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Effect of Azone and other penetration enhancers on the percutaneous absorption of dihydroergotamine

Esmail M. Niazy, Abdulla M. Molokhia and Abubaker S. El-Gorashi

College of Pharmacy, King Saud University, Riyadh (Saudi Arabia)

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Summary

In vitro drug diffusion studies were conducted, utilizing improved Franz diffusion cells, to estimate the effects of Azone and other additives (deoxycholic acid, dimethyl formamide, lecithin and 2-pyrrolidone) on the percutaneous absorption of dihydroergotamine (DHE). Rabbit skin obtained from the dorsal area was used as a barrier membrane. Among various absorption promoters tested, Azone was the most effective agent for enhancing the percutaneous absorption of DHE; the extent of enhancement ranged from 11-fold with 1.0% Azone coapplied with DHE, to 334-fold when skin was pretreated with 6.0% Azone. It was found that lecithin increased the transdermal delivery of DHE by 13.5-fold over the control. On the other hand the formulation containing 6.0% dimethyl formamide (DMF) did not show any significant difference from the control. In contrast, other additives tested including deoxycholic acid and 2-pyrrolidone showed a decrease in the amount of drug transported across the skin as compared to the control.

Introduction

Development of transdermal drug delivery system has been the focus of many research projects in recent years. However, the majority of drugs do not appear to penetrate the skin at a rate sufficiently high for therapeutic efficacy. The rate-limiting step for topical preparations intended for systemic effects is penetration of the drug through the skin to the site of absorption. The stratum corneum is generally recognized as the principal skin barrier to entry of foreign agents and to loss of water. Currently, the transdermal route is applicable to only a few drugs, but the use of suitable penetration enhancers will likely expand the list.

Penetration enhancers, accelerants or promoters have been extensively investigated over the past two decades. The literature on penetration enhancers was recently reviewed (Woodford and Barry, 1987). Penetration accelerants are substances that combine with or dissolve in the stratum corneum. They might produce increased penetration by causing the stratum corneum to swell and/or leach out some of the structural components, thus reducing the diffusional resistance and increasing the permeability (Hadgraft, 1984; Barry, 1983; Elfbaum and Laden, 1968). However, the mechanism of action of these promoters is imperfectly understood.

Correspondence: E.M. Niazy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

Based on the data generated from our previous study (Niazy et al., 1989), propylene glycol was found to be the most effective medium for the transdermal formulation of dihydroergotamine (DHE), which is widely used in the prevention and/or treatment of migraine (Little et al., 1982). However, the amount of DHE which penetrated the skin from propylene glycol was too little to achieve therapeutic efficacy. Therefore, a way to enhance the penetration of DHE through the skin was necessary in order to develop a clinically acceptable transdermal dosage form of the drug.

The purpose of this study is to investigate the effect of various penetration promoters: 1-dodecylazacycloheptan-2-one (Azone), deoxycholic acid, dimethyl formamide, lecithin, and 2-pyrrolidone on enhancing the transdermal delivery of DHE.

Materials and Methods

Materials

Dihydroergotamine mesylate (Sandoz Pharmaceuticals, E. Hanover, NJ, U.S.A.), propylene glycol (Fisher Scientific Company, Fair Lawn, NJ, U.S.A.), Azone (Nelson Research, Irvine, CA, U.S.A.), deoxycholic acid (Fluka AG, Buchs, Switzerland), dimethyl formamide, propylhydroxy-4-benzoate and 2-pyrrolidone (E. Merck AG, Darmstadt, F.R.G.), lecithin, sodium chloride, glycine, and hydrochloric acid (BDH Chemicals Ltd., Poole, U.K.) were used without further purification. Methanol (E. Merck AG, Darmstadt, F.R.G.) and acetonitrile (BDH Chemicals, Ltd., Poole, U.K.) were HPLC grade.

Preparation of skin for diffusion experiments

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 Inc. Co. Ltd., Japan) as close as possible to the skin without damaging it. The skin was examined under a strong magnifying lens for damage or diseased conditions. Any skin in which the barrier was disrupted was not used in the study. The dorsal area of the skin was utilized in this investigation.

