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Transdermal permeation of vasopressin. II. Influence of Azone on in vitro and in vivo permeation

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Summary

Different enhancers were evaluated by measuring in vitro diffusion of aqueous vasopressin solution through excised rat skin after pretreatment with enhancers. Azone was found to be the most effective enhancer studied, increasing the flux about 15 times. Concentrations of Azone higher than 3% v/v did not show any further enhancement of flux. The enhancement of flux by Azone in hairless mouse skin was more dramatic (about 70 times), suggesting that the mechanism of enhancement in hairless mouse skin might be different than in the rat. The effect of Azone was evaluated in vivo using adult vasopressin-deficient Brattleboro rats. Urine was collected at time intervals after topical administration of vasopressin, and urine volume, osmolality and radioactivity were determined. Significant reduction in urine volume and increase in osmolality over a 24 h period confirmed the enhancement of vasopressin absorption in presence of Azone found in vitro.

Introduction

Recently, interest in the use of peptide and protein drugs as therapeutic agents for both existing and new clinical conditions has soared. Most of these drugs are highly potent; and their elimination half-life is very short, being of the order of seconds to minutes. This necessitates controlled administration for a long period of time to attain sufficient concentration at the site of action. The generally large molecular size of peptides, along with their low lipid solubility, affects their ability to and the mechanism by which they cross biological and synthetic membranes. The oral administration of these drugs is precluded by their digestion in the stomach and small intestine. They are usually administered by parenteral routes such as i.m. or s.c. which are inconvenient for long-term therapy. In an attempt to improve patient compliance, other non-parenteral routes of administration such as nasal, pulmonary, rectal, buccal, vaginal, transdermal, and ocular routes are being investigated in those years. Some of the widely studied peptide drugs that have been used in therapy for a considerable period of time are: insulin, vasopressin, oxytocin, and human growth hormone. In this study, the feasibility of the transdermal route for vasopressin was investigated. Initial studies (Banerjee and

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Ritschel, 1989) indicated that vasopressin permeation through the rat skin is in the order of 3 $ng/cm^2 \cdot h$.

Replacement therapy with vasopressin has shown (Cheng et al., 1982) that to attain a certain level of antidiuresis, the vasopressin release rate must be at least 100 ng/day. In humans, assuming a steady state concentration of 2.5 pg/ml (Drug Information, 1986) and distribution volume of 9.1 liter, the desired zero-order input rate must be about 63 ng/h. Hence, in order to use skin as a therapeutically effective route, vasopressin permeation must be enhanced by some means.

Permeation enhancers have been reviewed elsewhere (Hadgraft, 1984; Barry, 1983; Idson, 1985; Woodford and Barry, 1986; Ritschel and Sprockel, 1988). The major group of permeation enhancers are such organic solvents as alcohols, acetone, glycols, dimethylsulfoxide (DMSO), dimethylacetamide, dimethylformamide, *N,N*-diethyl-*m*-toluamide (NNDMT) and pyrrolidones. Recently, Azone (1-dodecylazacycloheptan-2-one) has received considerable interest (Stoughton, 1982; Stoughton and McClure, 1983) as a permeation enhancer. It is a clear, colorless liquid, insoluble in water and generally acts at a concentration of 1–10%; although for erythromycin, the largest molecule studied, concentrations as high as 20% were needed to achieve maximum effect. It has been successfully formulated with a wide range of compounds for topical or transdermal applications (Nelson, 1985). Azone seems particularly suitable for enhancing the permeation of polar water-soluble compounds. The molecular weight of the drug appears to have no effect on enhancement by Azone, although this aspect was not studied in a systematic manner. The mechanism of action of Azone is not yet known. Because of its remarkable enhancement properties, Azone was used in this study after preliminary screening of different enhancers.

The correlation between pharmacologic response and plasma drug concentration is not well established for most peptide drugs. Hence, pharmacodynamics of peptides is a more accurate reflection of the performance of their delivery systems than plasma concentration. In the present study, enhancement of skin permeation by Azone was evaluated in vitro in rats and hairless mice and in vivo in Brattleboro rats.

Materials and Methods

Materials

Vasopressin was purchased from Sigma. Tritium labeled vasopressin was obtained from New England Nuclear. Azone was a gift from Nelson Research and Development Co. Transcutol and Labrafil were gifts from Gattefosse Corp. Dimethyl acetamide (DMAC) and DMSO were obtained from Fisher Scientific Company. NNDMT was supplied by Eastman Kodak Co. Propylene glycol was purchased from J.T. Baker Chemical Co.

Skin

Excised rat skin was prepared as described before (Banerjee and Ritschel, 1989). The effect of Azone was also evaluated using full thickness excised skin from female outbred BALB/C hairless mice (Harlan) of 16 weeks of age.

