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Original article

Formulation of a transdermal patches using the hydrogel and assesses the efficiency of this transdermal patch in diabetic rats

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Abstract

Aim and Background: *The objective of the study was to develop a transdermal formulation of insulin, determine its pharmaceutical characteristics and finally to test its efficacy in diabetic animals. Out of the several nanoparticles available, transfersomes were selected as the carrier system for insulin. The transfersomes were produced by the hand-shaking method using soy lecithin phospholipid and sodium deoxycholate as the surfactant.*

Materials and Methods: *Type-1 diabetes mellitus was induced in wistar rats of either sex with streptozotocin 60mg/kg i.p. Animals with blood glucose level above 250mg/dl were considered for the study. The diabetic animals were divided into 3 groups of 6 animals each. Blood samples were withdrawn from tail vein and blood glucose and insulin was estimated.*

Results: *A significant gradual reduction of blood glucose was recorded after subcutaneous administration of insulin in diabetic rats.However, no significant reduction in blood glucose was evident in diabetic animals that were applied with transdermal patch of insulin upto two hours. A mild reduction in blood glucose level was recorded at 24 and 48 hours.*

Conclusion: *In the present study we conclude that the However, a significant hypoglycemic effect of the transdermal patch of transfersomes containing insulin was not evident in diabetic animals in the present study. By overcoming the pharmaceutical drawbacks in the preparation of the transdermal patch, it can be hoped that the transdermal delivery of insulin can be successfully developed as a viable alternative approach for insulin administration.*

Key Words: *Transdermal, Insulin, Diabetes Mellitus*

Introduction

Diabetes mellitus has progressed into a pandemic in the last few decades and claimed to be the fifth foremost cause of death in developing and developed countries. According to the International Diabetes Federation (IDF), diabetes mellitus has affected 382 million people which are expected to be 592 million in 2035[1]. Diabetes mellitus is a chronic disorder in a state of hyperglycaemia, characterized by dysfunction in the metabolism of protein, carbohydrate and fats.

Diabetes mellitus is broadly classified into two categories like type 1 and type 2 diabetes mellitus (T1 DM and T2 DM). In T1DM there is complete deficiency of insulin whereas, different degree of insulin resistance, increase in the production of glucose and impaired insulin secretion are the causes of T2 DM. Insulin is the only remedy in T1 DM and in T2 DM patients whose diabetic state is not adequately controlled by using oral anti-diabetic agents. Even

though many combinations and timed release preparation of insulin are available, all of them have to be administered subcutaneously. The difficulties of multiple injection, physiological stress and cost also add to the emotional burden of the patient.

Several nano particles like chitosan[3]liposomes and pharmacosomes are available. Out of which transfersomes, a flexible vesicle that permeates the skin and follows a natural water gradient across the epidermis has been employed to prepare a hydrogel of insulin[4]. With this background, an attempt has been made in the present study to prepare insulin containing transfersomes, study their characteristics and incorporate them into a transdermal patch. It has also been considered to study the efficiency of the insulin transdermal patch in diabetic rats.

Materials and Methods

Preparation of insulin transfersomes for Transdermal patch

Human insulin (Torrent Pharmaceuticals), soy lecithin, sodiumdeoxycholate, phosphate buffer, Lowry's Reagent, Folin's reagent,carbopol and distilled water were used for the preparation oftransfersomes.

Preparation of transfersomes:

Transfersomes were formulated by conventional hand shakingmethod with some modifications as described in literature *(*Ghanbarzadeh*et al.* 2013)[5].Phospholipid (soylecithin) and surfactant (sodium deoxycholate)are mixed with chloroform in the ratio 1:1:1 in a round bottom flask.They were constantly hand shaken and maintained on a water bath at60ºC until a thin layer formed after solvent evaporation.0.347mg (10 IU) of human mixtard was dissolved inphosphatebuffer in1:1 ratio drop by drop in a water bath maintained at 60ºC.Resulting composition was added to the tranfersomes and was hydrated.The vesicles were allowed to swell for 2 hours at roomtemperature.The resulting transfersomes were sonicated at room temperaturefor 30 minutes using a bath sonicator.

Hydrogel preparation

Hydrogel of the insulin encapsulated transfersomes was prepared by using 1% carbopol as gelling agent. Encapsulated transfersomes was added to carbopol and dissolved in distilled water. The resulting mixture was heated at 50ºC until it attained the gel form and was stirred to achieve a uniform consistency and stored in a vial.

