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Transdermal delivery of glibenclamide and glipizide: *in vitro* permeation studies through mouse skin

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The purpose of this investigation was to study the feasibility of transdermal delivery of glibenclamide and glipizide. *In vitro* permeation of these drugs was studied through mouse skin using various penetration enhancers like Tween®-20, polyethyleneglycol-400, ethanol and d-limonene by simultaneous application of drug and enhancer solution or by pretreatment of the skin with neat enhancer. The partition coefficient values indicated that both drugs partition well into the skin. Glipizide did not show any skin metabolism, while glibenclamide showed a minimal metabolism during *in vitro* skin metabolism studies. The flux values (μg/cm^2/h) of both drugs significantly ($p < 0.05$) increased in the presence of penetration enhancers. The glibenclamide flux values ranged from 1.39 ± 0.13 without enhancer, to 19.01 ± 2.14 in a combination of 50% ethanol and 5% d-limonene. Glipizide flux values ranged from 3.01 ± 0.74 without enhancer, to 62.97 ± 7.10 in a combination of 50% ethanol and 5% d-limonene. Skin retention and solubility of both drugs increased with all penetration enhancers compared to control. The target permeation rates for glibenclamide and glipizide were calculated to be 193.8 and 184.8 μg/h respectively. The present study showed that the target permeation rates for both drugs could be achieved with the aid of enhancers by increasing the area of application in an appreciable range.

1. Introduction

Sulfonylureas are an important class of compounds currently available for treating hyperglycemia in non-insulin dependent diabetes mellitus. They increase the release of endogenous insulin as well as its peripheral effectiveness [1]. Many sulfonylureas like glibenclamide and glipizide have been associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heart burn, anorexia and increased appetite after oral therapy [2, 3]. Since these drugs are usually intended to be taken for a long period, patient compliance is also very important [4]. Transdermal drug delivery offers many advantages such as reduced side effects, improved patient compliance, elimination of first-pass effect, sustained drug delivery and interruption or termination of treatment when necessary [5]. In the present study, we have investigated the feasibility of transdermal application of glibenclamide and glipizide.

2. Investigations, results and discussion

The apparent partition coefficient (APC) values of glibenclamide and glipizide are listed in Table 1. Various systems like n-octanol/phosphate buffer, pH 7.4 (PB), isopropylmyristate (IPM)/PB, full thickness skin/PB and stratum corneum/PB were used to relate partition coefficients to transdermal permeation of drugs. The APC values are a good indication that both drugs partition well into the skin (log APC values of octanol/PB for glibenclamide and glipizide are 0.32 and 0.36, respectively) [6]. To some extent, the APC in octanol/PB and IPM/PB systems reflect the partitioning of the drugs into the intercellular spaces and into cells respectively. A comparatively high APC value in octanol/PB system indicates that both drugs permeate across the skin, possibly through an intercellular pathway [7]. Frequently, the skin is viewed as a bilayer carrier with a lipid horny layer in series with the aqueous viable tissue of the dermis-epidermis layer [8]. The comparison between the amount of drug partitioned between full thickness skin/stratum corneum and PB shows that both drugs have a stronger affinity to the lipid stratum corneum than to aqueous tissue.

The Fig. shows the stability of glibenclamide and glipizide in PB alone and epidermal, dermal and skin extracts. No significant metabolism was observed for glipizide during *in vitro* skin metabolism studies upto 8 h in any of the extracts. Glibenclamide was stable in epidermal and dermal extracts; but showed a minimal yet significant metabolism in skin extract (about 14.08 ± 1.98 in 8 h, $p < 0.05$). However in some studies, *in vitro* skin metabolism of drugs has been studied only upto 2 h [9] during which both drugs were highly stable. In an earlier study, a therapeutic concentration required to satisfactorily reduce blood glucose level was attained after topical application of glibenclamide gel [10]. Accordingly, in the present study a sufficient flux of glibenclamide required to achieve the target permeation rate is observed. Therefore, metabolism will not affect the transdermal application of glibenclamide and both drugs can be potential candidates for transdermal delivery.

