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Effects of some non-toxic penetration enhancers on in vitro heparin skin permeation from gel vehicles

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Abstract

The effects of some penetration enhancers with low toxicity such as Transcutol, propylene glycol dipelargonate (DPPG), soybean lecithin and d-limonene on the in vitro percutaneous absorption of heparin sodium salt through human skin were investigated. Using the pretreatment technique, all the enhancers tested increased heparin skin permeation with the exception of Transcutol. Measuring heparin flux from Carbopol gels containing such promoters showed that soybean lecithin, DPPG and d-limonene were able to enhance heparin skin penetration while Transcutol was not. To explain the mechanism of the effective promoters, the heparin diffusion and partitioning coefficients from the gels containing the enhancers were calculated. The results indicated that soybean lecithin and DPPG could act by increasing the heparin diffusion coefficient while d-limonene seemed to exert its enhancement effect on heparin skin/vehicle partitioning.

Key words: Heparin sodium salt; Skin permeation; Nontoxic penetration enhancer; In vitro permeation

1. Introduction

Heparin sodium salt is a macromolecule (average Mol. Wt 17000) commonly used for the topical therapy of vascular permeability diseases, superficial thrombotic and other pathological symptoms (Tauschel et al., 1984). Notwithstanding its high molecular weight, heparin has been reported to penetrate human skin from gel formulations, although to a low extent (Schaefer et al., 1982).

One of the most commonly used ways to increase the topical efficacy of drugs is to facilitate their penetration through the skin by using penetration enhancers (Barry, 1983).

In a previous paper of ours (Bonina and Montenegro, 1992), we pointed out that the heparin's high molecular weight did not affect the efficiency of some skin penetration enhancers, such as propylene glycol with Azone and *N*-methylpyrrolidone.

One of the major limitations of such enhancers is their potential toxicity and skin irritancy (Hadgraft, 1989).

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In this paper we investigated the ability of some penetration enhancers with low toxicity and therefore suitable for clinical use, such as d-limonene, Transcutol (diethylene glycol monoethyl ether), DPPG (propylene glycol dipelargonate) and soybean lecithin, to increase in vitro heparin skin permeation.

In the literature, it has been reported that these enhancers increase the skin permeation of many different drugs: d-limonene and Transcutol improved the skin permeation of anti-inflammatory drugs (Okabe et al., 1989) and theophylline (Touitou et al., 1991), respectively, while soybean lecithin and DPPG increased the skin penetration of procaterol (Mahjour et al., 1990) and morphine (Rojas et al., 1991), respectively. Furthermore, the low toxicity of these enhancers has already been well established: soybean lecithin and d-limonene have been approved by the FDA for food use while Transcutol and DPPG have been accepted for cosmetic use (Gattefossè, 1982).

The enhancer effects on heparin flux through excised human skin were studied first, by using the pretreatment technique, and then from Carbopol gels which represent the most commonly used formulation for topical heparin administration.

2. Materials and methods

2.1. Materials

[^{3}H(G)]Heparin sodium salt, with a specific activity of 0.49 mCi mg^{-1} was supplied by NEN (U.K.). d-Limonene was obtained from Carlo Erba, Italy. Transcutol and DPPG were a gift from Gattefossè (France). Soybean lecithin was purchased from Sigma Chemicals (St. Louis, MO). Carbopol 934 was supplied by Biochim (Italy). All other materials were of analytical grade.

2.2. Preparation of aqueous gel formulations

The heparin gel composition is reported in Table 1. The gels were prepared as previously described (Bonina and Montenegro, 1992). The enhancers were dissolved in ethanol or in water depending on their solubility. The gels were stored at room temperature for 24 h under air-tight conditions prior to use.

Table 1
Heparin gel composition (% w/w)

Constituents	Gel code				
	A	B	C	D	E
Carbopol 934	0.8	0.8	0.8	0.8	0.8
Ethanol	50	50	50	50	50
Triethanolamine	0.9	0.9	0.9	0.9	0.9
d-Limonene	–	1	–	–	–
Transcutol	–	–	18	–	–
DPPG	–	–	–	5	–
Lecithin	–	–	–	–	1
Heparin [a]	0.1	0.1	0.1	0.1	0.1
Distilled water	48.2	47.2	30.2	42.2	47.2

[a] [^{3}H(G)]Heparin sodium salt was added to unlabelled heparin sodium salt to give an activity of 4 μCi mg^{-1} of heparin.

2.3. In vitro skin permeation experiments

Samples of adult human skin (mean age 42 ± 9 years) were obtained from breast reduction operations. Membranes consisting of stratum corneum and epidermis (SCE) were obtained from whole skin samples, stored and assessed for barrier integrity as previously described (Bonina and Montenegro, 1992). In vitro heparin skin permeation was evaluated using Franz cells (LGA, Berkeley, CA) with a receptor volume of 3.2 ml and a skin surface area available for diffusion of 0.75 cm^2. The receptor compartment contained normal saline which was stirred and thermostated at 35 ± 1°C during all the experiments.

