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Research paper

Passive and iontophoretic permeation of glipizide

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Abstract

In vitro iontophoretic delivery of glipizide across the pigskin was investigated. The experiment was carried out at three different donor drug concentrations using cathodal iontophoresis (current density 0.5 mA cm^{-2}) with corresponding passive controls. At all concentration levels, iontophoresis showed enhanced permeation rate compared to passive controls ($P < 0.01$). For passive permeation, the steady-state flux significantly increased with the increase in donor drug concentration ($P < 0.01$). Passive process followed zero-order profile while the profile was nonlinear in iontophoresis. Competition by chloride ions released in the cathode compartment could be the reason. Flux enhancement was highest at the lowest drug load and lowest at the highest drug load. The target flux of glipizide was calculated to be 0. 4147 μmol h^{-1}. As the highest flux obtained was 0.2727 μmol cm^{-2} h^{-1}, it can be said that glipizide is a promising candidate for iontophoretic delivery.

Keywords: Glipizide; Iontophoresis; Pigskin; Current density; *In vitro*

1. Introduction

Clinical benefits, industry interest and regulatory precedence had predicted a strong market for transdermal drug products since the first batch was launched in 1981. Since then, a large number of drugs, especially the drugs of prolonged therapy had been screened for transdermal administration, though only few could be commercially exploited. Globally, the occurrence of diabetes is increasing at an alarming rate. A study shows that in 1990 it was the 16th leading cause of global mortality, accounting for 5,71,000 deaths [1]. Type-2 diabetes represents about 98% of all diabetes cases, in population older than 45 years of age. Medication-related problems leading to morbidity and mortality are quite common with the disease [2]. For diabetic patients, medication becomes an integral part of life and noncompliance of therapy may lead to chronic complications.

Glipizide is an oral hypoglycemic agent, used in the treatment of non-insulin dependent diabetes mellitus [3,4]. The drug has a plasma half-life of 3–5 h [3,5] and needs frequent administration. Moreover, its oral use is often associated with severe hypoglycemic symptoms like nausea, vomiting, heartburn, anorexia and increase in appetite [4]. Transdermal delivery can bypass the first pass metabolism and deliver the drugs in rate-controlled manner, which is desirable in anti-diabetic therapy [6]. Attempts have already been made to develop anti-diabetic drugs into transdermal dosage forms. In 1997, Takahshi and co-workers had investigated some sulfonylureas for transdermal administration and reported promising results [7]. Later, Mutalik and co-workers [8] had studied the skin-permeability of glipizide in mouse skin with the objective of its transdermal development. Ammar and co-workers had also attempted to enhance the permeation rate of glipizide using various enhancers [9]. This study investigated the iontophoretic permeability of the drug through excised pigskin to assess its potential for the development of a patient-controlled active transdermal system.

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2. Materials and methods

2.1. Materials

Hitachi high-performance chromatograph with a reversed phase Kromasil 300-4C-18, 10 μm column (150 × 4 mm internal diameter) equipped with Hitachi L-7110 pump, L-7400 UV detector and Winchrome-99 software was used. Iontophoretic direct current source (Model No. PSU 2510/Lab, digital display, current 0–10 mA, voltage 0–25 V) was purchased from C-Tech Mumbai, India. Iontophoretic diffusion cell was fabricated by Navin Scientific Glass Products, Bangalore.

Ethanol, methanol, sodium hydroxide, potassium dihydrogen orthophosphate, octanol and hydrochloric acid were obtained from S.D. Fine-Chem., Mumbai, India. Silver/silver chloride electrode was prepared as per the standard procedure [10]. Silver rods (99.99% pure, 1.0 mm thickness) were used as connecting wire. All the reagents/chemicals used were of analytical grade. Experiments were conducted with ultra pure water (resistivity 18.2 MΩ cm) obtained from Milli-Q academic system (Millipore Pvt. Ltd., Bangalore, India).

2.2. Preparation of skin membrane

From a local abattoir, ears were obtained from freshly slaughtered pigs. The skin was separated carefully from the underlying cartilage with a scalpel. After separating the full thickness skin, the fat adhering to the dermal side was removed using isopropyl alcohol. The average thickness was found to be 0.95 mm. Finally the skin was washed with tap water and stored at refrigerator in aluminum foil packing and was used within 2 days [11].

