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EFFECT OF VEHICLE AND DRUG CONCENTRATION ON TRANSDERMAL DELIVERY OF DIHYDROERGOTAMINE USING EXCISED ANIMAL SKIN

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ABSTRACT

Dihydroergotamine (DHE) is widely used in the treatment of migraine as I.M. injection of 1.0 mg/ml. Absorption of DHE averaged 23% when taken orally and the drug is subjected to extensive first-pass effect. The physico-chemical and pharmacokinetic characteristics of DHE such as small dose, low M.W. and extensive hepatic metabolism, suggests that this drug is a possible candidate for transdermal delivery.

The purpose of this study was to investigate the effect of vehicle and dose variation on the percutaneous absorption of DHE. In-vitro diffusion studies were conducted utilizing improved Franz diffusion cells. The rabbit skin obtained from the dorsal area was employed as a barrier membrane.

The percutaneous absorption of DHE was measured after application of 16.0 mg/ml of DHE solution from 5 different vehicle: propylene glycol, water, polyethylene glycol 400, liquid paraffin and glycerin. It was found that propylene glycol was the most effective medium for the transdermal formulation of DHE. The amount of the drug absorbed during the 24-hours period from the propylene glycol base was considerably larger than that absorbed from any other vehicle used in the study. Water was the slowest delivering medium for the drug. The data showed that as the dose of DHE applied to the skin increased, the amount of the drug permeated through the membrane also increased which suggests passive diffusion mechanism for the percutaneous absorption of DHE.

INTRODUCTION

Transdermal drug delivery has gained increased popularity in the past decade (1-6), possibly due to the successful introduction, in July 1981, of Transderm-Scoplamine (7) for prevention of motion sickness. One year later, three transdermal therapeutic systems of nitroglycerine for prevention and/or treatment of angina pectoris successfully received FDA approval for marketting (8). Recently, transdermal therapeutic systems of colonidine for treatment of hypertension (9) and oestradiol for treating menopausal symptoms (10) were approved for marketing. Currently, a number of other drugs such as the antihistaminic azatadine (11), the β-blockers timolol

(12) and Bupranolol (13), the analgesic fentanyl (14), and testosterone, for testosterone deficiency in hypogonadal men, (15) are undergoing investigation as possible candidates for transdermal delivery.

Transdermal therapeutic systems are designed to achieve a continuous and controlled absorption of the drug to the systemic circulation without passing through the liver where extensive metabolism of the drug can take place. In addition, such dosage form will reduce the adverse action caused by the dose dumping effect which often occurs after oral and parenteral dosing.

Dihydroergotamine (DHE) mesylate, 9,10-Dihydro-12-hydroxy-2-methyl-5 α-benzyl-ergotaman-3,6,18-trione methanosulphonate ($C_{33}H_{37}N_5O_5$, CH_3SO_3H=677.8) is an alpha adrenergic blocking agent with a direct stimulating effect on the smooth muscle of the peripheral and cranial blood vessels (16). Due to this effect, DHE is widely used in the prevention and/or treatment of migraine as an I.M. injection of 1 mg/ml (17, 18). Absorption of DHE ranged from 20-53% when taken orally, but 96% of the orally absorbed dose was subjected to first-pass effect (19). The mean apparent half-life of DHE was 2.37±0.29 hours (17).

In view of the physicochemical and pharmacokinetic characteristics of DHE (e.g., small dose, low M.W., lipid solubility and an extensive first-pass effect), it seems that there is potential for investigating the feasibility of incorporating DHE into a transdermal therapeutic system.

The importance of the vehicle on percutaneous absorption is well documented (20-23). No vehicle can completely force a drug to go through the skin if for physicochemical reasons the drug can not penetrate through the membrane. However, for drugs with some potential for skin penetration, the vehicle can play an important role in transdermal delivery of drugs by changing the affinity of the drug for the vehicle and/or altering the property of the membrane. As a result, partitioning of the drug into the skin and consequently the rate of transport may be increased.

In the present study the transdermal delivery of DHE from 5 different vehicles: glycerin, liquid paraffin, polyethylene glycol (PEG 400), propylene glycol and water was measured, in order to seek the most appropriate vehicle for the transdermal formulation of DHE. In addition the effect of concentration as a factor controlling the rate of percutaneous absorption was also investigated.