To facilitate quantitation, all the experiments were performed using [^{3}H(G)]heparin sodium salt (Stuttgen et al., 1990). Pretreatment was performed by applying 100 μl of enhancer on the skin surface. The following enhancers were studied: Transcutol/ethanol 20:80, DPPG/ethanol 5:95, d-limonene/ethanol 1:99, lecithin 1% water suspension. After 12 h, the excess enhancer was wiped off with tissue paper, 400 μl of heparin sodium salt aqueous solution (1 mg/ml, 4 μCi/ml) was applied to the stratum corneum surface and the experiment was run for 24 h. Samples of the receiving solution were withdrawn

at intervals, mixed with Instagel scintillation cocktail (Packard, U.S.A.) and counted on a Beckman LS 9800 series liquid scintillation counter to assess the heparin content. The sample volumes were replaced with fresh solutions. Heparin sodium salt permeation parameters without applying any enhancer on the skin have been previously determined (Bonina and Montenegro, 1992). In a further series of experiments, Carbopol gels (200 mg) containing the enhancer being tested were applied on the skin surface and the same procedure as described above was followed.

2.4. Calculations

Heparin flux through the skin was calculated by plotting the cumulative amount of drug penetrating the skin against time and determining the slope of the linear portion of the curve and the x-intercept values (lag time) by linear regression analysis. 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 (enhancement factor, E.F.) was determined by comparing heparin flux in the presence and absence of enhancers:

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

3. Results and discussion

Heparin flux values obtained after skin pretreatment with the enhancers are listed in Table 2. As may be noted from the E.F. values, d-limonene, soybean lecithin and DPPG increased heparin skin penetration compared with the control while Transcutol did not produce any significant flux enhancement.

As may be noted from Table 2, the heparin lag time value from the control solution was quite short. Other authors (Schaefer et al., 1982; Stuttgen et al., 1990) reported that, in in vitro skin permeation experiments, heparin showed a rather short lag time and that the steady state was achieved rapidly.

Table 2
Heparin steady-state flux, lag time and enhancement factor after skin pretreatment with different enhancers

Enhancer [a]	Flux ± S.D. [b] ($\mu g\ cm^{-2}\ h$)	Lag time (h)	E.F.
Control [c]	0.034 ± 0.010	1.98	1.00
LIM/EtOH 1:99	0.638 ± 0.099	0.70	18.76
TSC/EtOH 20:80	0.046 ± 0.008	2.36	1.35
SL	0.516 ± 0.087	0.43	15.18
DPPG/EtOH 5:95	0.342 ± 0.052	0.11	10.06

[a] LIM, d-limonene; EtOH, ethanol; TSC, Transcutol; SL, soybean lecithin 1% water suspension.
[b] $n = 3$.
[c] Data previously reported (Bonina and Montenegro, 1992). The skin was left in contact for 12 h with normal saline in the receptor phase and no enhancer in the donor side.

Lag time values after skin pretreatment with the enhancers were lower than those obtained with the control solution with the exception of Transcutol which did not significantly modify the heparin lag time value. A lag time reduction after in vitro skin pretreatment with effective enhancers has already been reported by several authors for other drugs (Chow et al., 1984; Sugibayashi et al., 1985).

Since the most common topical heparin dosage forms are aqueous Carbopol gels, we believed it would be worth investigating the effectiveness of

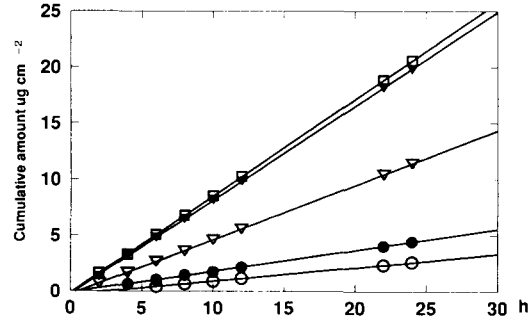

Fig. 1. Effect of different enhancers on heparin skin permeation from Carbopol gels. (●) Control; (△) d-limonene; (○) Transcutol; (□) DPPG; (▲) soybean lecithin.

Table 3
Heparin steady-state flux through excised human skin, lag time and enhancement factor from Carbopol gels containing different enhancers

Gel code	Flux ± S.D. [a] ($\mu g\ cm^{-2}\ h$)	Lag time (h)	E.F.
A [b]	0.189 ± 0.059	0.50	1.00
B	0.488 ± 0.053	0.58	2.58
C	0.122 ± 0.043	2.57	0.65
D	0.863 ± 0.063	0.14	4.57
E	0.834 ± 0.078	0.20	4.41

[a] Each experiment was run in duplicate on three different donors.
[b] Data previously reported (Bonina and Montenegro, 1994).

these enhancers in increasing heparin in vitro skin permeation from this type of formulation.

A plot of heparin cumulative amount permeated through the skin from Carbopol gels with and without enhancers as a function of time is reported in Fig. 1.