2.3. Procedure of passive permeation

The *in vitro* passive permeation studies were conducted using vertical type diffusion cell having a receptor compartment capacity of 10 ml. The excised skin was mounted between the half-cells with the dermis in contact with receptor fluid (phosphate buffer, pH 7.4) and was equilibrated for 1 h. The area available for diffusion was about 1.21 cm^2. The donor cell was covered with an aluminum foil to prevent the evaporation of vehicle. The fluid in the receptor compartment was maintained at 37 ± 0.5 °C. Under these conditions, the temperature at the skin surface was approximately 32 °C. Two milliliters of the glipizide suspension was placed in the donor compartment. The entire assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred continuously using a magnetic bead. Samples (1 ml) were withdrawn from the receptor compartment at hourly interval for a period of 8 h and assayed for drug content. Fresh phosphate buffer was added to replace the withdrawn sample volumes [4].

2.4. Procedure of iontophoretic diffusion

For iontophoresis, diffusion cell was modified as suggested by Glikfield et al. [12]. The apparatus consisted of a lower (receiver) compartment and two small upper compartments connected to the receiver through skin. The receiving chamber was large (25 ml) with two parallel ports on the top and a sampling port on the side. Two upper ports were actually cylindrical extension of receiving chambers which got converted into separate compartments when skin attached to the bottom of two small cylindrical glass tubes were slipped into them. The inner and outer tubes stayed attached by glass joints forming two separate chambers with skin as the base. Both the skin touched the receptor fluid at the same depth and each chamber housed one electrode. Donor solution (2 ml) was filled in one compartment and 2 ml of water in the other. Silver/silver chloride electrode was inserted into the donor compartment whereas silver plate was inserted into anodal chamber, which served as a return electrode. The assembly was put in a magnetic water bath (Spectralabs, Mumbai) to maintain the temperature at 37 ± 0.5 °C. Once the power supply was switched on, current flowed from anode to cathode and forces of electro-osmosis and electro-migration were established. Direct current (0.5 mA cm^{-2}) was used throughout the experiment. The receptor fluids (5 ml) were withdrawn at hourly interval and replaced with fresh buffer to maintain sink condition. The study was carried out for a period of 8 h. The samples were assayed by HPLC.

2.5. Solubility determination

In small glass vials phosphate buffer (PB) pH 7.4 and ethanol were taken in varying proportions and solubility was determined by making saturated solutions. The drug was gradually added till the solutions turned cloudy, indicating the presence of un-dissolved glipizide. The solutions were kept at rest for 24 h to assist the attainment of equilibrium with the un-dissolved drug particles. The supernatants were decanted and filtered through filter paper (Whatman No. 42) [13]. The filtrates were suitably diluted to measure the concentrations by HPLC.

2.6. Estimation of the drug

Glipizide was estimated by HPLC as suggested by Dhawan and co-workers [14]. For making standard graph, working standards were prepared in PB (5–80 μg ml^{-1}) and injected into the column (20 μl). The column was eluted with the mobile phase consisting of phosphate buffer (50 mM KH_2PO_4) and acetonitrile (65:35, adjusted to pH 3.5 by orthophosphoric acid) and the detection wavelength was 276 nm. Delivered at a flow rate of 1 ml min^{-1}, the retention time recorded was 14.67 min. The plots of peak area versus respective concentration of glipizide were found to be linear with the correlation coefficient (r) of 0.9989.

2.7. Partition coefficient

Octanol and PB were mutually saturated by shaking in a separating funnel, allowed to stand for 24 h and separated.

Standard solution of the drug was prepared in this pre-saturated PB. Octanol (10 ml) was added to equal volume of this standard drug solution in a separating funnel and was kept for 24 h at 37 °C with intermittent shaking. Finally, the PB layer was separated, clarified by centrifugation and assayed for drug content [13].

2.8. Data analysis

The cumulative amount permeated was plotted against time, and the slope of the linear portion of the plot was taken to be the steady-state flux. Permeability coefficient and diffusion coefficient were calculated using following formulas:

$$K_P = J_{SS}/C_d \quad (1)$$

$$D = K_P h/K \quad (2)$$

where K_P represents permeability coefficient, J_{ss} steady-state flux, C_d concentration of drug in donor compartment, D diffusion coefficient, K skin/vehicle partition coefficient and h thickness of the skin.

Flux enhancements were calculated by dividing iontophoretic steady-state flux with the corresponding passive steady-state flux.

2.9. Statistical analysis

To find the statistical significance of iontophoresis, two-way ANOVA was carried out using the concentration levels and process as the two parameters. The effect of concentration on steady-state flux was separately evaluated by one-way ANOVA followed by Bonferroni's test [15].

