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# Penetration enhancer effects on in vitro percutaneous absorption of heparin sodium salt

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## Summary

The effects of a number of penetration enhancers such as Azone, *N*-methylpyrrolidone, propylene glycol and oleic acid on the percutaneous absorption of heparin sodium salt through excised human skin were investigated. Franz-type diffusion cells were used for in vitro permeation experiments. Using skin pre-treated with the penetration enhancers, *N*-methylpyrrolidone, Azone and propylene glycol promoted heparin flux while oleic acid failed to do so. Studies of aqueous heparin solutions containing such enhancers confirm the enhancement effect of *N*-methylpyrrolidone and Azone while propylene glycol solutions do not increase heparin flux through the skin. Notwithstanding the high molecular weight of heparin, it does not seem to constitute a limiting factor for the efficiency of *N*-methylpyrrolidone and Azone.

## Introduction

Drug diffusion through the horny layer, which usually constitutes the rate-limiting or slowest step in the penetration process, can occur either through intercellular spaces, cells (transcellular pathway) or via a transappendageal route (eccrine and sebaceous glands and hair follicles) (Hadgraft, 1983). The relative importance of the various possible routes depends on many factors, among which the molecular weight may play an important role.

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Large polar molecules, which have very low diffusion coefficients through the stratum corneum and low partition coefficients from water, are supposed to pass through the skin mainly by means of the transappendageal route (shunt diffusion) (Barry, 1986). Because of the low fractional areas and volume of the appendages, it is unlikely that great amounts of large molecules could penetrate the skin (Idson, 1975).

One way of improving low drug permeability through human skin is to use penetration enhancers which reversibly modify the barrier properties of the skin (Barry, 1983). Although many investigators have studied a large number of skin penetration enhancers such as Azone, dimethyl sulphoxide, *N*-methylpyrrolidone, propylene gly-

col and oleic acid, there is a scarcity of data on the ability of enhancers to promote skin penetration of very large molecules.

Only recently have reports appeared of the ability of some penetration enhancers to increase skin permeation of large polar molecules like vasopressin (Mol. Wt 1084) (Banerjee and Ritschel, 1989) or macromolecules such as insulin (Mol. Wt 6000) (Priborsky et al., 1987).

The main objective of the present study was to investigate the ability of the penetration enhancers Azone, propylene glycol, oleic acid and *N*-methylpyrrolidone to increase in vitro percutaneous absorption through human skin of heparin sodium salt which is a macromolecule (average Mol. Wt 17000) commonly used in formulations for topical therapy of vascular permeability diseases, superficial thrombotic and other pathological symptoms (Tauschel et al., 1984).

By means of this study, it may be possible to evaluate whether the high molecular weight of heparin represents an upper limit to the ability of enhancers to promote skin permeation of macromolecules.

## Materials and Methods

*Materials*

[$^3$H(G)]Heparin sodium salt, with a specific activity of 0.49 mCi/mg, was supplied by NEN (U.K.). [$^3$H]Water (spec. act. 5 mCi/ml) was obtained from Amersham (U.K.). Azone was a gift from Whitby Research Inc. (Richmond, VA). Propylene glycol (PG), oleic acid (OA) and *N*-methylpyrrolidone (NMP) were obtained from Sigma Chemicals (St. Louis, MO). All other materials were of analytical grade.

*Skin membrane preparation*

Samples of adult human skin (mean age $36 \pm 8$ years) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at $60 \pm 1$°C for 2 min (Kligman and Christophers, 1963), after which stratum corneum and epidermis (SCE) were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at approx. 25% relative humidity (RH). The dried samples were wrapped in aluminium foil and stored at $4 \pm 1$°C until use. Preliminary experiments were carried out in order to assess SCE samples for barrier integrity by measuring the in vitro permeability of [$^3$H]water through the membranes using the Franz cells described below. The value of the permeability coefficient ($K_p$) for tritiated water was found to be $1.6 \pm 0.2 \times 10^{-3}$ cm/h which agreed well with those for tritiated water reported by others using human SCE samples (Bronaugh et al., 1986; Scott et al., 1986).

*Diffusion experiments*

Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz-type diffusion cells supplied by LGA (Berkeley, CA). The exposed skin surface area was 0.75 cm$^2$ and the receptor volume was 3.2 ml.