MATERIALS

Dihydroergotamine mesylate (Sandoz Pharmaceuticals, E. Hanover, N.J., U.S.A.), glycerin (Riedel-Dehaen AG, Seelze-Hannover, F.R.G.), liquid paraffin (Hopkin and Williams Ltd., Essex, England), polyethylene glycol 400 (Fluka AG, Buchs, Switzerland), propylene glycol (Fisher Scientific Company, Fair Lawn, N.J., U.S.A.), propylhydroxy-4-benzoate (E. Merck AG, Dermstadt, F.R.G.), sodium chloride, glycine, and hydrochloric acid (BDH Chemicals Ltd., Poole, England)

were used without further purification. Methanol (E. Merck AG, Dermstadt, F.R.G.) and acetonitrile (BDH Chemicals Ltd., Poole, England) were HPLC grade.

METHODS

Preparation of rabbit skin:

Male white New Zealand rabbits weighing 3.0-3.5 Kg were selected. After sacrificing the animal, the skin was carefully removed leaving the fat tissue behind. The hair was clipped (Daito Electric Machine Ind. Co. Ltd., Japan) as close as possible to the skin without damaging it. The skin was examined under a high-powered magnifying lens for damage or diseased conditions. The skin in which the barrier was disrupted was not used in the study. Then, the dorsal area of the skin was immediately cut into pieces (5X10 cm), wrapped in freezer bags and stored in a freezer. Before starting an experiment, the frozen skin sample was thawed at room temperature and washed with warm water.

In-vitro diffusion technique:

In-vitro diffusion studies were carried out utilizing improved Franz diffusion cells (Crown Glass Company, Somerville,N.J.,U.S.A.). The cell consists of the cap (donor compartment), body (receptor compartment), clamps and a stirring bar (Figure 1). The donor compartment had a volume of 15.0 ml, and an effective permeation area of 3.14 cm^2. The skin was tightly secured between the receptor and

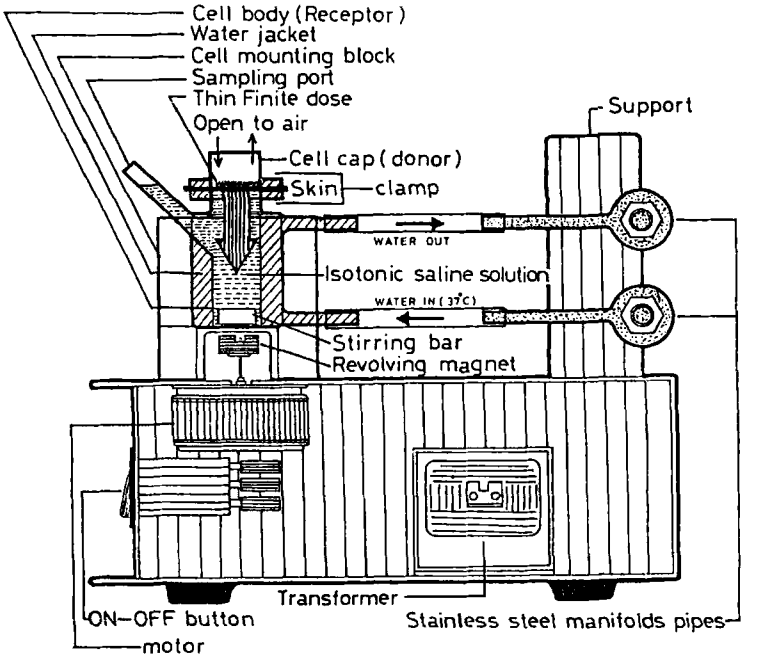

FIGURE 1

Schematic diagram of Franz diffusion cell drive console.

the donor compartments. Any excess of the membrane was trimmed using scissors and forceps. The receptor compartment was filled with isotonic saline solution. The temperature in the receiving compartment was maintained at 37°C by thermostatically controlled water enters the lower part of the water jacket surrounding the cell body and circulates through the upper part. The temperature of the liquid bathing the membrane was maintained by the agitating action of teflon-covered magnetic stirring bar. The cell cap was open to

the air, exposing the epidermis to the ambient condition of laboratory environment and allowing quick application of the dose to the skin. Samples were collected through the sampling port. Nine of these cells were inserted in 9-clear acrylic mounting blocks which allow viewing of dynamics within the cell in especially designed Franz diffusion cell drive console. The console is connected to a constant temperature water pump (Haak Company, Frankfurt, F.R.G.) by stainless steel manifold pipes which distribute and remove warm water in continuous circulation through the water-jacket surrounding each cell. Each cell mounting is equipped with a single-speed (600 rpm) magnet driving motor which drives the stirring bar placed in the diffusion cell.

