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Transdermal Delivery of Repaglinide from Solid Lipid Nanoparticles in Diabetic Rats: In Vitro and In Vivo Studies

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Abstract

Transdermal patches loaded with Solid Lipid Nanoparticles has been used as a suitable dosage to maintain the blood glucose level in diabetic patients. Repaglinide was taken as model antidiabetic drug get incorporated in Solid lipid Nanoparticles (SLNs) by a hot homogenization method using cephalin and lecithin as lipids and Tween 80 as stabilizer. The prepared nanoparticles evaluated for particle size measurement, poly dispersity index, Zeta potential, entrapment efficiency, *and In vitro- In vivo* release studies so as to ensure the quality Solid lipid Nanoparticles. Report of Scanning Electron Microscopy showed that the SLN particles were spherical shape and has size range between 85 – 120 nm, the poly dispersity indexes was found to be 0.148 to 0.227. The zeta potential range between - 27.1 ± 2.5 to −36.1 ± 2.1 mV. The entrapment efficiency (EE %) and drug loading capacity (DL %) was 80.4 ± 4.2 % to 92.3 ± 7.2 % respectively. Repaglanide SLN was incorporated on transdermal patches and performed ex-in vivo release studies. Blood glucose level was calculated by using Diabetic rat, blood glucose levels of rats decreased and it could be sustained at such levels over 48 hrs. SLN loaded transdermal patches showed more suitable controlled release kinetics for protein delivery.

Keywords:

Solid lipid Nanoparticles, Repaglinide, In vivo studies, diabetic, transdermal patches.

INTRODUCTION

Recent advances in nanoparticulate systems for improved drug delivery display a great potential for the administration of wide variety of active pharmaceuticals[1]. The main challenge in transdermal drug delivery is to overcome the inherent barrier of skin. There is evidence that the rate limiting step in transdermal transport occurred at the stratum corneum. Many approaches have been used to enhance the penetration of drugs through skin. Some of the transdermal delivery attempts resulted in showing success in delivering glucose responsive doses (approximately 20–50 *m*IU/mL) of insulin over short periods[2]. The role of these systems in the long-term treatment of diabetes, however, remains debatable. Especially questionable are those methods involving physical or chemical Disruption of the skin, which might cause chronic pathological changes. The transdermal route in particular is an attractive candidate for the steady and sustained delivery of insulin into the blood. Although the stratum corneum poses a significant barrier for protein absorption, once the protein passes through this barrier, the transdermal route offers several unique advantages. Firstly, proteolytic degradation of drug is low because the skin contains relatively few proteases. Secondly, painless, noninvasive, and patient-friendly application of patches offers good patient compliance. Thirdly, patches are also easy to remove in the event of hyperinsulinemia[3].

The use of drug carriers as vehicle for transdermal delivery is a good strategy[4]. Drug carrier could modify the physiochemical properties of the encapsulated molecules and facilitate the percutaneous delivery. Recently, they are some report about liposomes[5], microemulsions, polymeric nanoparticles loaded transdermal films[6].

SLN have been proven a better alternative carrier system than conventional systems. They produce prolonged release and protect the drug against chemical degradations. Compared to polymeric nanoparticles, they possess some distinctive effect apart from the lower cytotoxicity due to the absences of solvent and relatively low cost for exipients and large scale-up production is possible by the simple process of homogenization[7].

Repaglinide[8] is an oral blood glucose lowering drug of Meglitinide class used in the management of type 2 diabetes mellitus (NIDDM). It lowers blood glucose levels by stimulating the release of insulin from pancreas. This action is dependents upon stimulating beta cells in the pancreatic islets. Insulin release is glucose dependent and diminishes at low glucose concentrations. Repaglinide closes ATP-dependent potassium channels in the beta cell membrane by binding at characterizable sites. The potassium channel blockade depolarizes the beta cell, which leads to an opening of calcium channels. Their resulting increased calcium influx induces insulin secretion.

Repaglinide, a fast and short-acting meglitinide analog was chosen as the drug candidate since it is indicated for the development of a dosage form with increased GRT. It has a very short half-life (1 h), low bioavailability (50%) and poor absorption in the upper intestinal tract. It is completely metabolized by oxidative biotransformation and direct conjugation with glucuronic acid after either an IV or oral dose. Moreover it produces hypoglycemia after oral administration[9].

MATERIALS & APPARATUS

Repaglinide obtained as gift sample from Tri-Star formulation Pvt. Ltd. Puducherry. Streptozotocin (STZ) was purchased from Merck Specialist Pvt Ltd, Mumbai. Lecithin, cephalin was obtained from Sd Fine Chemicals, Mumbai. Tween 80, Methocel K100M was purchased from Merck chemicals, Mumbai. All reagents used were of analytical grades.

Preparation and characterization of Repaglanide solid lipid Nanoparticles:

Preparation and characterization of solid lipid nanoparticles loaded Repaglinide was reported in V.Vijayan *et al*. /JITPS 2010, Vol.1 (8), 320-328. The success formulation of solid lipid nanoparticles using for further studies.