The receiver compartment contained normal saline which was stirred and kept at $35 \pm 1$°C during all the experiments. All the experiments were performed using [$^3$H(G)]heparin sodium salt (NEN). Stuttgen et al. (1990), using [$^3$H(G)]-heparin sodium salt (NEN) in human skin permeation studies, affirmed that no separation of bound tritium from the heparin molecule was observed.

Preliminary screening of enhancers was conducted by pretreating the excised skin for 12 h with 100 $\mu$l of enhancer. The following enhancers were studied: Azone, PG, PG:Azone 9:1, OA, NMP, PG:NMP 80:20. The 12 h pretreatment period was chosen because, as reported by Goodman and Barry (1989) and Sugibayashi et al. (1985), Azone does not appear to be effective when applied over short periods during in vitro pretreatment studies. After 12 h, the excess enhancer was wiped off with tissue paper, 400 $\mu$l of heparin sodium salt aqueous solution (concentration 1 mg/ml, 1 $\mu$Ci/ml) was applied to the stratum corneum (SC) surface and the experiment was run for 24 h. Samples of the receiving

solution were withdrawn periodically, mixed with Instagel scintillation cocktail (Packard, U.S.A.) and counted on a Beckman LS 9800 series liquid scintillation counter. The sample volumes were replaced with fresh solutions. The same kind of experiment was carried out leaving the skin untreated as control to determine heparin sodium salt permeation parameters. On the basis of the results obtained with the pretreatment technique, heparin solutions containing various percentages of NMP, Azone and PG were prepared. The composition of the solutions is reported in Table 2. Different percentages of ethanol were added to the solutions containing Azone in order to solubilize this enhancer in the vehicle. To assess whether ethanol incorporation could affect heparin flux, experiments were carried out applying 50:50 water:ethanol solution to the skin. Heparin flux from this last solution was not found to differ significantly from that determined from aqueous solution. After having applied enhancer solutions to the skin, samples from the receiving compartment were withdrawn at intervals during the 24 h run and the same procedure as described above was followed.

*Calculation of results*

The cumulative amount of heparin sodium salt penetrating the skin was plotted against time. A linear profile (steady state) was observed only during the 24 h period and the slope of the linear portion of the curve and the $x$-intercept values (lag time) were determined by linear regression. Drug flux ($\mu g/cm^2$ per h), at steady state, was calculated by dividing the slope of the linear portion of the curve by the area of the skin surface through which diffusion took place.

The effectiveness of penetration enhancers was determined by comparing heparin flux in the presence and absence of enhancers. This was defined as the enhancement factor (E.F.).

$$\text{E.F.} = \frac{\text{heparin flux at steady state in the presence of enhancers}}{\text{heparin flux at steady state in the absence of enhancers}}$$

## Results and Discussion

Heparin mean flux values at steady state from control solution were found to be $0.034 \pm 0.010$ $\mu g/cm^2$ per h. Schaefer et al. (1982), determining the in vitro heparin flux value through human skin from a gel containing 0.1% of radiolabeled heparin, obtained a flux value of $6.9 \times 10^{-12}$ $mol/cm^2$ per h. This value, assuming that the mean molecular weight of heparin was 17000, corresponds to 0.117 $\mu g/cm^2$ per h and is about 3-fold higher than that observed in our experiments. Mean heparin flux values determined after pretreatment of skin with enhancers are listed in Table 1. As shown in Table 1, Azone, propylene glycol and NMP were able to increase the flux of heparin sodium salt through adult human skin while oleic acid failed. NMP showed the best E.F. (10.09) using the pretreatment technique. When skin was pretreated with Azone or PG:Azone 9:1, the flux of heparin increased 3.41- and 7.47-fold, respectively, compared to the control solution. Thus, propylene glycol showed a synergic effect on Azone's ability to promote skin permeation of heparin, as pointed out previously by several investigators for other drugs (Touitou and Abed, 1985; Sheth et al., 1986). In contrast, propylene glycol did not display a synergic effect on NMP enhancement, therefore skin pretreatment with PG:NMP 9:1 did not increase heparin flux as compared to NMP alone. Heparin fluxes after skin pretreatment with PG:Azone 9:1 or NMP were quantitatively comparable. Lag time values with different enhancers were close to those obtained with the control solution although other investigators observed shorter lag times after the enhancers had been used to pretreat skin in in vitro experiments (Chow et al., 1984; Sugibayashi et al., 1985). The lack of lag time reduction could be ascribed to the initial rapid penetration of heparin sodium salt through the skin. Schaefer et al. (1982) and Stuttgen et al. (1990) reported that, in in vitro permeation experiments, heparin showed a rather short lag time and that a steady state was reached rapidly.