The intrinsic transdermal permeation rate of glibenclamide and glipizide from the aqueous saturated solution was not adequate to meet respective target permeation rates. Therefore, several common and safe enhancers like Tween®-20, Polyethyleneglycol-400 (PEG), ethanol and d-limonene have been used to improve the penetration of drugs [11–16]. Table 2 and 3 lists the permeation parameters, drug retention in the skin and solubility studies of drugs in the first set (simultaneous application of drug and enhancer solution) and in the second set (pretreatment of the skin with neat enhancer) of experiments respectively. In the first set

Table 1: Apparent partition coefficients of glibenclamide and glipizide in different systems

System	Apparent partition coefficients	
	Glibenclamide	Glipizide
n-Octanol/PB	2.11 ± 0.63	2.30 ± 0.18
IPM/PB	0.50 ± 0.05	0.28 ± 0.12
Full thickness skin/PB	0.13 ± 0.01 mg/g	0.14 ± 0.01 mg/g
Stratum corneum/PB	0.56 ± 0.02 mg/g	0.64 ± 0.01 mg/g

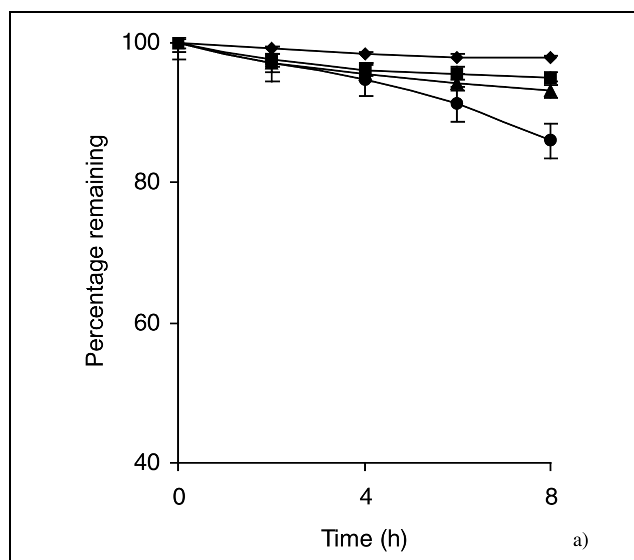
All values are expressed as mean ± SE; n = 3–6
PB: Phosphate buffer, IPM: Isopropylmyristate

Table 2: Permeation parameters, drug retention in the skin and solubility results of glibenclamide and glipizide in the presence of penetration enhancers

Penetration enhancers	Drug	J_{Max} (μg/cm²/ h)	Permeability coefficient (cm/h)	Amount of drug in skin (μg/mg)	Solubility (mg/ml)
Control	GLB	1.39 ± 0.13	0.138 ± 0.048	0.18 ± 0.07	0.010 ± 0.001
(PB alone)	GPZ	3.01 ± 0.74	0.018 ± 0.002	1.46 ± 0.23	0.165 ± 0.010
Tween-20 5%	GLB	1.76 ± 0.57	0.125 ± 0.023	0.25 ± 0.09	0.014 ± 0.001*
	GPZ	8.92 ± 2.62*	0.042 ± 0.005*	1.58 ± 0.18	0.212 ± 0.011*
PEG 5%	GLB	1.84 ± 0.49	0.120 ± 0.008	0.29 ± 0.10	0.015 ± 0.002*
	GPZ	9.05 ± 0.45*	0.039 ± 0.002*	1.82 ± 0.27	0.230 ± 0.021*
Ethanol 5%	GLB	2.05 ± 0.67	0.118 ± 0.007	0.38 ± 0.10	0.017 ± 0.002*
	GPZ	10.66 ± 1.73*	0.026 ± 0.002*	2.01 ± 0.14	0.371 ± 0.005*
Ethanol 50%	GLB	12.91 ± 2.34*	0.008 ± 0.001	2.14 ± 0.25*	1.678 ± 0.650*
	GPZ	51.66 ± 5.20*	0.015 ± 0.001	4.37 ± 0.44*	3.450 ± 0.04 0*
d-Limonene 5% +	GLB	19.01 ± 2.14*	0.008 ± 0.001	2.91 ± 0.22*	2.145 ± 0.370*
Ethanol 50%	GPZ	62.97 ± 7.10*	0.006 ± 0.001*	4.84 ± 0.33*	10.04 ± 0.080*