3. Results and discussion

Skin permeability of a drug is strongly influenced by its physicochemical parameters. According to Doh and coworkers [16], drug candidates for transdermal delivery should have molecular weight around 200–500 Da. Glipizide having molecular weight of 445 fits into the category but two of its properties, solubility and pK_a, are not favorable for transdermal permeation. Glipizide is slightly soluble in water and hence to deliver it at adequate concentration, the binary vehicle of PB–ethanol was used. In the composition of 30:70 (PB:ethanol), glipizide attained maximum solubility (Table 1). However, to ascertain the effect of donor concentrations on the permeation rate, studies were carried out at three concentration levels (1.4809, 7.2503 and 13.0528 μmol/mL^{-1}) for which PB:ethanol compositions were 80:20, 50:50 and 30:70, respectively.

The octanol–water partition coefficient is a measure of the relative lipophilicity of a compound. The experimentally determined partition coefficient (octanol/PB) was found to be 2.3122 ± 0.1316, which indicated that the drug had greater affinity towards the lipids compared to phosphate buffer. The results of skin/vehicle partition coefficients are depicted in Table 2. An apparent contradiction was noticed here. The system with highest drug concentration resulted in lowest partition coefficient and the system with lowest drug concentration showed highest partition coefficient. However, the lipophilicity of a drug moiety is an intrinsic character and cannot be changed. In this case also the skin's capacity to accommodate the drug was more or less the same but the concentration of the drug was different in different donor vehicles. Since the skin vehicle partition coefficient is the ratio of the drug concentration in the skin to that in the vehicle (after equilibrium), the vehicle with the higher drug concentrations resulted in lower partition coefficient values.

Table 1
Solubility of glipizide in different phosphate buffer-ethanol binary vehicles

Vehicle composition PB:ethanol	Solubility (μmol ml^{-1})			
	Trial 1	Trial 2	Trial 3	Mean ± SD
100:0	00.369	00.352	00.363	00.361 ± 0.008
90:10	00.660	00.631	00.574	00.621 ± 0.043
80:20	01.463	01.509	01.470	01.481 ± 0.025
70:30	02.673	02.431	02.711	02.605 ± 0.152
60:40	06.109	05.512	05.802	05.808 ± 0.298
50:50	07.222	07.460	07.069	07.250 ± 0.197
40:60	09.898	11.540	10.438	10.625 ± 0.836
30:70	13.893	12.302	12.963	13.053 ± 0.799
20:80	09.179	09.489	10.544	09.737 ± 0.715
10:90	06.681	07.152	06.842	06.891 ± 0.239
0:100	02.289	02.226	02.913	02.476 ± 0.379

At pH 7.4 mammalian skins are negatively charged and ionic forms have low affinity toward skin. Glipizide, being an acidic drug with the pK_a value of 5.9, was largely ionized [17] which reduced its affinity toward the skin [18]. In our initial study, when the drug was delivered from aqueous saturated solution (data not shown), intrinsic permeability was found to be very low. Because of poor aqueous solubility and ionization, high concentration gradient could not be developed which was the driving force of mass transfer in passive permeation. In iontophoresis, the ionized moieties are actively propelled through natural pore pathways of skin while the unionized fraction could pass the unbroken horny layers by passive permeation resulting in enhanced permeation [19]. To simulate the physiological condition, the diffusion cell was modified where both the

Table 2
Partition coefficient of glipizide from phosphate buffer-ethanol binary vehicles

Composition of vehicles PB:ethanol	Amount of drug in skin (μmol/cm^2)	Partition coefficients	
		Skin/vehicle	Octanol/vehicle
80:20	2.249 ± 0.235	18.399 ± 0.374	1.259 ± 0.102
50:50	3.417 ± 0.457	04.473 ± 0.136	0.387 ± 0.041
30:70	3.383 ± 0.103	02.590 ± 0.082	0.256 ± 0.033

Each point represents the mean ± S.D. of three observations.

electrodes were placed on the same side of skin. The receiving chamber with PB reflected the body.

Iontophoretic systems should have conductive medium, which supports the movement of ions. The binary vehicle PB:ethanol was conductive of electricity and supported the movements of ions. In all the systems the drug was delivered as suspension. For drugs of low solubility this is particularly necessary as the amount required for prolonged maintenance often exceeds the quantity that could be delivered as true solutions. In suspensions, the loss due to permeation is supplemented by the presence of solid drugs in the reservoir and thermodynamic activity could be maintained constant [20]. Moreover, thermodynamic activity is a function of percentage saturation in the vehicle and high thermodynamic activity results in higher partitioning into the stratum corneum [21]. Hence maximum fluxes can be achieved from suspension, which represents the highest saturation level.