Permeation studies:

To determine the effects of vehicle on penetration of DHE through the rabbit skin and to find the most effective medium for the transdermal formulation of DHE, several vehicles including propylene glycol, glycerin, water, propylene glycol 400, and liquid paraffin were tested as the medium for the formulation of DHE.

One hundred sixty milligrams of accurately weighed DHE mesylate were added to each of 10.0 ml volumetric flasks containing different vehicles. The final concentration of DHE solution in each vehicle was 16.0 mg/ml, from which 1.0 ml was applied to the skin in the donor compartment. One cell was used as a reference where 1.0 ml of drug-free vehicle was applied to the skin. Two ml samples from the

receptor phase were withdrawn from each cell at 3, 6, 9, 12 and 24 hours after initiating of the experiment and filtered through 0.45 μm Millipore filter, type HA (Millipore Corporation, Bedford, MA, USA). The sample taken for the assay from the diffusion cells was immediately replaced by fresh isotonic saline solution. Care was taken to avoid air bubbles remaining under the skin. Samples were sometimes diluted as necessary and assayed for DHE.

In order to determine the effect of DHE concentration on its percutaneous absorption, and to find the optimum concentration of DHE which could be used in the transdermal dosage form, four experiments were carried out using four different concentrations of DHE solutions (8.0, 16.0, 30.0 and 50.0 mg/ml) in the propylene glycol base. Experimental procedures were kept the same as described earlier.

Assay method for DHE:

Concentration of DHE in the various samples were determined using an HPLC assay method previously developed in our laboratory (24).

RESULTS AND DISCUSSION

The permeation data of DHE obtained from different vehicles are presented in Table 1 and 2. The average amount of DHE which penetrated the skin from water base during the 24 hours was found

TABLE 1

Amount of DHE Penetrated through the Skin from Different Vehicles

Time Interval (hrs)	DHE in μg* ±S.E.+ which penetrated skin				
	Propylene Glycol	PEG 400	Glycerin	Liquid Paraffin	Water
0 - 3	0.25±0.02	0.12±0.02	0.12±0.01	0.22±0.06	0.04±0.01
3 - 6	0.33±0.03	0.37±0.10	0.20±0.08	0.25±0.07	0.04±0.01
6 - 9	0.35±0.04	0.37±0.03	0.24±0.02	0.96±0.18	0.043±0.002
9 - 12	0.63±0.19	0.53±0.15	0.24±0.03	0.79±0.05	0.09±0.01
12 - 24	5.69±1.70	1.68±0.41	0.47±0.17	1.92±0.47	0.09±0.007
0 - 24	7.25±1.80	3.05±0.29	1.27±0.26	4.14±0.56	0.3 ±0.03

* Average of four cells.

\+ Standard error (S.E.).

to be 0.3 μg and the maximum rate of absorption was 0.02 μg/hr and occurred during the 9-12 hours time interval. Around 1.27 μg of DHE delivered through the skin during 24-hr after application of glycerin as medium containing the drug with a maximum rate of 0.07 μg/hr achieved during 9-12 hours time interval. The absorption rate of DHE from PEG 400 reached the maximum rate of 0.13 μg/hr

TABLE 2

Comparison Between Percutaneous Absorption Rate of DHE from Different Bases

Midpoint of Time Interval (hrs).	Cumulative Rate of Percutaneous Absorption* DHE µg/hr ± S.E.				
	Propylene Glycol	PEG 400	Glycerin	Liquid Paraffin	Water
1.5	0.08±0.01	0.04±0.008	0.04±0.003	0.07±0.02	0.01±0.004
4.5	0.10±0.01	0.08±0.02	0.05±0.01	0.08±0.003	0.01±0.003
7.5	0.10±0.006	0.09±0.01	0.06±0.01	0.11±0.02	0.01±0.002
10.5	0.13±0.02	0.12±0.02	0.07±0.008	0.07±0.004	0.02±0.002
18.0	0.30±0.08	0.13±0.01	0.05±0.01	0.08±0.01	0.01±0.001