All values are expressed as mean ± SE; n = 3. * $p < 0.05$ compared to control
J_{Max}: maximum flux, PB: phosphate buffer, GLB: glibenclamide, GPZ: glipizide

of experiments, 50% ethanol and a combination of 50% ethanol and 5% d-limonene significantly ($p < 0.05$) increased the flux of glibenclamide compared to the average flux from the control (without enhancer). The fluxes of glipizide from all penetration enhancer solutions of the drug were significantly higher ($p < 0.05$) than that obtained from the control. In the second set of experiments all the enhancers significantly ($p < 0.05$) increased the flux of glibenclamide and glipizide compared to respective controls. For both drugs, the flux values were slightly higher in the second set of experiments where the skin was pretreated with neat enhancer. These observations are in accordance with the earlier findings and clearly demonstrate that both experimental designs lead to similar results [17]. The activity of enhancers increased in the following order: Tween-20, PEG, ethanol and d-limonene. Tween-20 and PEG exhibited weaker permeation enhancing effect than ethanol and d-limonene. These results are in accordance with earlier findings where Tween-20 and PEG failed to achieve high flux values of drugs [11, 12]. Tween-20 acts as skin permeation enhancer only for the most hydrophilic compounds [12]. Glibenclamide and glipizide are lipophilic which accounts for the low flux values observed with Tween-20. Similarly PEG is also hydrophilic; hence PEG could not achieve high flux values either. Ethanol has been observed to increase the permeation of a wide range of drugs through human and animal skin when used as a part of a cosolvent system with water. Therefore, higher flux values were observed with ethanol in comparison to Tween-20 and PEG [13, 18]. d-Limonene, a hydrocarbon terpene, has been re-



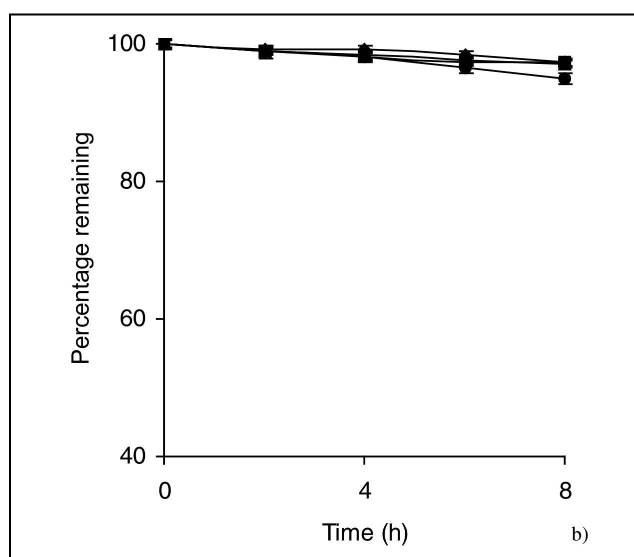


Fig.: *In vitro* skin metabolism of a) glibenclamide and b) glipizide. (—◆— phosphate buffer, —■— epidermis extract, —▲— dermis extract and —●— skin extract)

Table 3: Permeation parameters of glibenclamide and glipizide following pretreatment of skin with neat enhancer

Penetration enhancers	Drug	J_{Max} (μg/cm²/ h)	Permeability coefficient (cm/h)
Control	GLB	1.49 ± 0.13	0.148 ± 0.048
(PB alone)	GPZ	3.01 ± 0.74	0.018 ± 0.002
Tween 20	GLB	3.36 ± 0.60*	0.333 ± 0.020*
	GPZ	9.20 ± 0.43*	0.056 ± 0.001*
PEG	GLB	3.57 ± 0.68*	0.347 ± 0.025*
	GPZ	10.46 ± 0.84*	0.063 ± 0.001*
Ethanol	GLB	4.01 ± 0.44*	0.400 ± 0.002*
	GPZ	11.69 ± 0.65*	0.071 ± 0.001*
d-Limonene	GLB	7.26 ± 0.76*	0.725 ± 0.002*
	GPZ	15.08 ± 1.57*	0.091 ± 0.002*

