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Differences in penetration-enhancing effect of Azone through excised rabbit, rat, hairless mouse, guinea pig and human skins

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Abstract

The transdermal delivery of dihydroergotamine (DHE), from propylene glycol formulations with and without 6.0% laurocapram (Azone), and the penetration enhancing effect of Azone were evaluated in vitro on excised rabbit, rat, hairless mouse, guinea pig and human skins utilizing improved Franz diffusion cells. The steady-state flux of DHE from the propylene glycol formulation without Azone were 0.045, 0.270, 0.395, 0.128 and 10.035 $\mu g/cm^2$ per h across excised human, rat, guinea pig, rabbit and hairless mouse, respectively. Under the influence of the enhancer, Azone increased DHE penetration through excised skin of the various species used in this study in the following order: rabbit skin > human skin > rat skin > guinea pig skin > hairless mouse skin. The maximum enhancement factor of Azone (251.47) was obtained across rabbit skin and the minimum enhancing effect (14.44) was observed in the case of hairless mouse skin. The enhancement factor of Azone across human skin was 54.56. These results show that animal skins are poor models for human skin under the conditions used. The lag time of DHE, from the propylene glycol formulation containing 6.0% Azone, through human skin was longer than the lag times across all other skin species tested in this investigation.

Keywords: Percutaneous absorption; Dihydroergotamine; Azone; Laurocapram; Penetration enhancer; Enhancement factor; Human skin; Animal skins

1. Introduction

The transdermal route for systemic drug delivery has received considerable attention in recent years (Shaw, 1984; Wellstein et al., 1986; Banerjee and Ritschal, 1989; Niazy, 1991; Kim et al., 1993; Ruland et al., 1994). However, a major problem encountered in this route of administration is the low permeability of the skin. One way to reduce this problem and improve the bioavailability after topical application of drugs is to include a penetration enhancer in the transdermal formulation. Penetration enhancers, accelerants or promoters are thought to interact with some components of skin causing the stratum corneum to swell and/or leach out some of the structural components and thus increase drug penetration through the barrier

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membrane (Elfbaum and Laden, 1968; Barry, 1983; Hadgraft, 1984; Beastall et al., 1988; Carelli et al., 1993; Hirvonen et al., 1994).

One of the most promising permeation promoters is laurocapram, 1-dodecylazacycloheptan-2-one (Azone) which was patented by Nelson Research in 1983. Azone is non-irritant to human skin, even in undiluted form (Stoughton, 1982), reversible in its action (Adachi et al., 1988) and hardly absorbed through human skin (Wiechers et al., 1988). Wotton et al. (1985) showed that a single dose of Azone is capable of enhancing the transdermal delivery of subsequent doses of metronidazole for at least 5 days. This indicates that Azone partitions into the stratum corneum and has little tendency to diffuse into the epidermis, which is an obvious advantage in enhancer technology. The results of pharmacological and toxicological studies demonstrate that Azone possesses a safe toxicological profile comparable to that observed with nutritional compounds (Stoughton and McClure, 1983). It has been reported that Azone enhances the transdermal delivery of various therapeutic agents of varying polarity (Stoughton, 1982; Stoughton and McClure, 1983; Niazy et al., 1989; Mahjour et al., 1989; Banerjee and Ritschal, 1989; Ruland et al., 1994).

In a previous study we have shown that dihydroergotamine (DHE), which is used in treating migraine, is a good candidate for transdermal administration (Niazy et al., 1990). In the present investigation, in order to determine the enhancing effect of Azone on various types of skin we have evaluated the percutaneous absorption of DHE through excised rabbit, rat, hairless mouse, guinea pig and human skins from a propylene glycol formulation containing 6.0% Azone and from the same formulation without Azone.

2. Materials and methods

Dihydroergotamine mesylate (Sandoz Pharmaceuticals, E. Hanover, NJ, USA), propylene glycol (Fisher Scientific Company, Fair Lawn, NJ, USA), Azone (Nelson Research, Irvine, CA, USA), sodium chloride, glycine and hydrochloric acid (BDH Chemicals Ltd., Pool, UK), propyl 4-hydroxybenzoate (E. Merck AG, Darmstadt, FRG) were used without further purification. Methanol and acetonitrile (BDH Chemicals Ltd., Pool, UK) were HPLC grade.