Preparation of transdermal patches[7]

For preparation of transdermal patches Methocel K100M were used as a film forming agent. The polymer was soaked in water for overnight, and then 50mg of prepared SLN were incorporated and mixed uniformly. Suspension was casted on a glass mould, after drying the patches were cut into small pieces and stored in between the sheet of wax paper in desiccator for further studies.

IN-VITRO *STUDIES*

Drug content analysis

The patches (n=3) of specified area were weighted and dissolved in 100ml methanol. The solution was filtered through membrane filter and drug content were analysis by HPLC.

Preparation of skin[11]

Male Wistar rats weighing 200-260 g for the in vitro permeation studies. The hair of rats in abdominal region was removed with a depilatory, and examined for integrity using a lamp-inspecting Method. The subcutaneous fat and connective tissue were carefully removed by scalpel. Finally obtained skin was rinsed with physiological saline and stored at -20 □ in an aluminium foil.

Ex-* in vivo *permeation study

The skin samples were mounted carefully on Franz-type diffusion Cells with the stratum corneum side up with an effective Diffusion area of 1.72 cm2. The receiver compartments were filled with 15 ml of physiological saline to ensure sink condition. The diffusion cells were maintained at (37±0.5) ◦C with stirring at 100rpm throughout the experiment. A 3 cm^2 transdermal patch of Repaglinide was mounted onto skin surface.

5 ml of the sample was collected from the medium at predetermined time Interval of (0.5, 1, 2, 4, 6, 8, 10 and 12 h) and replace same volume of fresh physiological saline. All the samples were filtered through a membrane filter and analyzed by HPLC.

INVIVO STUDIES

Preparation of animal for studies

Male Wistar rats weigh 200-260 g were housed with free access to a standard diet and water for three days, and then they were fasted overnight before experiment. The hair on the backside of the rats was removed with a depilatory cream

(Anne French, Wyeth Limited, Hyderabad, India). Prior to the day of the experiment. Animals were divided into each 3 groups (n=4) of normal and diabetes rats. The rats were treated as following:

Group I (control) placebo patch prepared by Methocel without SLN.

Group II Repaglanide oral administration contain 2mg drug.

Group III transdermal patch (3 cm^2) contains repaglinide SLN.

Induction of diabetes in rats[12]

Diabetic was induced by injecting 60 mg/kg of STZ dissolved in 0.1M citrate–citrate sodium buffer (pH 4.5) intraperitoneally. After allowing the diabetic rats for stabilization over 72 hours. The blood glucose level was estimated by using repaglinide transdermal patches. Blood samples were collected from the tail vein before and after treatment to determine blood glucose levels. These samples were taken at scheduled times. Blood glucose levels were obtained by Using the once touch glucometer (Roche, Germany).

Statistical analysis

Results are expressed as mean ± SEM values. Statistical significances were evaluated using student t test. A value of $p<0.05$ was considered significant.

RESULTS

Loading of SLN on transdermal patches:

The SLN containing Repaglinide loaded in Methocel K100M transdermal patches. This polymer is highly hydrophilic, so the lipophillic SLN are not soluble and structure of nanoparticles remain intact. The drug content of each patch was found to be uniform and ranged between 85.27 ± 0.12 to 92.06 ±0.22 % (1.98mg/patch).

Ex- *in vivo* skin permeation studies:

The result of ex in vivo skin permeation of SLN from transdermal patches is shown in fig: 1. the cumulative amount of drug release from SLN made by lecithin, cephalin, and combination were reported as 240.06±3.02, 254.12± 0.42 and 269.12 ± 2.16 µg/cm^2 respectively .The release was shown in fig no 1.