* Average of four cells.

during 12-24 hours time interval and the mean total amount of DHE transported through the skin was about 3.05 µg. The amount of DHE penetrated the skin using liquid paraffin as base was found to be 4.14 µg during the 24-hr time period with a maximum rate of 0.11 µg/hr achieved during 6-9 hours time interval. A total of 7.25 µg of DHE permeated through the skin during the 24 hrs from the propylene glycol base. The highest rate of penetration from propylene glycol was 0.3 µg/hr, which occurred during the 12-24 hrs time interval.

Figure 2 is histogram illustrating the amount of DHE delivered through the skin in 24 hrs from different vehicles. The cumulative

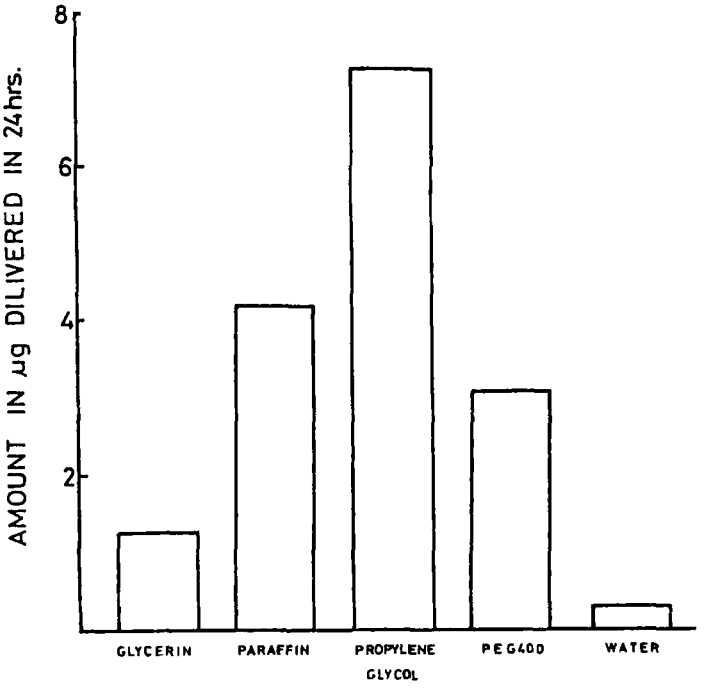

FIGURE 2

Histogram showing the effect of different vehicles on the penetration of DHE through rabbit skin.

rates of DHE absorption observed for: water, glycerin, PEG 400, liquid paraffin and propylene glycol are plotted against the mid points of the sampling intervals in Figure 3. From the histogram and the rate profiles drawn in these figures it is clear that the highest permeation was obtained when propylene glycol was used as medium for the transdermal formulation of DHE. Water was the slowest delivering medium for the drug. The amount of DHE transported through the skin from PEG 400 during the 24 hrs (3.05 µg) was about 2-fold more than the amount delivered using glycerin as vehicle for the drug. However, the total amount of DHE crossed the