2.1. Preparation of skin for diffusion experiments

The dorsal skin of male white New Zealand rabbits (3.0–3.5 kg), Wistar male rats (270–300 g), Hartely male guinea pigs (450–500 g) and male HL strain hairless mice (35–40 g) were utilized in this investigation. After killing the rabbits and other animals by spinal dislocation, the skin was carefully removed leaving the fat tissue behind. The hair (except hairless mice) was clipped (Daito Electric Machine Inc. Co., 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 this study. Human cadaver chest skin (female age 23 years) was obtained through King Khalid University Hospital, Riyadh, Saudi Arabia and used within 4 h post-mortem. Each piece of excised skin was of full thickness (i.e. epidermis with stratum corneum and dermis).

2.2. In-vitro permeation studies

The in-vitro diffusion technique used in this investigation was described in detail in our previous study (Niazy et al., 1990). A system employing nine Franz diffusion cells (Crown Glass Company, Somerville, NJ, USA) was used in the permeation experiments. 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 first test formulation was prepared by dissolving 1.6 g DHE mesylate in a 100.0 ml volumetric flask containing propylene glycol. This formulation was used to evaluate the transdermal delivery of DHE through excised rabbit, rat, hairless mouse, guinea pig and human skins. In order to compare the enhancing effect of Azone on penetration of DHE through different skin types, Azone was added at a concentration of 6.0% to the propylene glycol/

DHE formulation. The final concentration of DHE in each test formulation was 16.0 mg/ml. Of each tested formulation, 1.0 ml was applied to the skin in the donor compartment, which was sufficient to cover the exposed surface area of the skin (3.14 cm^2). One cell was used as reference where the drug-free formulation was applied to the skin.

2.3. Analysis of samples

Drug concentrations in the receptor compartment were determined, after withdrawing 2.0 ml samples at 3, 6, 9, 12 and 24 h post application of the dose to the skin, using a simple, rapid and sensitive high-performance liquid chromatographic assay (HPLC) method, previously developed in our laboratory (Niazy et al., 1988). Chromatographic separation was achieved on a C_{18} reversed phase column; the mobile phase consisted of methanol, acetonitrile and glycine buffer (0.5:3.5:6.0); the eluent was monitored with a detector equipped with a mercury lamp which provided 254 nm excitation and with a 300–400 nm emission filter. With this eluting solvent system the drug and the internal standard (propyl 4-hydroxybenzoate) were well separated from interfering skin constituents. The retention times for DHE and the internal standard were 8 and 10 min, respectively. The average recovery of DHE from 6 replicate samples of different concentration (5–30 ng/ml) were 92.2 $\pm$ 3.37%. The reproducibility of the assay was evaluated by comparing the linear regression analysis of the three standard plots obtained over a 1 week period. The coefficient of variation of the slopes of the three lines was 11.68%. The minimum detectable concentration of DHE by this method was 2 ng/ml.

2.4. Data analysis

The skin flux was determined from Fick's law of diffusion:

$$J_s = dQ_r/Adt$$

where J_s is the steady-state skin flux in $\mu g/cm^2$ per h, dQ_r is the change in quantity of material passing through the membrane into the receptor compartment in μg, A is the active diffusion area in cm^2 and dt is the change in time. The steady-state flux of DHE through the various types of skin was calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area versus time plot. The lag time was determined by extrapolating the linear portion of the curve to the abscissa. The enhancement factor (E.F.) of Azone on transdermal delivery of DHE through the various skins utilized in this study was calculated as the ratio of DHE flux with the penetration enhancer to the flux without penetration enhancer.

2.5. Statistical analysis

The steady-state fluxes of DHE through excised human and animal skins from two propylene glycol formulations were evaluated statistically using analysis of variance (ANOVA). Duncan's multiple range test was applied (if ANOVA indicated significant difference) to find the source of possible differences between the various fluxes of DHE across the different types of skin utilized in this study. Differences between fluxes from the same formulation were considered statistically significant for P-value ≤ 0.05. Analysis of all the data was performed using a statistical software package (Statistical Analysis System, SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

Fig. 1 shows the penetration profiles of DHE through excised rabbit, rat, hairless mouse, guinea pig and human skin after application of 16.0 mg dose of DHE from a propylene glycol formulation containing 6.0% Azone and from the same formulation without Azone. Table 1 presents the DHE fluxes, across the various excised skins utilized in this investigation, from the formulation with 6.0% Azone and the formulation without Azone. Table 1 also shows the values of the enhancement factor (E.F.) of Azone on the transdermal delivery of DHE through the various skins used in this investigation.