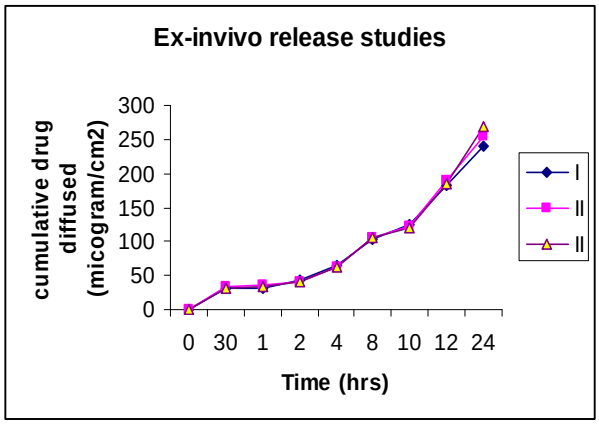

Fig no: 1 Ex-invivo diffusion studies

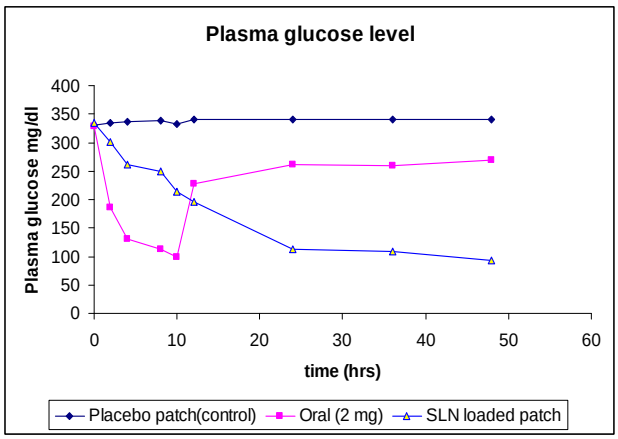

Fig no: 2 Plasma glucose level in diabetic rats

Hypoglycemic effects

The reports showed decreased blood glucose level by using transdermal patches loaded SLN when compared with oral administration of Repaglinide (2mg) in both normal and diabetes rats. The time course profile of blood glucose response in rats were shown in table no: 1 & fig no:2. The blood glucose level in normal rats drastically reduced in orally administered drug upon 10 hrs and was reported as 54.08 ± 0.22 mg/ dl and 35.40 ±0.04 mg/dl for 48 hrs in the case of transdermal patches containing SLN. In STZ induced diabetes rats, the blood glucose level gradually reduced upto 98.48 mg/dl at t=10 hrs from 328.67 mg/dl. Transdermal patches containing SLN produced maximum drop of blood glucose at 92.74 mg/dl at 48 hrs. Neither placebo patch applied showed hypoglycemic effect.

Table no: 1 Blood glucose level in rats

Time (hrs)	*Normal rats (mg/dl)*			*Diabetes rats (mg/dl)*		
	Placebo patch(control)	*Oral (2 mg)*	*SLN loaded patch*	*Placebo patch(control)*	*Oral (2 mg)*	*SLN loaded patch*
0	84.17±0.12	84.02±0.34	83.98±0.24	331.67±0.02	328.67±0.10	334.67±0.10
2	85.33±0.32	70.60±0.82	80.16±s0.76	334.33±0.02	185.42±0.32	301.18±0.62
4	85.17±0.22	68.12±0.96	77.00±0.14	336.10±0.14	130.33±0.40	262.27±0.02
8	85.92±0.53	60.42±0.16	71.18±0.38	338.67±0.12	112.60±0.62	249.00±0.22
10	85.04±0.72	54.08±0.22	69.66±0.38	332.45±0.98	98.48±0.60	212.92±0.60
12	85.86±0.24	72.18±0.42	65.20±0.72	340.10±0.22	228.10±0.34	196.68±0.02
24	85.10±0.41	76.92±0.44	52.38±0.34	340.48±0.62	261.87±0.10	112.34±0.06
36	84.98±0.92	77.64±0.10	40.42±0.04	339.62±0.74	259.44±0.22	108.62±0.88
48	85.62±0.74	78.06±0.04	35.40±0.04	340.09±0.02	269.19±0.02	92.74±0.02

Valves are expressed as mean ± SEM, n=4, $p<0.05$.

DISCUSSION

Since transdermal delivery of Repaglinide SLN showed prominent result suggesting that SLN could penetrate through stratum corneum, epidermis and dermis to reach the blood circulation. The penetration was due to utrafine particles size of SLN and its lipid content. The phospholipids vesicles could penetrate rapidly when compared to other vesicles.

This study demonstrated the significant quantity of Repaglinide can be delivered into blood stream from a single transdermal SLN patch over extended period of time. The ex in vivo permeation studies predicted that the high cumulative amount of drug permeated by using SLN made by mixture of lecithin and cephalin. In vivo experimentation proved that SLN permeation from transdermal patch was more at 48 hrs compared to initial hours.

It was reported that dropping of blood glucose level were prolonged by transdermal patches upto 48 hrs. The slow and sustained hypoglycemic response could be due to slow release of drug from SLN. In orally treated group, the hypoglycemic effect was reduced upto 10 hrs, which could be due to its short biological half life. The oral route produced severe hypoglycemia in initial hours, whereas there was no such effect in the case of transdermal patches. The pharmacokinetic parameters obtained with transdermal SLN patches were significantly ($p<0.05$) different from orally treated group. This could be a fast absorption and short half life. Whereas SLN through transdermal route showed slow release of drug from lipid vesicles and maintained peak plasma concentration over a prolonged period. Transdermal SLN system would also protect the formulation from dehydration and accidental damage, leakage of drug from SLN.

CONCLUSION

This solid lipid nanoparticles system successfully delivered Repaglinide transdermally, as evidenced by a significant sustained decrease in blood glucose in normal rat and those with diabetes. These result support the feasibility of developing transdermal repaglinide for human applications. The transdermal delivery of solid lipid nanoparticles contained Repaglinide was safety, economic and chronic delivery for diabetes patients.

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