All values are expressed as mean ± SE; n = 3. * $p < 0.05$ compared to control
J_{Max}: maximum flux, PB: phosphate buffer, GLB: glibenclamide, GPZ: glipizide

ported to provide higher penetration enhancing activity for lipophilic drugs and accordingly, flux values with d-limonene are high compared to the other enhancers tested [19]. Among the two sets of experiments, higher flux values were observed with 50% ethanol alone and a combination of 50% ethanol and 5% d-limonene. Berner et al reported a linear relationship between the skin permeation of nitroglycerin and the transdermal flux of ethanol, which implies that ethanol penetrates through the skin and changes the permeation properties of the skin. It is also suggested that when less than 70% ethanol is used, permeation of both ethanol and drug is enhanced by hydration of the entire stratum corneum [13, 18]. As 50% v/v ethanol is used in the present study, the local concentration of ethanol would probably be high in the stratum corneum and viable tissues, which may have led to an increase in the solubility of drugs in the skin. This would alter the partitioning of drugs between vehicle and the stratum corneum (and perhaps the viable tissues) causing an increase in drug flux. Some researchers have demonstrated the formation of new pores in the stratum corneum at higher ethanol fractions [13, 20].

The combination of 50% ethanol and 5% d-limonene showed higher flux values for both drugs than 50% ethanol alone. Similar results were reported by Obata et al., who attributed the higher enhancing activity for the permeation of diclofenac by limonene to its higher thermodynamic activity in 40% ethanol-buffer solution [21].

The effect of permeation enhancers was further evaluated by calculating the permeation coefficients. In the first set of experiments, the increase in the flux values was associated with a decrease in the permeation coefficients. This observation is in accordance with the earlier one, where low permeation coefficients were found with testosterone and its derivatives when the concentration of ethanol was increased [13].

In the present study, gibenclamide showed less skin permeation than glipizide. A lower drug solubility or lower diffusion coefficient in the barrier and cutaneous metabolism of glibenclamide might be responsible for this observation.

Skin retention of both drugs was found to be significantly high ($p < 0.05$) with 50% ethanol alone and a combination of 50% ethanol and 5% d-limonene. High transdermal flux generally results in retention of larger quantity of drug in the skin [22]. In a previous report the skin concentration after topical application of piroxicam was related to its flux across the skin [23]. The present results also indicate that the amount of the drug retained in the skin is related to the transdermal flux.

Glibenclamide and glipizide showed low solubility in PB; but the addition of penetration enhancers (5% v/v) increased the solubility significantly ($p < 0.05$). In the present study, drug suspensions have been tested and hence drug solubility might not play any role on permeation enhancement as maximum thermodynamic activity is reached as soon as the solubility limit is exceeded. Therefore the penetration enhancing effect could mainly be attributed to the alteration of skin properties by enhancers which is further supported by the results of second set of experiments. Tween-20 has been reported to extract the lipids from the skin, thus modifying the composition of the membrane and favoring the permeation [24]. The permeation enhancing effect of ethanol might result from the formation of pores in the stratum corneum, conformational changes of keratanized protein and partial lipid extractions [11]. Terpenes enhance the percutaneous absorption by increasing the stratum corneum lipid fluidity and perturbing the barrier integrity of epidermis [25].

The target skin permeation rate for both drugs was calculated by the following eq. [26] using available pharmacokinetic data [27, 28]:

$$J \cdot A = Cl \cdot C_p \cdot W$$

where J is the flux, A is the area of application, C_p is plasma concentration (170 and 110 μg/l for glibenclamide and glipizide, respectively) and W is the weight of the subject (60 kg). Clearance rate, Cl, (0.019 and 0.028 l/h/kg for glibenclamide and glipizide, respectively) was calculated using the equation [26]

$$t_{1/2} = (\ln 2V)/Cl$$

where $t_{1/2}$ is the half-life (10.5 and 4.85 h for glibenclamide and glipizide, respectively) and V is the volume of distribution (0.3 and 0.2 l/kg for glibenclamide and glipizide, respectively).

The target permeation rates for glibenclamide and glipizide were calculated to be 193.8 and 184.8 μg/h, respectively. The flux values obtained with the aid of enhancers indicate that the target permeation rates for both drugs can be achieved (especially with 50% ethanol or a combination of 50% ethanol and 5% d-limonene) within an appreciable range of application area.