Passive and iontophoretic permeation profiles of glipizide at different donor concentrations are shown in Figs. 1 and 2. The passive profiles were linear at all concentration levels indicating that the permeation kinetics was more or less of zero order. In the passive process both rate and extent of permeation increased with increasing donor drug concentration ($P < 0.01$). This was expected as increase in the donor drug concentration enhanced the concentration gradient, which was the driving force of mass transport [22]. In contrast, iontophoretic profiles were less linear indicating the involvement of multitude of factors. Raising the concentration from 1.48 to 7.25 μmol ml^{-1}, permeation rate increased ($P < 0.01$) but no significant increase was found when the concentration was raised to 13.05 μmol ml^{-1} ($P > 0.05$). This was in agreement with the hypothesis that increase in the drug concentration increases iontophoretic delivery up to a certain point, but at still higher concentrations, the flux may become independent of concentration [10]. In iontophoresis though ionic repulsion is the dominant force, convective flow of solutes toward the direction of current influences the permeation rate. Permeability of skin also changes under influence of current [23].

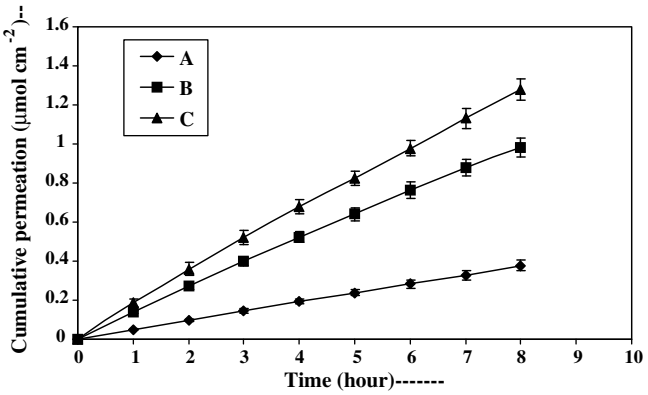

Fig. 1. Permeation profile of glipizide (passive) at different donor concentrations. Each point represents the mean ± S.E. of three observations. A (1.48 μmol ml^{-1}), B (7.25 μmol ml^{-1}) and C (13.05 μmol ml^{-1}) represent donor concentrations.

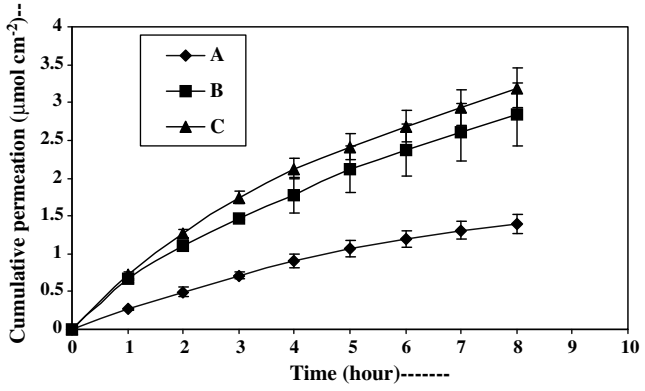

Fig. 2. Permeation profile of glipizide (iontophoresis) at different donor concentrations. Each point represents the mean ± S.E. of three observations. A (1.48 μmol ml^{-1}), B (7.25 μmol ml^{-1}) and C (13.05 μmol ml^{-1}) represent donor concentrations.

The total flux of a solute during iontophoresis is the sum of fluxes due to electro-repulsion, convective flow, and passive diffusion [19]. Glipizide (pK_a 5.9) at pH 7.4 acquires a negative charge due to ionization of sulfonyl group and was delivered from cathodal chamber. Since the isoelectric point of the skin varies between 3 and 4, at physiological pH, the volume flow was directed toward the cathode. Hence at pH 7.4, only passive and electro-repulsive fluxes were likely to contribute to the overall permeation. Electro-osmotic flow may even oppose the permeation from the cathodal compartment [24]. The iontophoretic profiles showed the initial permeation was high but the permeation rate declined in the later hours. This was unexpected as the voltage gradually dropped with time and hence the magnitude of electro-osmotic opposition was expected to be lesser in the later part of the study. The opposite result suggested the involvement of a factor that negatively influenced the permeation as time progressed. It is possible that during the passage of current, the cathodal electrode (Ag/AgCl) received a steady flow of elec-