Taking into account the results for the pretreatment technique, we prepared different heparin solutions containing Azone, PG and NMP in

TABLE 1

*Steady-state flux, lag time and enhancement factor of heparin after skin pretreatment with enhancers*

| Enhancer | Flux ± S.D. ($\mu g/cm^2$ per h) | Lag time (h) | E.F. |
|---|---|---|---|
| Control [a] | 0.034 ± 0.010 | 1.98 ± 1.11 | 1.00 |
| Azone | 0.116 ± 0.030 | 2.51 ± 0.31 | 3.41 |
| PG | 0.196 ± 0.024 | 1.00 ± 0.44 | 5.76 |
| PG-Azone 9:1 | 0.254 ± 0.008 | 2.14 ± 0.20 | 7.47 |
| OA | 0.046 ± 0.017 | 2.12 ± 0.17 | 1.35 |
| NMP | 0.343 ± 0.152 | 2.91 ± 1.23 | 10.09 |
| NMP-PG 20:80 | 0.324 ± 0.132 | 2.68 ± 0.83 | 9.53 |

[a] Control: 12 h preincubation of skin in contact with normal saline in the receptor phase. There was no enhancer on the donor side. $n = 3$.

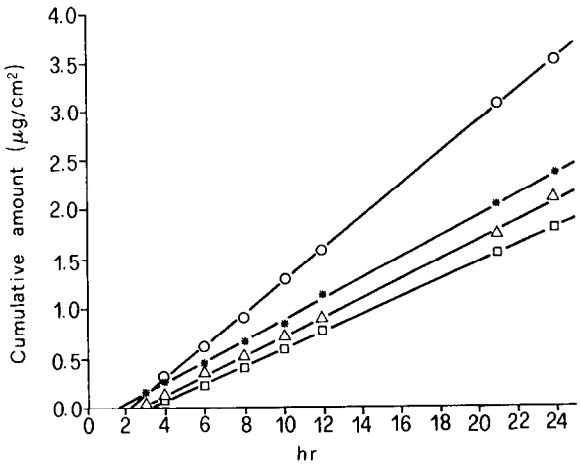


Fig. 1. Effect of different PG percentages on heparin percutaneous absorption from solutions containing 5% Azone. (○) 20% PG, (△) 40% PG, (□) 60% PG, (*) control.

order to determine their ability to increase heparin flux from solutions. Solution composition and percutaneous absorption parameters are listed in Table 2. As shown in Table 2, heparin flux from a hydroalcoholic solution containing 5% Azone was higher than from the control solution. Since the incorporation of PG synergistically influences the effectiveness of Azone, we prepared 5% Azone solutions containing various percentages of PG (20, 40, 60%). As can be seen in Fig. 1, a synergic effect was observed only for the solution containing 20% PG (EF = 4.8): an optimum PG concentration to increase the efficacy of Azone has already been noted by others (Priborsky et al., 1987). Probably, a concentration of PG over 20% produces a decrease in thermodynamic activity and disfavors heparin partitioning in the skin. In any case, the synergic effect of PG was not pronounced as was expected on the basis of the data obtained with the pretreatment technique.

TABLE 2

*Steady-state flux, lag time and enhancement factor of heparin from solutions containing different percentages of enhancers*