Invivo study of Transdermal Patch

The experiment was carried out in 24 number of Wistar rats of either sex weighing 180-220 g. The animals were housed in a controlled environment (2 animals in each cage), with food and water available *ad libitum* on natural day and night cycle. The animals were randomly allocated to four different groups. The experimental protocol was approved by the institutional animal ethics committee**.**

Induction of Diabetes

The Rats were fasted for 12 hours with water available *ad libitum*.Streptozotocin was freshly prepared in a 0.1 M citrate buffer (pH: 4.5) and injected in a dose of 60mg/kg i.p to the experimental animals. Blood was withdrawn from tail vein and the blood glucose was estimated at 24 hour intervals using a glucometer. Animals whose blood glucose values were above 250 mg/dl for three consecutive days were considered for the study.

The diabetic animals were divided into four groups of six animals each.

Group-I: Control (saline)

Group-II: Diabetic Control

Group-III: Animals was treated with insulin subcutaneously in a dose of 2IU/Kg.

Group-IV: Diabetic animals were selected for transdermal application of insulin. In these animals, the hair over the nape of neck was removed using 'Veet' hair removal cream on the previous day of transdermal patch application.

Biochemical Analysis:

Blood Glucose estimated by GOD- POD method and Plasma insulin level was estimated by CLIA method

Statistical Analysis

The data was subjected to analysis by employing SPSS 17 software. One way ANOVA and Bonferroni's multiple comparison tests was carried out to analyze the results.

Results:

Administration of streptozotocin to Wistar rats resulted in prompthyperglycaemia after 72 hours. The blood sugar level in normal controlgroup of animals was 149.8±14.8 mg/dl which was increased above 500mg/dl in streptozotocin treated animals. (Table:1)

In vehicle treated diabetic animals there was no significant changein blood glucose level over the period of observation.When insulin (2 I.U/Kg) was administered subcutaneously todiabetic rats there was a gradual and significant reduction in bloodglucose level at 30 minutes (432.5±42.73 mg/dl), 60 minutes (201.6±24.77mg/dl) and at 120 minutes (71± 6.5 mg/dl).When transdermal patches of insulin were applied to diabetic rats,there was no significant reduction in blood glucose level up to 120minutes. When the measurement was extended to 24 hours and 48 hoursthere was a mild reduction in blood glucose level, when compared toinitial value, however not statistically significant. (Table:1).

Particulars	**Blood Sugar Levels (mgs/dL)**					
	0mins	**30mins**	**60mins**	**120mins**	**24hrs**	**48hrs**
Group-I	149.8± 14.8	140.6 ± 14.8	155.5± 15.5	156.3± 15.5	-	-
Group-II	505.8±50.5@	495.6 ± 44.4	523.72± 50.9	501.4± 45.8	-	-
Group-III	514.0±30.88@	432.5±42.73*	201.6±24.77**	71±6.5**	-	-
Group-IV	465.6 ± 46.7@	521.4 ± 51.6	515.6± 45.3	535.7± 55.6	383.5±35.8	429.3±42.5

@- P < 0.001, compared to normal control value at '0' minute.

*- P < 0.05 and **- P < 0.001 compared to corresponding value at '0'minute.

Each value represents the mean ± SEM of 6 animals.

Changes in insulin values in different treatment groups

In normal rats the plasma insulin levels did not vary much from the initial value (0.56±0.22 IU/ml), when measured at 60 minutes (0.66±0.14 IU/ml). Similarly in diabetic control animals (which did not receive any exogenous insulin) also, the plasma insulin levels were not significantly different when measured at 0 minutes (0.63±0.08 IU/ml) and 60 minutes (0.55 ± 0.15IU/ml). When diabetic animals were treated with subcutaneous injection of insulin (2IU/Kg), there was a marked elevation of plasma insulin after 60 minutes (6.09 ±0.88 IU/ml) when compared to the initial level (1.54±0.6IU/ml). (Table:2) When diabetic animals were treated with transdermal patch of insulin, the plasma insulin levels which was 0.20±0.10 IU/ml at 0 minute, significantly increased to 0.61±0.15 IU/ml at 60 minutes and remained in the same range (0.68±0.15IU/ml)up to 120 minutes. However, the plasma insulin values returned to baseline value after 24 hours. (Table:2).

Particulars	**Plasma insulin mIU/L (Mean ± SEM)**			
	0mins	**60mins**	**120mins**	**24hrs**
Group-I	**0.56±0.22**	0.66±0.14	-	-
Group-II	0.63±0.08	0.55±0.15	-	-
Group-III	1.54±0.60	6.09±0.88**	-	-
Group-IV	0.20±0.10	0.61±0.15*	0.68±0.15**	0.18±0.0.09

Each value represents the mean ± SEM of six animals.