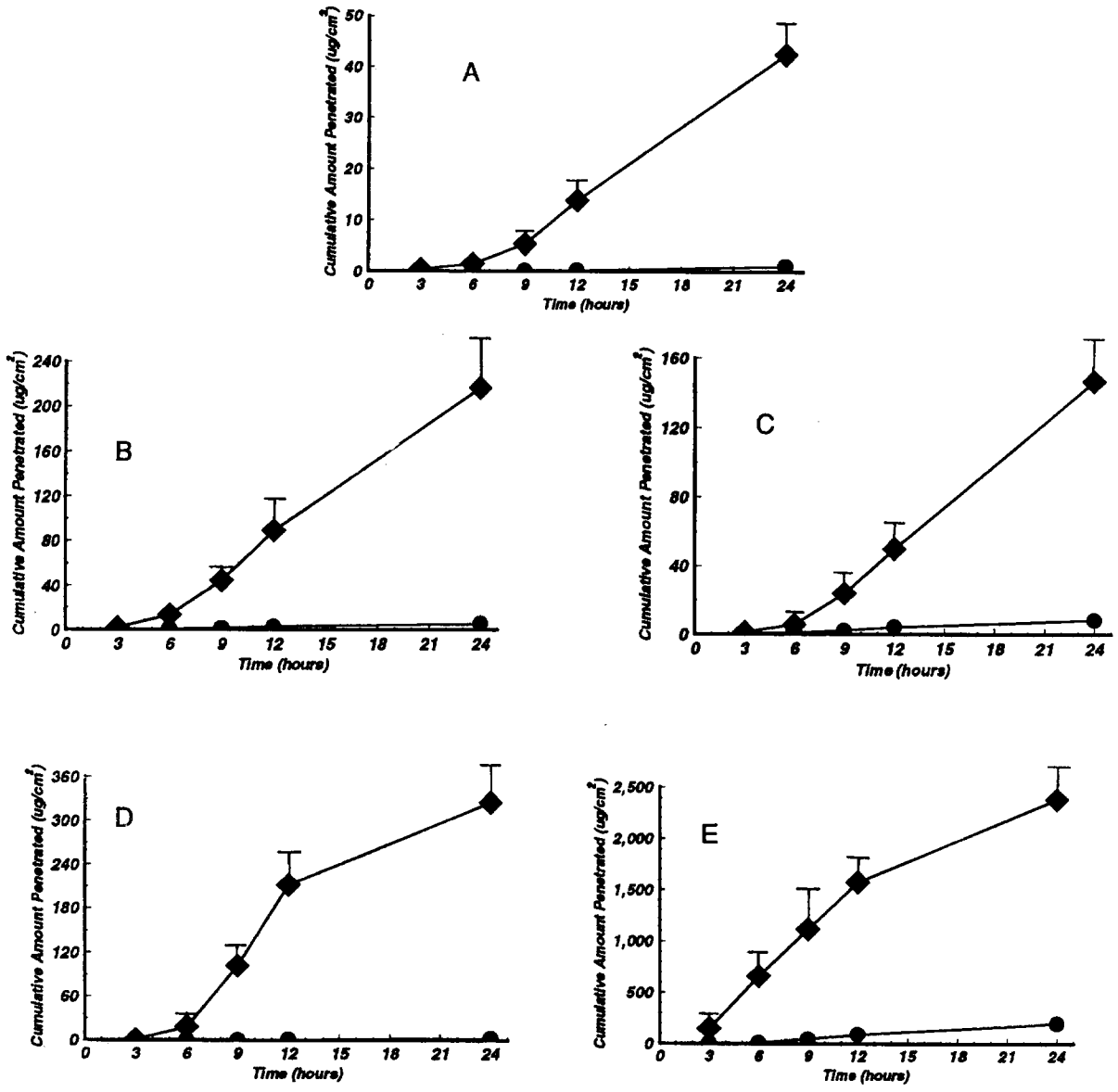

Fig. 1. Penetration profiles of DHE across excised human and animal skins after application of formulations with 6.0% Azone (◆) and without Azone (●). Points and vertical bars represent the mean and S.D. (n = 4), respectively. (A) Human skin; (B) rat skin; (C) guinea pig skin; (D) rabbit skin; (E) hairless mouse skin.