| Vehicle composition (% v/v) | | | | | Flux ± S.D. [a] ($\mu g/cm^2$ per h) | Lag time (h) | E.F. |
|---|---|---|---|---|---|---|---|
| PG | Azone | NMP | Ethanol | $H_2O$ | | | |
| – | – | – | – | 100 | 0.034 ± 0.010 | 1.98 ± 1.11 | 1.00 |
| – | 5 | – | 80 | 15 | 0.104 ± 0.006 | 1.55 ± 0.98 | 3.06 |
| 20 | 5 | – | 55 | 20 | 0.163 ± 0.011 | 2.18 ± 0.65 | 4.80 |
| 40 | 5 | – | 45 | 10 | 0.096 ± 0.006 | 2.60 ± 1.06 | 2.82 |
| 60 | 5 | – | 25 | 10 | 0.086 ± 0.027 | 3.13 ± 0.89 | 2.53 |
| 20 | 1 | – | 59 | 20 | 0.060 ± 0.014 | 2.49 ± 0.73 | 1.76 |
| 20 | 10 | – | 50 | 20 | 0.174 ± 0.003 | 1.78 ± 0.46 | 5.12 |
| 20 | – | – | – | 80 | 0.039 ± 0.007 | 2.06 ± 0.81 | 1.15 |
| 40 | – | – | – | 60 | 0.032 ± 0.015 | 2.75 ± 0.54 | 0.94 |
| – | – | 5 | – | 95 | 0.205 ± 0.019 | 3.24 ± 1.36 | 6.03 |
| – | – | 10 | – | 90 | 0.282 ± 0.015 | 2.35 ± 0.71 | 8.29 |
| – | – | 20 | – | 80 | 0.249 ± 0.008 | 2.86 ± 0.99 | 7.32 |
| 20 | – | 10 | – | 70 | 0.046 ± 0.017 | 2.06 ± 0.21 | 1.35 |
| 40 | – | 10 | – | 50 | 0.028 ± 0.009 | 2.18 ± 0.34 | 0.82 |

[a] $n = 3$.

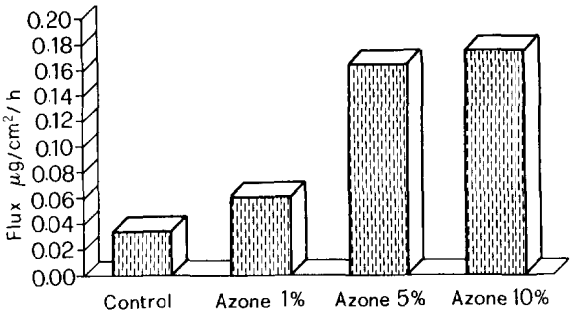


Fig. 2. Effect of different Azone percentages on heparin flux from solutions containing 20% PG.

As Azone concentrations in the range of 1–10% have been reported to be the most effective (Wiechers and De Zeeuw, 1990), we prepared different solutions containing 1, 5 and 10% Azone with similar proportions of PG (20%) in order to evaluate the optimum Azone concentration (see Table 2). As shown in Fig. 2, heparin flux from solutions containing different percentages of Azone increased compared to the control solution. No additional or reduced heparin permeation was observed at Azone concentrations above 5%. This pattern is in agreement with the data reported by other authors (Vaidyanathan et al., 1987; Banerjee and Ritschel, 1989) who observed a plateau in Azone concentration for percutaneous absorption of hydrophilic compounds.

In contrast to what was expected on the basis of the data obtained in pretreatment experiments, 20 and 40% PG aqueous solutions did not increase heparin flux compared to the control solution. To assess the enhancement effect of NMP, we used 5, 10 and 20% NMP aqueous solutions. As shown in Table 2, a significant increase in heparin flux was observed, particularly when using 10% NMP. Thus, NMP is as effective in the pretreatment as in solution.

Priborsky et al. (1987), studying the effect of some enhancers on in vitro percutaneous absorption of insulin from aqueous solutions through newborn pig skin, emphasized the need for PG to promote NMP action, therefore we added 20 and 40% PG to 10% NMP solution. Measurements on these last solutions showed that NMP failed to enhance heparin flux (see Fig. 3). In a study of

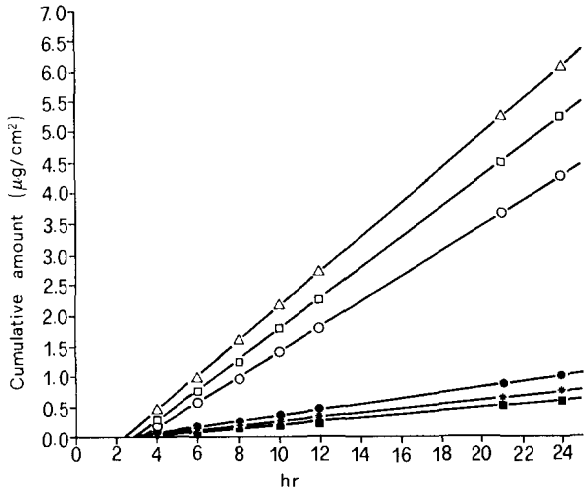


Fig. 3. Effect of NMP and PG on heparin skin penetration from solutions containing different percentages of enhancers. (○) 5% NMP, (△) 10% NMP, (□) 20% NMP, (●) 10% NMP-20% PG, (■) 10% NMP-40% PG, (*) control.

the effect of NMP on metronidazole, Hoelgaard et al. (1988) reported a similar pattern and explained this effect of NMP as being due to slow release from the PG vehicle to the skin, presumably because of a specific enhancer-vehicle interaction.