Heparin percutaneous absorption parameters from Carbopol gels are reported in Table 3. As may be noted, heparin flux from the Carbopol gel without enhancers (control gel) (Bonina and Montenegro, 1992) was greater than from the control aqueous solution.

Other workers (Nishihata et al., 1988) observed higher drug percutaneous absorption from aqueous gels as compared to aqueous solutions containing the same drug concentration. In our case, this effect could be explained by a likely increase of heparin thermodynamic activity in the aqueous control gel as compared to aqueous solutions, since the gel contained ethanol which decreases heparin solubility. However, the heparin flux determined in our experiments from Carbopol control gel was close to that reported by Schaefer (1982) for 0.1% heparin gels.

As can been seen in Table 3, d-limonene, DPPG and soybean lecithin increased heparin skin permeation from Carbopol gels compared with the control gel while Transcutol gels did not show any heparin flux enhancement, thus confirming the ineffectiveness of this enhancer already observed with the pretreatment technique.

The literature contains conflicting reports as to whether Transcutol increases skin permeability. So, Transcutol showed an enhancing effect on the in vitro skin permeation of prostaglandin (Watkinson et al., 1991) and theophylline (Touitou et al., 1991) while it was not able to promote morphine flux through the skin (Rojas et al., 1991). Vasopressin is the only compound with a high molecular weight (1084) for which Transcutol has been assayed as a skin penetration enhancer, however, this promoter did not produce any significant increase in percutaneous penetration (Banerjee and Ritschel, 1989). This last report together with the lack of Transcutol enhancement efficacy in our study suggest that the high molecular weight could represent one of the limitations for Transcutol effectiveness. The enhancement factors determined for the heparin gels containing the enhancers studied (reported in Table 3) demonstrate the appreciable effectiveness of soybean lecithin and DPPG which produced a similar heparin skin penetration increase (E.F. 4.41 and 4.57, respectively) and were more effective than d-limonene (E.F. 2.58).

Lag time values after application to the skin of heparin Carbopol gels containing the enhancers tested were lower for soybean lecithin, not significantly different for DPPG and higher for d-limonene and Transcutol as compared to the control gel. Penetration enhancers may act by altering the drug diffusion coefficient (D_m) or by modifying the drug stratum corneum/vehicle partitioning (K_m).

In an attempt to explain the enhancer mechanisms which proved effective in our study, we calculated heparin K_m and D_m values from the gels containing the effective promoters.

The diffusion coefficient was calculated based on the equation (Flynn et al., 1974):

$$D_m = h^2/6t_L$$

where h is the barrier thickness which was taken as equal to 16.8 μm, assuming that the stratum corneum is the main rate-limiting barrier (Bronaugh et al., 1982), and t_L denotes the lag time.

The stratum corneum/vehicle partition coefficient was obtained from the equation:

$$K_m = P_m h/D_m$$

Table 4
Heparin skin permeation parameters from Carbopol gels containing d-limonene, soybean lecithin and DPPG

Enhancer	Parameter		
	K_p (cm h^{-1})	K_m	D_m (cm^{-2} h^{-1})
Control [a]	18.9×10^{-5}	0.34	9.4×10^{-7}
Limonene	48.8×10^{-5}	1.01	8.1×10^{-7}
Lecithin	83.4×10^{-5}	0.59	2.3×10^{-6}
DPPG	86.3×10^{-5}	0.43	3.4×10^{-5}

[a] Data previously reported (Bonina and Montenegro, 1994).

where P_m is the permeability coefficient calculated by dividing the steady-state flux by the drug concentration in the gels.

These parameters, as calculated for heparin skin permeation, are reported in Table 4. As may be noted, d-limonene seems to exert its enhancement activity mainly by increasing heparin partitioning into the stratum corneum. Williams and Barry (1990) reported that terpenes can act by a dual mechanism of action: increasing the diffusion coefficient or the partitioning into the skin, due to the presence of the enhancer in the membrane. In the case of heparin, the second mechanism seems to be predominant. In contrast, soybean lecithin appears to act by increasing the heparin diffusion coefficient without significantly affecting heparin partitioning into the stratum corneum. This mechanism is consistent with the findings of other authors (Mahjour et al., 1990) who reported that the soybean lecithin enhancement effect was mainly due to the reduction of skin resistance to drug permeation and not to an increase in drug partitioning.

As for DPPG, its enhancement mechanism may be explained by an increase in heparin diffusion coefficient. A similar DPPG effect on drug skin penetration has been reported by Rojas et al. (1991), who observed that the DPPG vehicle increased the morphine diffusion coefficient and shortened lag time values.

In conclusion, among the penetration enhancers with low toxicity tested in this study, DPPG, soybean lecithin and d-limonene increased heparin in vitro skin penetration using both the pretreatment technique and Carbopol gels, while Transcutol did not show any enhancement effect.

On the basis of the heparin diffusion and partition coefficients from the gels containing the effective enhancers, DPPG and soybean lecithin seem to act by increasing the drug diffusion coefficient while d-limonene may predominantly affect the amount of drug in the stratum corneum.

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