In vitro skin permeation

In vitro permeation was studied using single-chambered Franz diffusion cells (Banerjee and Ritschel, 1989). Preliminary screening of enhancers was done by pretreating the excised skin with the solvent or enhancer (Shetti et al., 1986). One hundred μl of undiluted enhancer was applied to the stratum corneum. After 4 h, the excess solvent was wiped off with tissue paper, and 200 μl of vasopressin solution (49.5 μg/ml), 8 μCi/ml) was applied thereafter. The enhancers studied were: Azone, DMSO, DMAC, NNDMT, propylene glycol, Labrafil and Transcutol. Labrafil is a glycolysed ethoxylated glyceride produced from natural vegetable oil by alcoholysis, whereas Transcutol is purified diethylene glycol monoethyl ether obtained by distillation. Both have been used as enhancers in topical preparations (Gattefosse, 1982).

After Azone was found to be the best enhancer, solutions with 4 different concentrations (3%, 5%, 10% and 25%, v/v) of Azone were prepared. Propylene glycol was used to solubilize Azone in

water. The concentration of vasopressin in all solutions was 54.25 μg/ml (4 μCi/ml). A control was also prepared without Azone. Of these solutions 200 μl were applied on the stratum corneum side without any pretreatment of the skin. In hairless mouse skin, only the solution with 5% Azone was studied against control. Data analysis was done as previously described (Banerjee and Ritschel, 1989).

In vivo evaluation

In vivo enhancement of vasopressin absorption through skin in presence of Azone was studied in Brattleboro rats (Blue Spruce Farms). Eight rats were used in a cross-over design separated by 5 days of rest period. Following clipping of the dorsal hair under light ether anaesthesia, a flexible Teflon ring (3 cm diameter) was glued to the skin with a medical grade adhesive (Dow Corning). 100 μl of vasopressin solution (59.5 μg/ml, 7 μCi/ml) with and without Azone was applied evenly on the centre (1 cm diameter). After covering the ring with aluminum foil (Reynolds Metals Co.), the rat was wrapped with an elastic bandage, and a rubber tubing was placed around the neck to prevent it from chewing the bandage. The rat was placed in a metabolism cage, and urine was collected in graduated centrifuge tubes or Erlenmeyer flasks every 4 h for 48 h.

The animals were housed at 22–25 °C under a 12-h light and dark cycle. They had free access to powdered rat chow (Ralston Purina Co.) and tap water ad libitum.

With each urine sample, urine volume, osmolality and radioactivity were determined. Before the beginning of the study, the rats were acclimatized in the metabolism cages for 1 week; and baseline urine volume and osmolality in 24 h were determined for 5 days. Urine volume was measured in centrifuge tubes or graduated cylinders. Osmolality was determined in an osmometer (Model 3L, Advanced Instruments) using 0.25 ml samples. Means of duplicate runs were taken. Radioactivity was measured by liquid scintillation counting of 500 μl or less urine (depending on the volume of sample) in a scintillation counter (Model LS 7000, Beckman Instruments) after mixing with 5 ml cocktail fluid 3a70B (Research Products International).

Results and Discussion

In the preliminary screening of enhancers, the mean flux values from duplicate runs are shown in Fig. 1. Among the enhancers used, Azone was undoubtedly the best, DMSO was second, followed by Labrafil, DMAC and NNDMT. Transcutol did not change the permeation significantly. The lag time and flux at each concentration of Azone are reported in Table 1. No radioactivity was detected in the control up to 24 h. All the solutions had similar proportions of propylene glycol (70–80%). It is possible that propylene glycol inhibited vasopressin permeation in the control. The concentration of Azone had no significant effect on the flux and lag time of vasopressin. None of the differences were statistically significant although lag time tended to be lower at higher concentrations of Azone. When compared to the control flux values from a previous study (Banerjee and Ritschel, 1989), the flux increased about 15 times in presence of Azone. Skin permeation-time profile of vasopressin through hairless mice skin in presence of Azone is shown in Fig. 2. In presence of Azone, the steady state is reached early and then the curve levels off because of exhaustion of the supply of vasopressin in the donor phase. The flux was calculated from the early hours (up to 12 h). Table 2 shows the permeation data. Permeation enhancement by Azone

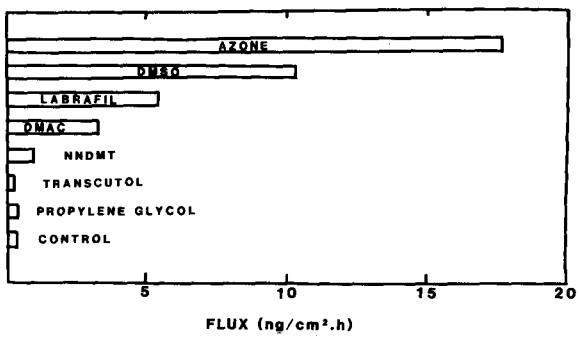

Fig. 1. Mean flux ($n = 2$) of vasopressin through rat skin after pretreatment with different enhancers.