*- P < 0.01 and ** - P < 0.001 compared to respective '0' minute insulin value.

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Discussion

The area of drug delivery has been changing over the years. Theresearchers are constantly working on various systems to improve theperformance of drug delivery to maximize therapeutic activity andminimize side effects. Each delivery system has its own uniquepharmaceutical and physiological characteristics and must be chosenaccording to the characteristics of the drug which it is going to carry. Anumber of delivery systems are being developed for anti- diabetic drugsand insulin[6, 7]. The discovery of insulin is one of the greatest breaks -through in the medical history for patients with type 1 diabetes mellitus otherwise which would have been a fatal metabolic disorder. The initial researchesfocused on purification of insulin obtained from bovine and porcinepancreas. In last five decades the research outlook shifted in developing rapidand long acting insulin analogues[8].

The Diabetic Control and Complication trial (DCCT) focuses onthe importance of Intensive Insulin Therapy (IIT) in type 1 diabetesmellitus patients to prevent micro and macro vascular complications.But Intensive Insulin Therapy always resulted in hypoglycaemia, amajor hurdle in achieving a glycaemic target[9]. The new methods of insulin delivery aim to deliver insulin withminimal invasiveness in an accurate and painless manner to increase thepatient's compliance[10].

In the present study soy lecithin has been used as the phospholipidand sodium deoxycholate has been used as the surfactant for their betterbiocompatibility and high entrapment efficiency (Maurya *et al*, 2010)[10].The examination by optical microscopy indicated the formation of welldefinedspherical, transfersome vesicle. Further, the surface morphology and the three dimensional nature of developed transfersomes wasconfirmed by the results of Scanning electron microscope. Sphericalvesicles of transfersomes, without aggregation were confirmed. The meansize of transfersomes was found to be 130 nm.

The insulin loaded transfersomes were subjected to various tests toconfirm the physical and chemical stability. The results of the physicalstability studies revealed that the insulin vesicles were free fromaggregations or fusion for a period of 30 days. The UV absorbance of theinsulin entrapped transfersomes was found to be uniform when studied atdifferent time intervals. This observation confirms that thechemical composition of the transfersomes remain stable and unalteredeven after 30 days.

A variety of topical formulations have been developed bypharmaceutical companies to treat local indications. However the utilityof cutaneous application to treat systemic diseases had many limitations.But the advent of the transdermal preparation of scopolamine to treatmotion sickness has revolutionized the mode of drug delivery over theskin to treat systemic ailments. Many other drugs like estradiol, lidocaine,testosterone and fentanyl are also available as transdermal patch fortherapeutic use. Only a few attempts have been made to prepare insulinfor transdermal delivery. In the present study a simple transdermal patchcontaining insulin transfersomes has been prepared. The formulation ofthe patch is in conformation with the standard transdermal patchesavailable. The patch consists of a backing layer, over which a hydrogelcontaining insulin transfersomes was evenly spread.A rate controlling membrane for uniform delivery has beenassembled over it. A bio-adhesive layer is covering the layer which canbe removed before the application of the transdermal patch over the skin.The prepared transdermal patches were measuring 5 x 3.5 cm loaded with10 IU of insulin.The next stage of the study attempted to investigate the efficacy ofthe prepared insulin transdermal patch in diabetic animal models.

The changes in the plasma insulin levels in diabetic rats after theadministration of either subcutaneous insulin or transdermal patch insulinwere also monitored at different intervals in the present study. A sharpincrease (almost five fold) in plasma insulin could be detected in animalstreated with subcutaneous injection of insulin (Table.2). In the animalsapplied with transdermal patch of insulin, the plasma insulin wasmeasured at 60 minutes, 120 minutes and after 24 hours. There was asignificant increase in plasma insulin levels at 60 minutes and 120minutes, however, which returned to baseline value after 24 hours.

Considering the higher efficiency of transfersomes Malakar *et al* (2012)[4]prepared insulin encapsulated transfersomes. This gel preparation directlyapplied over the skin had been studied for its effect on diabetic animals.

The present study is a pilot attempt to prepare an efficient carriersystem like transfersomes for transdermal delivery of insulin. Howeverthe results have been encouraging. Even though the plasma insulinlevels were significantly elevated after transdermal patch application, thisresulted in a significant reduction in blood glucose level.

Conclusion

In the present study we conclude that a significant hypoglycaemic effect of the transdermal patch of transfersomes containing insulin in diabetic animals. By overcoming the pharmaceutical drawbacks in the preparation of the transdermal patch, it can be hoped that the transdermal delivery of insulin can be successfully developed as a viable alternative approach for insulin administration.

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