In vitro permeation procedure

The in vitro diffusion technique used in this investigation was described in detail in our previous study (Niazy et al., 1989). Franz diffusion cell drive console (Crown Glass Company, Somerville, NJ, U.S.A.) was utilized in the permeation experiments. Nine of the improved Franz diffusion cells were inserted into this console. The skin was tightly secured between the receptor and the donor compartment. The area of the skin available for permeation was 3.14 cm^2. The temperature of the isotonic saline solution bathing the dermal layer in the receiving compartment was maintained at 37 °C.

To study the effect of Azone on penetration of DHE through the skin and to determine the optimal concentration which produces the maximum enhancing effect, several experiments were performed using 1.0, 3.0, 6.0 and 9.0% (v/v) Azone which were added to a solution of DHE in propylene glycol containing 16.0 mg/ml of the drug. Additional experiments were carried out to determine whether pretreatment of the skin with Azone will produce penetration enhancement effect or the presence of Azone in the formulation is essential.

In order to compare the effect of other penetration accelerants on percutaneous absorption of DHE, various additives including deoxycholic acid, dimethyl formamide, lecithin, and 2-pyrrolidone were each added at a concentration of 6.0% (w/w) to the propylene glycol/DHE formulation (16.0 mg/ml).

Of each tested formulation, 1.0 ml was applied to the skin in the donor compartment. One cell was used as reference where 1.0 ml of propylene glycol containing 16.0 mg DHE was applied to the skin. Samples were collected at 3, 6, 9, 12 and 24 h post application. Concentration of DHE in the various samples was determined using an HPLC assay method previously developed in our laboratory (Niazy et al., 1988). In case of pretreatment of the skin with Azone, 0.5 ml of 12.0% (v/v) Azone in propylene glycol (to keep the same amount of Azone as in the 6.0% (v/v) experiment

without any pretreatment) was applied to the skin 4 h before applying 0.5 ml of 32.0 mg/ml DHE in propylene glycol to achieve a final dose of 16.0 mg of DHE in propylene glycol base. One cell was kept as a control where 0.5 ml of propylene glycol was applied to the donor compartment 4 h before applying 0.5 ml of the formulation containing 16.0 mg DHE. Other experimental procedures were kept the same.

Results and Discussion

The cumulative amounts of DHE penetrated the skin during the 24 h at different time intervals from propylene glycol formulations containing 0.0, 1.0, 3.0, 6.0 and 9.0% (v/v) Azone are shown in Table 1. This table also shows the results obtained from the skin pretreated with Azone. The total amount of DHE delivered through the skin during the 24 h period from the formulations containing 0.0, 1.0, 3.0, 6.0 and 9.0% Azone were 7.25, 83.14, 272.65, 1021.07, and 601.04 μg respectively. On the other hand the amount of DHE transported across the membrane was 2419.12 μg when the skin was pretreated with 6.0% Azone. It is clear from the data presented in Table 1 that Azone has considerably increased the amount of DHE permeated through the rabbit skin from all formulations tested in this study; the extent of enhancement, based upon 24 h penetration, varied from 11-fold with 1.0% Azone simultaneously present with DHE, to 334-fold with 6.0% Azone applied prior to DHE. The maximum effect of Azone from the coapplication experiments was observed

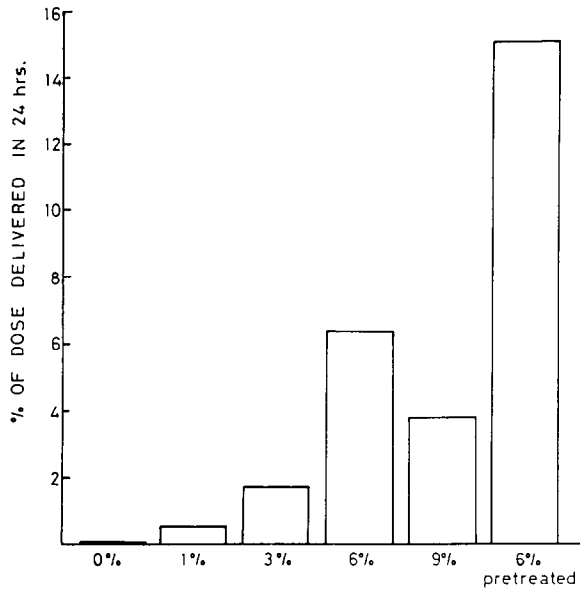

Fig. 1. Histogram showing the effect of different concentrations of Azone on the penetration of DHE through rabbit skin in 24 h.