TABLE 1

Steady state flux and lag time of vasopressin solutions containing different concentration of Azone through rat skin

Azone conc. (%, v/v)	Flux ($ng/cm^2 \cdot h$)	Lag time (h)
3.0	50.94 ± 24.86 [a]	4.88 ± 0.91
5.0	54.86 ± 15.80	5.40 ± 1.50
10.0	45.60 ± 2.70	3.97 ± 0.58
25.0	53.54 ± 12.13	3.54 ± 0.54

[a] Values are mean ± S.D., $n = 4$.

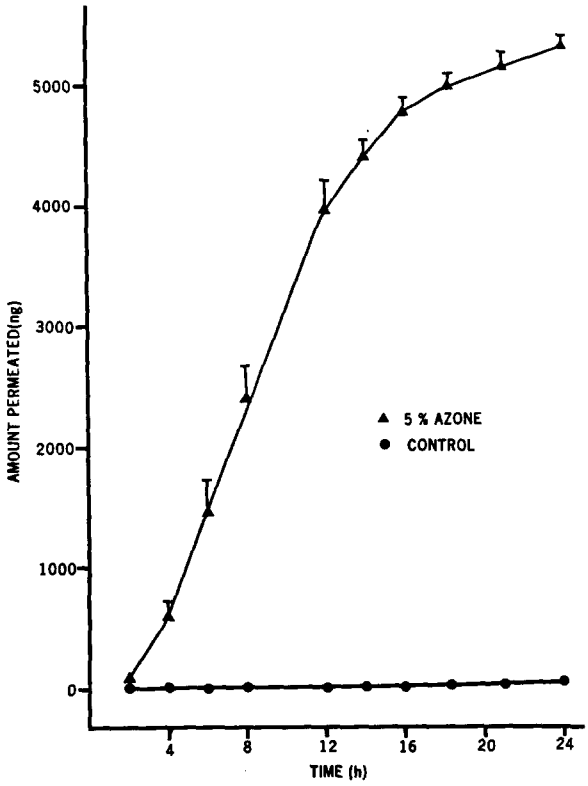

Fig. 2. In vitro skin permeation-time profile of vasopressin (mean ± S.E.M.) through hairless mouse skin.

TABLE 2

Permeation parameters of vasopressin through hairless mouse skin

Azone conc. (%, v/v)	Flux ($ng/cm^2 \cdot h$)	Lag time (h)
Control	2.07 ± 1.71 [a]	13.99 ± 2.55
5.0	134.07 ± 10.26	2.50 ± 0.746

[a] Values are mean ± S.D., $n = 3$.

TABLE 3

Permeability parameters in rat and hairless mouse in presence of Azone

Parameter	Control	5% Azone
Rat		
D_m (cm^2/h)	9.99×10^{-8}	10.4×10^{-8}
K_m	1.30	17.8
P_m (cm/h)	7.08×10^{-5}	1.01×10^{-3}
Hairless mouse		
D_m (cm^2/h)	0.944×10^{-8}	5.28×10^{-8}
K_m	3.60	41.6
P_m	3.82×10^{-5}	2.47×10^{-3}

in the hairless mouse was more pronounced (about 70 times) than in rats.

The diffusion coefficient of vasopressin through skin can be estimated from the lag time (t_L) (Flynn et al., 1974):

$$D_m = \frac{h^2}{6t_L} \quad (1)$$

where h is the barrier thickness. Assuming stratum corneum as the main rate-limiting barrier, h is 18.4 μm for rat and 8.9 μm for hairless mice (Bronaugh et al., 1982). Permeability coefficient (P_m) can be obtained by dividing the steady state flux (J_s) by donor phase concentration (C_s). Thus, skin/vehicle partition coefficient (K_m) can be calculated indirectly by the following equation:

$$K_m = \frac{P_m \cdot h}{D_m} \quad (2)$$

Table 3 reports these parameters for rat and hairless mice. In rat, Azone increased the skin/vehicle partitioning, but it has no effect on the diffusion coefficient of the drug; whereas, in hairless mouse, diffusion coefficient of vasopressin increased along with its skin/vehicle partitioning. This suggests that the mechanism of enhancement in the latter might be different. Hairless mice skin data show that enhancement by Azone occurs irrespective of the hair on the skin.