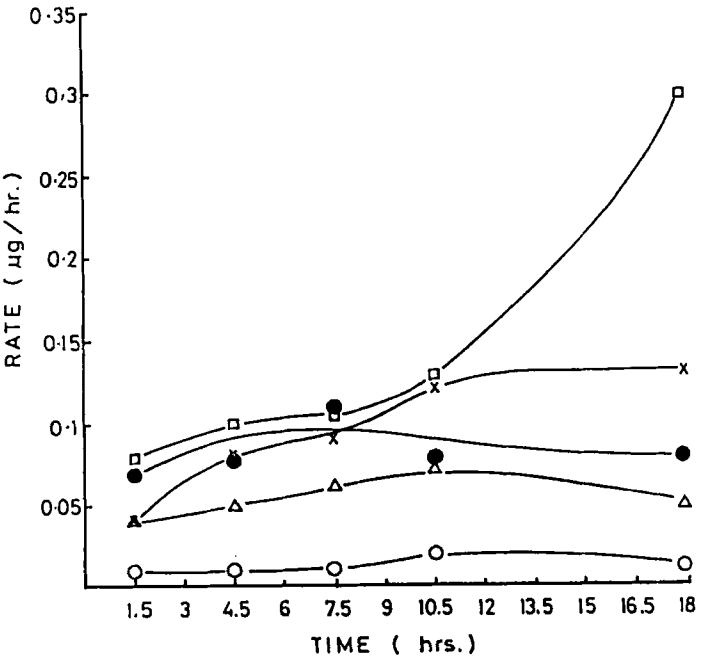

FIGURE 3

Effect of different vehicles on the rates of percutaneous absorption of DHE. Keys (○), water; (●), liquid paraffin; (△), glycerin;(X), PEG 4000; (□), propylene glycol.

skin from the liquid paraffin base was more than that from the PEG 400 base. Statistical analysis of the data using Student's t-test indicates that the difference in the results obtained from the different vehicles were significant ($P<0.05$).

There could be two possible explanations for the larger amount of DHE absorbed from propylene glycol base compared to the other vehicles tested in this study; propylene glycol is known to solvate the stratum corneum by unfolding the keratinized protein matrix, therefore, increasing the permeability of drugs and also known that

this vehicle penetrates the skin itself (22), thus it is possible that DHE may be carried into the skin with propylene glycol. It can be seen from Figure 2 that the amount of DHE delivered through the skin from PEG 400 was around 40% of the amount obtained by using propylene glycol. This result could be attributed to the poor penetration ability of PEG 400 (22, 25). In addition, this vehicle is less effective in maintaining hydration of the skin surface and it is known that dehydrated skin is more resistant to penetration (26). The low overall percutaneous absorption rate of DHE from glycerin base may be attributed to the weak penetration ability of glycerin through the skin itself (22). Table 1 shows that the amount of DHE penetrated across the skin from liquid paraffin base was about 14-fold more than the amount transported using water as medium for the drug. The lower value obtained for the water base may be due to the low solubility of the drug in this vehicle which may lead to low thermodynamic activity of DHE in the water base. In addition the lipophilic nature of the membrane could retard penetration of the drug from the water base. A similar result obtained by Touitou (27) for penetration of diazepam from water base due to the very low solubility of the drug in water. On the other hand, the relatively large amount of DHE delivered across the membrane from liquid paraffin base could be explained by the good solubility of the drug in this vehicle as well as the fatty nature of the membrane.

Table 3 represents the permeation of DHE from propylene glycol base using different drug concentrations. The total amount of the drug delivered through the skin during the 24 hrs period from the concentration; 8, 16, 30 and 50 mg/ml were 3.78, 7.25, 14.47 and

TABLE 3

Amount of DHE Penetrated through the Skin from Different Concentrations in Propylene Glycol Base

Time Interval (hrs)	DHE in μg±S.E. which penetrated the skin			
	8 mg/ml+	16 mg/ml*	30 mg/ml*	50 mg/ml*
0 - 3	0.17±0.003	0.25±0.02	0.25±0.005	0.33±0.02
3 - 6	0.14±0.04	0.33±0.03	0.56±0.05	1.21±0.26
6 - 9	0.21±0.05	0.35±0.04	1.23±0.14	5.77±1.21
9 - 12	0.45±1.12	0.63±0.19	1.27±0.08	4.07±1.08
12 - 24	2.63±1.13	5.69±1.70	10.85±1.14	27.62±4.20
0 - 24	3.78±1.45	7.25±1.80	14.47±1.50	38.98±6.31

+ Average of three cells.

* Average of four cells.

38.98 μg respectively. The data showed that as the concentration of DHE in the vehicle increased, the amount of the drug crossing the membrane also increased. The results suggest that the mechanism of DHE absorption through the skin was the passive diffusion. Since the saturation concentration of DHE in propylene glycol is 50 mg/ml, increased concentration did not result in enhanced permeation.