for the formulation containing 6.0% Azone. This formulation delivered the drug 140-fold more than the formulation without Azone. The pretreatment of the skin with Azone was found to be more efficient than coapplication of Azone in the vehicle. The cumulative amount transported across the pretreated skin, in 24 h, was more than two times higher than the amount delivered from the formulation containing 6.0% Azone without any pretreatment during the 24 h interval. This observation appeared to be in agreement with those reported by Chow et al. (1984), showing that pretreatment with Azone was more effective than

TABLE 1

Cumulative amount of DHE penetrated through the skin from propylene glycol base using Azone as enhancer

Time (h)	Control 0.0%	Formulation with Azone without any pretreatment				Skin pretreated with Azone 6.0%
		1.0%	3.0%	6.0%	9.0%	
3	0.25 ± 0.02	0.20 ± 0.03	0.36 ± 0.06	2.92 ± 1.32	3.59 ± 1.49	13.93 ± 2.56
6	0.59 ± 0.05	0.47 ± 0.05	0.99 ± 0.22	59.69 ± 12.82	42.94 ± 15.83	99.51 ± 49.90
9	0.93 ± 0.06	1.52 ± 0.43	4.77 ± 1.09	255.65 ± 70.15	160.47 ± 33.66	347.53 ± 94.11
12	1.56 ± 0.14	3.71 ± 0.69	7.24 ± 1.51	696.30 ± 87.02	355.62 ± 46.93	512.32 ± 61.53
24	7.25 ± 1.80	83.14 ± 17.79	272.65 ± 88.73	1021.07 ± 136.05	601.04 ± 83.36	2419.12 ± 98.72

Values in μg, mean of 4 cells ± S.E.M.

TABLE 2

Cumulative amount of DHE penetrated through the skin from propylene glycol base using different enhancers

Time (h)	Control	Deoxycholic acid	Dimethyl formamide	Lecithin	2-Pyrrolidone
3	0.25 ± 0.02	0.33 ± 0.1	0.9 ±0.29	0.82 ± 0.34	0.35 ± 0.03
6	0.59 ± 0.05	0.44 ± 0.09	2.56 ± 0.91	2.76 ± 1.08	0.46 ± 0.04
9	0.93 ± 0.06	0.67 ± 0.08	3.31 ± 0.99	6.62 ± 1.96	0.52 ± 0.05
12	1.56 ± 0.14	0.87 ± 0.12	4.70 ± 1.42	7.86 ± 1.89	0.80 ± 0.06
24	7.25 ± 1.80	1.97 ± 0.21	6.25 ± 2.08	98.06 ± 12.88	2.65 ± 0.88

Values in μg, mean of 4 cells ± S.E.M.

coexistence of Azone with triamcinolone acetonide. Table 1 also illustrates a marked reduction in the lag time for permeation observed with the skin pretreated with Azone and with propylene glycol formulations containing 6.0 and 9.0% Azone. Wotton et al. (1985) reported a significant reduction in lag time of permeation of metronidazole with the coexistence of Azone in propylene glycol. Fig. 1 is a histogram showing the percentage of DHE dose (16.0 mg) delivered through the skin as a function of varying Azone concentrations. The data showed that as the concentration of Azone in the formulation increased, the percentage of the dose crossing the membrane in 24 h also increased. However, when 9.0% Azone was added to the formulation, the percentage of the dose delivered was less than that of the 6.0% Azone. The presence of an optimum Azone concentration for enhancing DHE penetration is probably related to the combined effect of Azone on the animal membrane and on the physical properties of the formulation. A similar result was reported by Stoughton and McClure (1983) when they studied the effect of Azone concentration on penetration of sodium fusidate and other drugs. On the other hand, the highest percentage of the dose transported across the skin in 24 h (15.1%) was achieved with the pretreated skin experiment. Statistical analysis of data presented in Table 1 using Student's *t*-test indicates that the difference in the results obtained from the different formulations using Azone as enhancer were significant ($P < 0.05$).