In vivo study was performed to confirm the significant enhancement of vasopressin absorption in presence of Azone as found in vitro. Vasopres-

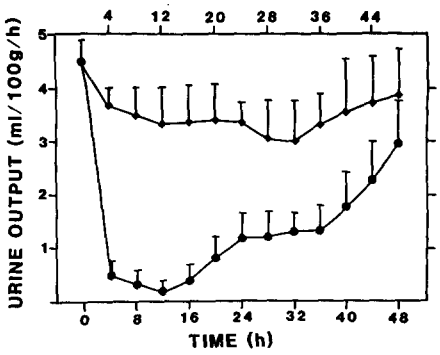

Fig. 3. Urine output (mean ± S.D.) in Brattleboro rats after topical application of vasopressin solution with (●) and without (◆) Azone.

sin delivery systems have been evaluated (Kruisbrink and Boer, 1984; 1986) in vivo in Brattleboro rats in the past. Brattleboro rats (Sokol and Valtin, 1982) are genetically deficient in vasopressin and secrete large volume of urine with low osmolality. Exogenous administration of vasopressin reduces their urine output and elevates urine osmolality (Boer et al., 1983) back to normal. Urinary output, urine osmolality and radioactivity excreted in the urine are represented graphically in Figs. 3–5, respectively. Radioactivity excreted after application of vasopressin solution alone was negligible or zero. Urinary output

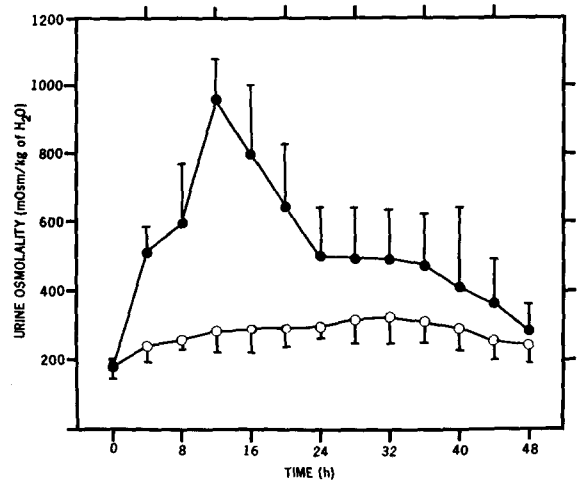

Fig. 4. Urine osmolality (mean ± S.D.) in Brattleboro rats after topical application of vasopressin solution with (○) and without (●) Azone.

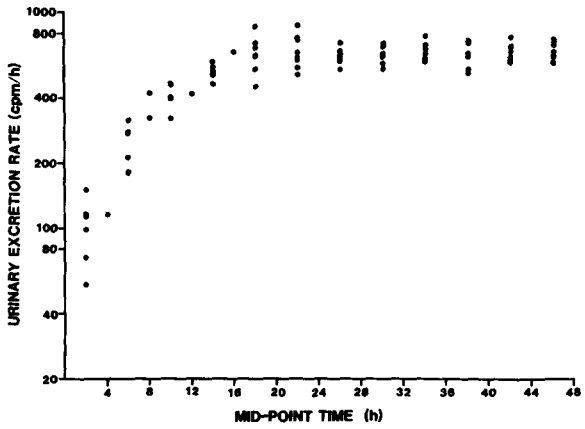

Fig. 5. Urinary excretion rate vs. mid-point time plot of radioactivity in Brattleboro rats after topical application of vasopressin solution containing Azone.

and urine osmolality data clearly show that in presence of Azone, vasopressin permeates the skin in significant amounts. The urinary excretion rate of radioactivity gradually reached a plateau by 12 h and remained constant thereafter although the effect on urine volume and osmolality decreased gradually after 24 h. This is probably due to a second stream of tritium excretion (contained in different tissues) not directly related to [^{3}H]vasopressin permeation (Kruisbrink and Boer, 1986).

Cheng et al. (1982), using osmotic minipumps for subcutaneous release, found a linear relation between the infusion rate of vasopressin and urine production of the Brattleboro rat but no correlation between infusion rate and urinary excretion of vasopressin. The gradual decrease in effect after 24 h could be due to permeation of Azone from the donor into the receptor phase. All the in vitro and in vivo studies were done under occlusive conditions to prevent evaporation of the solvent and consequent change in concentration. Hence it is difficult to speculate on the effect of Azone under non-occlusive conditions. It is possible that under occlusive conditions, Azone permeates the skin more readily. This will cause a reduced lag time under occlusion compared to non-occlusive conditions, but the steady state flux will remain unchanged. The release of Azone from the vehicle was not controlled. Controlled release of Azone along with controlled release of the drug is likely

to maintain the effect for longer than 24 h. This study demonstrates that transdermal delivery of medium size peptides such as vasopressin is feasible and future research in that direction is necessary.

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