In the present study the diffusion parameters of DHE from the propylene glycol base were also determined using the lag time method

TABLE 4

Cumulative Amount of DHE Penetrated through the skin from Propylene Glycol Base*

Time (Seconds)	Cumulative Amount of DHE in gm which penetrated the skin X + S.E.
10800	$0.33 \times 10^{-6} \pm 0.02 \times 10^{-6}$
21600	$1.54 \times 10^{-6} \pm 0.27 \times 10^{-6}$
32400	$7.30 \times 10^{-6} \pm 1.27 \times 10^{-6}$
43200	$11.37 \times 10^{-6} \pm 2.14 \times 10^{-6}$
86400	$38.98 \times 10^{-6} \pm 6.31 \times 10^{-6}$

* 50 mg dose of DHE

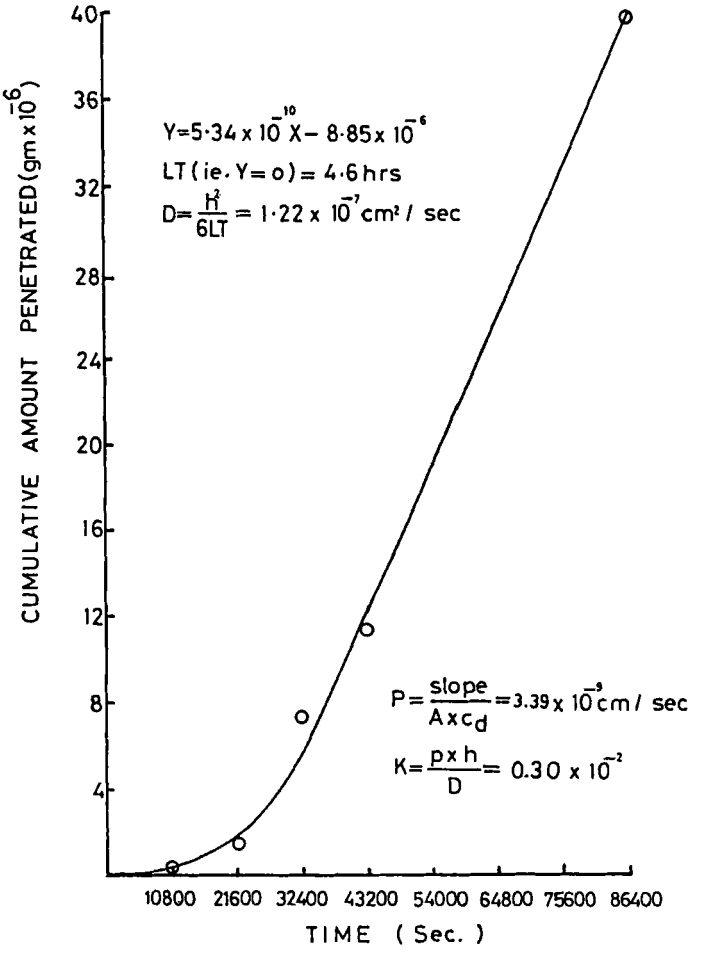

FIGURE 4

Typical permeation curve for DHE (50 mg dose) from propylene glycol base.

(28) and utilizing the data presented in Table 4. The steady state flux of DHE through the excised rabbit skin was achieved 7 hrs past application of 50.0 mg dose of DHE from the propylene glycol base as shown in Figure 4. The calculated value of the diffusion coefficient of DHE was 1.22×10^{-7} $cm^2/sec.$, which was higher than those reported for steroids by Scheuplein (29). The permeability coefficient of DHE was found to be 3.39×10^{-9} cm/sec. The value of the partition coefficient from propylene glycol base was 0.30×10^{-2}.

Based on the data generated from this study, propylene glycol was chosen as the primary medium for the transdermal formulation of DHE over all other vehicles tested in this investigation. A similar conclusion was derived by Wotton et. al. (23) for choosing propylene glycol as vehicle for metronidazole. Although propylene glycol appeared to be most effective medium for the transdermal formulation of DHE, the amount of the drug which penetrated the skin was too little to achieve therapeutic efficacy. Therefore, a way to enhance the penetration of DHE through the skin is necessary. Additional studies are currently underway to investigate the effect of various penetration promoters on the transport rate of DHE through the skin in order to develop a clinically acceptable transdermal dosage form of DHE.

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