminished. Once bolt, plasma glucose level in diabetic induced rats stopped the fluctuation during experiments (Figure 3) [8].

After application of a single transdermal patch containing insulin nanoparticle (2IU) to diabetic induced rats, plasma glucose was decreased to 96.27±0.02 from 334.17±0.1 mg/dl. Admit these treatments, plasma glucose level was normally maintained from 80.34±0.06 to 94.74±0.02 mg/dl for up to 60 h (Figure 3). Plasma insulin level of transdermal nanoinsulin patch treated rats fanfare drastically increased from 15.2±044 to 30.82±0.62 µIU/ml after 2 h, and then calmly fluctuated in plasma level, thereafter maintained normal insulin level upto 60 h. The control group of subcutaneous insulin injection showed an immediate rise in insulin level of 46.68±0, depleted to 32 µIU/ml within 2 h, after that it bears a drastic decrease to reach the initial level (Figure 4).

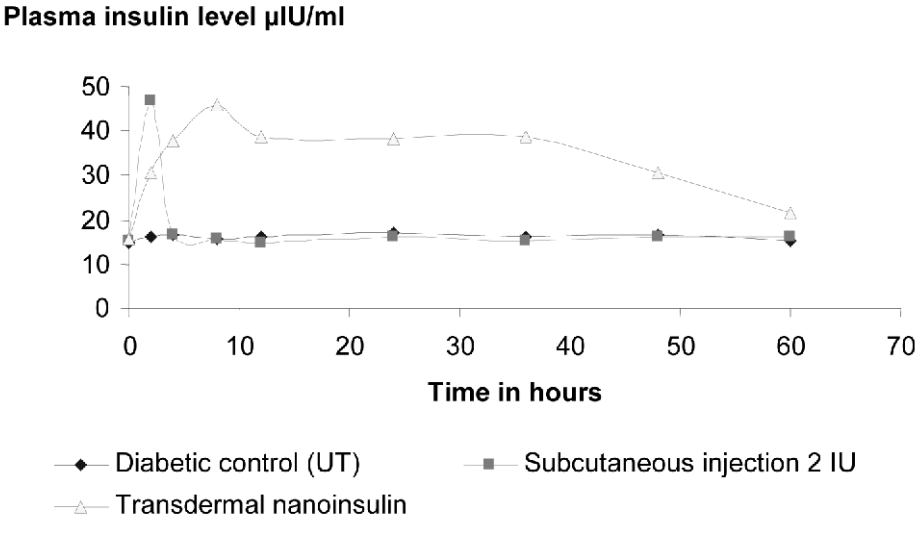

Figure 4. Effect of transdermal application of nanoinsulin on the plasma insulin level in diabetic rats.

3.3. Bioavailability parameters

The bioavailability parameters [9] like AUC, Cmax, Tmax, relative bioavailability of transdermal nanoinsulin patch was determined by Trapezoid methods which were bearded in Table 1. Relative bioavailability was determined by AUC of standard subcutaneous injection and AUC of transdermal nanoinsulin. AUC_0^{60} of plasma insulin level of transdermal nanoinsulin was 3153.36 µIU/ml/h as hang with subcutaneous insulin level 2614.08 µIU/ml/h and C_{max}, and T_{max} of transdermal nanoinsulin were 45.80 µIU/ml, at 8 h, respectively, compared with subcutaneous insulin injection (Table 2).

4. Discussion

Insulin nanoparticles were prepared with chitosan by polyelectrolyte complex formation method. These cationic polymers has allured a great attention in the insulin drug delivery, owing to its unique properties like biocompatibility, gutter toxicity and the power to boom the absorption of highly hydrophilic molecules athwart the epithelial layer of skin via a peculiar transport pathway [10]. Currently, polyelectrolyte complex formation (PEC) method is being largely used for the preparation of insulin nanoparticles, but the rest of the methods such as using organic chemicals, heat or vigorous agitation which may result in cutting of the proteins and formation of toxic byproducts are not common [11, 12]. Chitosan nanoparticles have good cellular guff and colloidal stability and affirmative particle size distribution, and nanoparticle with size of about 110 nm can penetrate through the stratum corneum and cross epidermis, and reach slowly to dermis.

The transdermal insulin nanoparticle was broach for safe and viable drug delivery of insulin. Hence the plasma glucose level decreased slowly after transdermal nanoinsulin in comparison with subcutaneous injection, echo the differences in insulin adsorption rates. The absorbed insulin nanoparticles might bunk in the dermis and calmly degrade the chitosan polymer and release free insulin *in situ*.

The bioavailability of transdermal nanoinsulin correlated with pharmacokinetic and pharmacodynamic response, which was clinched from plasma insulin and glucose level, compared to subcutaneous injected insulin. The transdermal biodegradable chitosan insulin nanoparticle acquired a therapeutic more cogent response that efficiently maintains blood glucose level in diabetic rats. Transdermal nanoinsulin has prolonged drug release over a long period compared with a subcutaneous insulin injection. Furthermore, it decoded the problems depict to poor absorption, more proteolytic degradation and improper drug delivery.

5. Conclusion

The polyelectrolyte complex formation method has drawn more attention for nanoparticle preparation of protein and peptides. This biodegradable insulin chitosan nanoparticle system successfully gemmed the insulin transdermaly, as evidenced by a significant reduction of plasma glucose level and elevation of the secretion of insulin in diabetic rats. These result support the feasibility of developing transdermal biodegradable nanoinsulin for human application at prolonged time period. This experiment proved that the transdermal nanoinsulin was apt to deliver a significant proportion of insulin from the transdermal patch over a prolonged period of time of 60 h.

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