The permeation data of DHE from propylene glycol base in presence of other additives are presented in Table 2. The cumulative amount of DHE transported across the skin during the 24 h period from the formulations containing 6.0% (w/w) deoxycholic acid, dimethyl formamide, lecithin and 2-pyrrolidone were 1.97, 6.25, 98.06 and 2.65 μg respectively. It can be seen that, except for lecithin which showed an enhancement in DHE penetration of 13.5-fold over the control, the other additives have no or negative effect on drug transport. Kato et al. (1987) reported that lecithin enhanced transdermal delivery of isosorbide dinitrate by 4.55-fold, theophylline by 12.25-fold and bunazosin by 56.15-fold, which are in agreement with our results. The total amount of DHE absorbed from propylene glycol base containing 6.0% (w/w) lecithin (98.06 μg) was almost the same as that delivered from the formulation containing 1.0% (v/v) Azone (83.14 μg) with no significant difference ($P > 0.05$). In contrast, the results obtained from using propylene glycol base containing 6.0% 2-pyrrolidone and 6.0% deoxycholic acid showed a decrease in the amount of DHE delivered through the skin as compared to the control ($P > 0.05$). This significant decrease in the transport of DHE may be due to complexation between the drug and deoxycholic acid and 2-pyrrolidone causing a decrease in the concentration of free DHE in the vehicle and thereby lowering the thermodynamic activity of the drug. On the other hand, the amount of DHE penetrated from the propylene glycol base containing 6.0% dimethyl formamide after 24 h did not significantly differ from the control ($P > 0.05$). This result may be attributed to the low concentration of DMF in the formulation. A previous report (Hadgraft, 1984) has indicated that concentration about 60.0% of DMF is required in order to significantly increase transdermal delivery of solutes. It can also be seen clearly from Table 2 that lecithin has considerably enhanced the penetration of DHE

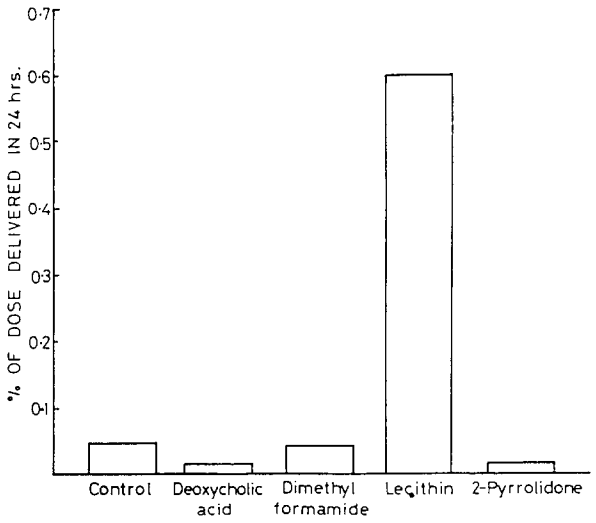

Fig. 2. Histogram showing the effect of different enhancers on penetration of DHE through rabbit skin in 24 h.

over the entire 24 h period. The dimethyl formamide formulation showed initial enhancing in the amount of DHE delivered through the skin which is similar to the enhancing effect achieved with the lecithin formulation during the first 6 h time interval. This was followed by slow increase in the permeation of the drug to give a cumulative amount similar to the control at the end of 24 h time period. The percentage of the dose of DHE transported across the skin from the formulations containing the various additives is illustrated as a histogram in Fig. 2. Based on the data generated from this study, we conclude that the incorporation of Azone in DHE formulation or the pretreatment to the skin by the same enhancer has dramatically increased DHE transdermal delivery. The sizable enhancement observed in this study warrants future clinical trials with DHE/Azone formulations from conventional dosage forms and from controlled release transdermal therapeutic systems.

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