The present study shows that glibenclamide and glipizide are suitable candidates for transdermal delivery. Based on the permeation parameters, higher concentrations of ethanol with or without d-limonene, may serve as a better system for transdermal delivery of both drugs.

3. Experimental

Glibenclamide and glipizide were gifts from Bal Pharma (P) Ltd., India. d-Limonene was purchased from E. Merck, Schuchardt. All the other chemicals used were of reagent grade. Membrane for the permeability studies was the dorsal section of full thickness skin from Swiss albino mice, 6–8 weeks old, whose hair had been previously removed with an electric clipper. Stratum corneum was prepared from the full thickness skin [9].

3.1. *Apparent partition coefficient determination*

The apparent partition coefficients (APC) of the drugs in n-octanol/PB and IPM/PB systems were determined by the method of Wells [29]. The APC of the drugs in full thickness skin/PB and stratum corneum/PB systems was determined by placing the skin (about 300 mg) or stratum corneum (about 50 mg, from 300 mg of skin) in a vial containing 10 ml of aqueous drug solution. The vial was gently tumbled for 24 h following which the concentration of drug in the aqueous solution was determined [8].

3.2. *In vitro skin metabolism of drugs*

The *in vitro* skin metabolism of glibenclamide and glipizide was performed in epidermis, dermis and skin extracts by the method described earlier [13]. Briefly, freshly excised mouse skin was mounted between the two half cells, with stratum corneum facing the donor half-cell and the dermis facing the receptor half-cell. Both donor and receptor compartments were filled with PB and epidermis and dermis extracts were separately collected after 24 h. Skin extract was prepared from freshly excised skin added to ten fold PB and homogenized for 15 min in an ice bath. The supernatant was obtained after centrifugation for 20 min at 9000 g. The drug solutions in epidermis, dermis and skin extracts were placed in a shaking water bath (50 rev/min). At different time intervals, the concentration of drug in each solution was measured considering the initial concentration of drug as 100%. The study was also carried out with phosphate buffer (PB) alone as control.

3.3. *In vitro skin permeation studies*

In vitro skin permeation studies were conducted using vertical type diffusion cells having a receptor compartment capacity of 20 ml. The excised skin was mounted on the diffusion cell and the receiver was filled with 20 ml PB. Three ml drug suspension in PB, with or without 5% v/v penetration enhancer, was placed in the donor compartment and sealed with Parafilm®. Sample solution from the receptor compartment was withdrawn

at regular intervals and assayed. For d-limonene, the donor compartment contained 3 ml drug suspension in PB containing 5% v/v d-limonene and 50% v/v ethanol. Fifty percent ethanol was used to solubilise d-limonene [25]. A permeation study with 50% v/v ethanol alone was also conducted. In order to confirm the permeation activity of enhancers, a second set of experiments was conducted [17]. The skin was mounted on the diffusion cell. This time, 100 μl of the appropriate penetration enhancer alone was applied to the skin for 2 h. Subsequently the residual enhancer was removed from the skin and drugs were applied as aqueous enhancer free suspensions. The experiment was then continued as in the first set. Flux and permeability coefficients were calculated [25]. At the end of the permeation experiment, the amount of the drug retained in the skin was determined [30].

3.4. Solubility studies

An excess amount of drug was added to PB, with or without 5% v/v penetration enhancer. The solution was immersed in a shaking water bath and allowed to equilibrate. After 24 h, the saturated solution was assayed after appropriate dilution with PB [13].
In all the experiments, the drug concentration in aqueous solution was determined spectrophotometrically at a maximum wavelength of 300 and 274 nm for glibenclamide and glipizide, respectively, after filtering through a 0.45-μ membrane (Nulge Nunc, UK). Statistical significance was analyzed by Student's t-test. Difference below the probability level 0.05 was considered to be statistically significant.

Acknowledgements: Authors are grateful to CSIR, India for providing Senior Research Fellowship to Mr. S. Mutalik. They are thankful to Dr. J. C. Jagetia, Head, Radiobiology, KMC, Manipal for providing some laboratory facilities and Bal Pharma, India for supplying glibenclamide and glipizide as gift samples. They are also thankful to Chetana M, Sulochana B and Merriline SM for help and cooperation.

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Received April 2, 2002
Accepted June 29, 2002

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