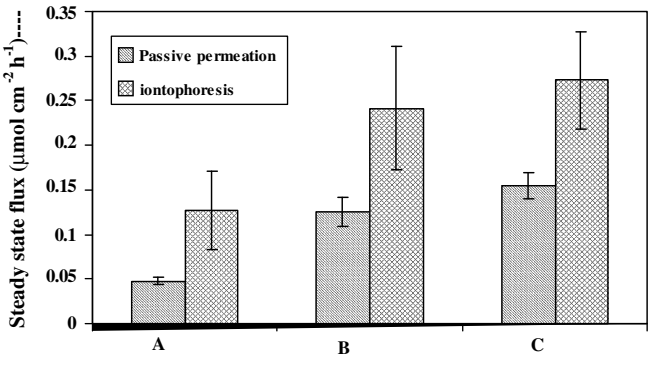

Fig. 3. Comparison of steady-state fluxes at different donor concentrations. Each point represents the mean ± S.D. of three observations. A (1.48 μmol ml^{-1}), B (7.25 μmol ml^{-1}) and C (13.05 μmol ml^{-1}) represent drug concentrations.

Table 3
Permeability and diffusion coefficients of glipizide in different systems for passive and iontophoresis

Donor concentrations (μmol/ml)	Permeability coefficient (cm h^{-1})		Diffusion coefficient (cm^2 s^{-1}) × 10^{-8}	
	Passive	Iontophoresis	Passive	Iontophoresis
01.481	0.0322 ± 0.0027	0.8595 ± 0.0436	48.6 ± 0.98	12.8 ± 0.11
07.250	0.0173 ± 0.0022	0.0333 ± 0.0687	10.7 ± 0.36	21.0 ± 0.17
13.053	0.0120 ± 0.0009	0.0280 ± 0.0546	13.2 ± 0.75	30.3 ± 0.59

Each point represents the mean ± S.D. of three observations.

Table 4
Steady-state flux and enhancement ratio in various systems

Donor concentrations (μmol/ml)	Steady-state flux (μmol cm^{-2} h^{-1})		Enhancement ratio (R)	Net benefit by iontophoresis (μmol cm^{-2} h^{-1})
	Passive	Iontophoretic		
01.481	0.0477 ± 0.0039	0.1273 ± 0.0436	2.6680	0.0796
07.250	0.1256 ± 0.0165	0.2417 ± 0.0687	1.9243	0.1161
13.053	0.1546 ± 0.0149	0.2727 ± 0.0546	1.7639	0.1181

Each point represents the mean ± S.D. of three observations.

tron, which resulted in the liberation of negatively charged chloride ions. As time progressed, the concentration of this newly released chloride ions was likely to increase in the cathodal compartment. Since the drug was negatively charged, chloride ions served as competitor. A chloride ion, being much smaller than the drug ion, was a powerful competitor, which reduced the transport efficiency of glipizide [25].

Fig. 3 depicts the enhancement in iontophoretic fluxes compared to the corresponding passive fluxes. Enhancement was highest at the lowest drug load and lowest at the highest drug load.

The values of permeation parameters are depicted in Table 3. Albeit steady-state flux is the most important and therapeutically relevant parameter and permeability coefficient is usually used for comparison purpose [26]. It was evident that the permeability coefficients decreased with increase in donor concentration.

The net benefit of iontophoresis over and above that of passive fluxes is depicted in Table 4. The iontophoretic contribution was found to be slightly more at higher donor concentrations. The varying proportion of phosphate buffer used in the different donor vehicles might be the reason. The buffer carrying negative charge PO_4^{2-} was a source of competitor co-ions for the drug. As the proportion of the buffer used was lesser at high donor concentrations, the drug ions vastly outnumbered the competitor co-ions. This might have resulted in higher electro-repulsive contribution. However, compared to passive permeations, iontophoretic permeations were significantly higher at all concentration levels ($P < 0.01$).

Glipizide is a potent medicine effective in low dosage range (5–15 mg). The noncompartmental analysis of the pharmacokinetic data indicated that 0.4147 μmol of drug must be supplied to the systemic circulation every hour to meet the demand of maintenance therapy for a 60 kg individual. As the highest flux obtained was 0.2727 μmol cm^{-2} h^{-1}, it can be said that glipizide is a promising candidate for iontophoretic delivery.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2008.01.010.

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