The values of lag times after skin application of heparin solutions containing the enhancers studied were not significantly different compared to the control solution. Hence, we observed the same trend as for pretreatment values.

The enhancement factors for NMP, Azone and PG : Azone, summarized in Fig. 4, show the ap-

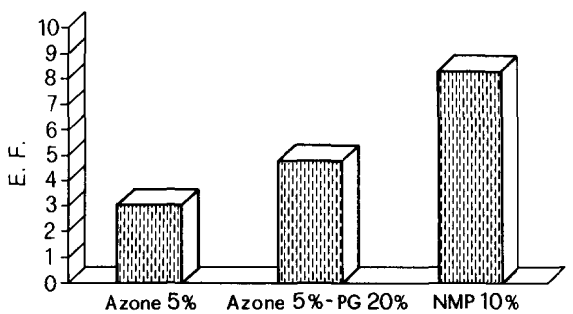


Fig. 4. Enhancement factors (E.F.) obtained for heparin solutions containing 5% Azone, 5% Azone-20% PG and 10% NMP.

preciable efficacy of 10% NMP solution and Azone 5%-PG 20% solution in increasing in vitro heparin flux. Penetration enhancers may act by altering the diffusion characteristics of the skin or by modifying the SC/vehicle partitioning behaviour of the drug.

The diffusion coefficient ($D_m$) of a permeant 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 that the stratum corneum is the main rate-limiting barrier, $h$ is 16.8 $\mu$m for human skin (Bronaugh et al., 1982). The permeability coefficient ($P_m$) can be evaluated by dividing the steady-state flux ($J$) by the donor phase concentration ($C_s$). Therefore, the SC/vehicle partition coefficient can be calculated indirectly from the equation:

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

These parameters, as calculated for the permeation of heparin, are listed in Table 3. As shown in Table 3, Azone increased SC/vehicle heparin partitioning but did not affect the diffusion coefficient of the drug.

Notwithstanding the suggestion of several authors (Goodman and Barry, 1986; Beastall et al., 1988; Bouwstra et al., 1989) that Azone exerts its effect on the drug diffusion coefficient by altering the packing of the bilayer tails within the intercellular space, other workers (Okamoto et al., 1988; Banerjee and Ritschel, 1989: Bhatt et al., 1991) have pointed out that for different drugs the mechanism of enhancement for Azone seems to be improved partitioning of the drug into the skin.

Recently, Ward and Tallon (1988), using small-angle X-ray diffraction and $^2$H-NMR measurements, have concluded that the mechanism by which Azone promotes the penetration of hydrophilic drugs is related to its ability to increase the water capacity of the stratum corneum lipid matrix, providing, in this way, a greater potential reservoir for hydrophilic drug molecule absorption and increased SC/vehicle partitioning for these molecules.

As for NMP, this enhancer is believed either to increase drug diffusion through the skin or, due to its strong solvent power, to improve partitioning into the skin (Barry, 1988). In our studies, the ability of NMP to increase heparin flux may be related to the solvent capacity of this enhancer which improves SC/vehicle heparin partitioning without affecting the diffusion coefficient (see Table 3).

In conclusion, NMP and Azone are capable of enhancing the in vitro percutaneous absorption of heparin despite its high molecular weight. Consequently, in our study, the high molecular weight of heparin did not constitute an upper limit to the efficacy of these enhancers.

The promising results obtained invite further studies to confirm the in vitro and in vivo efficiency of these enhancers in increasing the transdermal flux of heparin in topical formulations.

TABLE 3

*Heparin permeability parameters from aqueous solution in the presence of NMP and Azone-PG*

| Parameter | Control | 5% Azone-20% PG | 10% NMP |
|---|---|---|---|
| $P_m$ (cm/h) | $3.4\times10^{-5}$ | $16.3\times10^{-5}$ | $29.2\times10^{-5}$ |
| $K_m$ | 0.24 | 1.27 | 2.44 |
| $D_m$ (cm$^2$/h) | $2.3\times10^{-7}$ | $2.15\times10^{-7}$ | $2.01\times10^{-7}$ |

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