The steady-state flux of DHE from the propylene glycol formulation without Azone through excised human, rat, guinea pig, rabbit and hairless mouse skins were 0.045, 0.270, 0.395, 0.128 and 10.035 μg/cm^2 per h, respectively. Duncan's multiple range test indicated that the difference in the fluxes obtained across the different type of skins utilized in this investigation were statistically significant ($P \leq 0.05$). Therefore, it is clear from the previous data that the skin permeability to DHE of the different species used in this study decreased in the following order: hairless mouse >

Table 1
In-vitro steady-state fluxes of DHE through excised human and animal skins from two propylene glycol (P.G.) formulations and enhancement factor (E.F.) of Azone

Species	Flux ± S.D.[a] (μg/cm^2 per h)		E.F.
	P.G. formulation without Azone	P.G. formulation with 6.0% Azone	
Human	0.045 ± 0.013	2.455 ± 0.203	54.56
Rat	0.270 ± 0.026	11.343 ± 1.117	42.01
Guinea pig	0.395 ± 0.042	7.927 ± 0.569	20.07
Rabbit	0.128 ± 0.021	32.188 ± 2.757	251.47
Hairless mouse	10.035 ± 1.177	144.887 ± 23.516	14.44

[a] $n = 4$.

guinea pig > rat > rabbit > human. Human skin is much less permeable to DHE than any other species examined and the hairless mouse is particularly susceptible to DHE penetration. Similar observation had been reported by Catz and Friend (1990) for permeability of the contraceptive drug levonorgestrel from a saturated solution in ethyl acetate through the four skin types utilized in their investigation which showed the following trend: hairless mouse skin > hairless guinea pig skin > rat skin > human skin. It has long been recognized that the main barrier to percutaneous absorption is the stratum corneum and its thickness increases with animal size. In other words as the stratum corneum thickness increases, the transport of drug across the skin decreases. Our results appeared to be in agreement with the previous basic principles.

The transdermal flux of DHE from the propylene glycol formulation containing 6.0% Azone were 2.455, 11.343, 7.927, 32.188 and 144.887 μg/cm^2 per h across excised human, rat, guinea pig, rabbit and hairless mouse skins, respectively. It is clear from the data presented in Table 1 and Fig. 1 that Azone has considerably increased the flux of DHE through excised skin of the various species used in this study. As may be noted from the E.F. values listed in Table 1, Azone increased DHE penetration across human and animal skins in the following order: rabbit > human > rat > guinea pig > hairless mouse. Stoughton and McClure (1983) reported E.F. (12) for transdermal delivery of 8-bromo cAMP across hairless mouse skin using Azone as enhancer which is similar to our result for DHE. The extent of enhancement across guinea pig skin was 20.07. A similar enhancement effect for Azone was previously reported by Okamoto et al. (1988) for the transport of 6-mercaptopurine through guinea pig skin. Azone increased transdermal delivery of metronidazole through human skin by 25-fold more than the control (Wotton et al., 1985), which is less than the E.F. (54.56) observed across human skin in our study. Bronaugh et al. (1982) have shown that rat skin has a stratum corneum that is, in fact, as thick as that of human skin. Their finding may explain why the E.F. (42.01) of Azone on DHE transport through rat skin was close to the E.F. observed across human skin in this investigation. It can be seen from Table 1 that the maximum E.F. (251.47) was obtained across rabbit skin. The lag times for transdermal delivery of DHE, from the propylene glycol formulation containing 6.0% Azone, through human and hairless mouse skin were 6.9 and 3.9 h, respectively. The lag time across human skin was the longest lag time observed among the different skin species utilized in this study.

Based on the data generated from this study, we conclude that differences in percutaneous absorption of DHE does exist among the species examined in this investigation. In addition, there is interspecies variation which has been observed in the degree of enhancement of Azone on transdermal delivery of DHE. On the other hand, there exists a number of similarities between the penetration of the drug molecule through animal and human skin. The data obtained in this study from

hairless mouse skin is very discouraging while the results from rat skin are somewhat encouraging. However, there is no animal skin that completely mimics the penetration characteristics of human skin. Therefore, excised animal skin can be used to evaluate the release characteristics of the formulation and/or transdermal delivery device. However, correlation to predict penetration parameters of drug molecules through human skin must be made with utmost care within the scope of the method and species used. For more objective studies, percutaneous absorption using excised human skin remains the best option.

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References

Adachi, Y., Hosaya, K., Sugibayashi, K. and Morimoto, Y., Duration and reversibility of the penetration-enhancing effect of Azone. *Chem. Pharm. Bull.*, 36 (1988) 3702–3705.

Banerjee, P. and Ritschal, W., Transdermal permeation of vasopressin. II. Influence of Azone on in-vitro and in-vivo permeation. *Int. J. Pharm.*, 49 (1989) 199–204.

Barry, B., Properties that influence percutaneous absorption. In Swarbrick, J. (Ed.), *Dermatological Formulation: Percutaneous Absorption*, Dekker, New York, 1983, pp. 127–233.

Beastall, J., Hadgraft, J. and Washington, C., Mechanism of action of Azone as percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects. *Int. J. Pharm.*, 43 (1988) 207–213.

Bronaugh, R., Stewart, R. and Congdon, E., Methods for in vitro percutaneous absorption studies. II. Animal models for human skin. *Toxicol. Appl. Pharmacol.*, 62 (1982) 481–488.

Carelli, V., DiColo, G., Nanoripieri, E. and Serafini, M., Bile acids as enhancers of steroid penetration through excised hairless mouse skin. *Int. J. Pharm.*, 89 (1993) 81–89.

Catz, P. and Friend, D., Transdermal delivery of levonorgestrel. VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin and human skin. *Int. J. Pharm.*, 58 (1990) 93–102.

Elfbaum, S. and Laden, K., The effect of dimethyl sulphoxide on percutaneous absorption. *J. Soc. Cosm. Chem.*, 19 (1968) 119-127, 163-172, 841–847.

Hadgraft, J., Penetration enhancers in percutaneous absorption. *Pharm. Int.*, 5 (1984) 252–254.

Hirvonen, J., Rajala, R., Vihervaara, P., Laine, E., Paronen, P. and Urtti, A., Mechanism and reversibility of penetration enhancer in the skin – a DSC study. *Eur. J. Pharm. Biopharm.*, 40 (1994) 81–85.

Kim, C., Kim, J., Chi, S. and Shim, C., Effect of fatty acids and urea on the penetration of ketoprofen through rat skin. *Int. J. Pharm.*, 99 (1993) 109–118.

Mahjour, M., Mauser, B. and Fawzi, M., Skin permeation enhancement effects of linoleic acid and Azone on narcotic analgesics. *Int. J. Pharm.* 56 (1989) 1–11.

Niazy, E., Molokhia, A. and El-Gorashi, A., Quick and simple determination of dihydroergotamine by high performance liquid chromatography. *Anal. Lett.*, 21 (1988) 1833–1843.

Niazy, E., Molokhia, A. and El-Gorashi, A., Effect of Azone and other penetration enhancers on the percutaneous absorption of dihydroergotamine. *Int. J. Pharm.*, 56 (1989) 181–185.

Niazy, E., Molokhia, A. and El-Gorashi, A., Effect of vehicle and drug concentration on transdermal delivery of dihydroergotamine using excised animal skin. *Drug Dev. Ind. Pharm.*, 16 (1990) 1697–1715.

Niazy, E., Influence of oleic acid and other permeation promoters on transdermal delivery of dihydroergotamine through rabbit skin. *Int. J. Pharm.*, 67 (1991) 97–100.

Okamoto, H., Hashida, M. and Sezaki, H., Structure-activity relationship of 1-alkyl or 1-alkenylazacycloalkanone derivatives as percutaneous penetration enhancers. *J. Pharm. Sci.*, 77 (1988) 418–424.

Ruland, A., Kreuter, J. and Rytting, J. Transdermal delivery of the tetrapetide hisetal melanotropin [6–9]. I. Effect of various penetration enhancers: in-vitro study across hairless mouse skin. *Int. J. Pharm.*, 101 (1994) 57–61.

Shaw, J., Pharmacokinetics of nitroglycerine and clonidine delivered by transdermal route. *Am. Heart. J.*, 108 (1984) 217–222.

Stoughton, R., Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. *Arch. Dermatol.*, 118 (1982) 474–477.

Stoughton, R. and McClure, W., Azone: a new non-toxic enhancer of cutaneous penetration. *Drug Dev. Ind. Pharm.*, 9 (1983) 725–744.

Wellstein, A., Kuppers, H., Pitschner, H. and Palm, D., Transdermal delivery of bupranolol: pharmacodynamics and β-adrenoceptor occupancy. *Eur. J. Clin. Pharmacol.*, 31 (1986) 419–422.

Wiechers, J., Drenth, B., Jonkman, J. and deZeeuw, R., Percutaneous absorption, metabolism and elimination of the penetration enhancer Azone in humans after prolonged application under occlusion. *Int. J. Pharm.*, 47 (1988) 43–49.

Wotton, P., Mollgaard, B., Hadgraft, J. and Hoelgaard, A., Vehicle effect on topical drug delivery, III. Effect of Azone on the cutaneous permeation of metronidazole and propylene glycol. *Int. J. Pharm.*, 24 (1985) 19–26.