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Vehicle effects on in vitro heparin release and skin penetration from different gels

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Summary

The effects of different gel bases, such as Carbopol 934, Pluronic F-127 and Methocel J5MS, on both heparin release from gel formulations and its penetration through excised human skin were investigated. Heparin release rate from the gel examined increased in the order: Methocel < Pluronic < Carbopol and no inverse correlation existed between the heparin apparent diffusion coefficient and the gel viscosity values. Heparin steady state flux values through human skin were not affected by the different gel base used and were lower than the release rates from the gels. Furthermore, the ability of Azone and *N*-methylpyrrolidone to increase heparin skin penetration from gels was assessed. The addition of 5% Azone or 10% *N*-methylpyrrolidone to Carbopol gels increased heparin flux through the skin about 4-fold compared to control gels.

Introduction

The therapeutic efficacy of a topically applied drug depends on its ability to penetrate the skin and be accumulated in the deeper layers of the skin. The extent of this absorption varies depending on both the physicochemical properties of the penetrant and its formulation. The vehicle composition can affect both drug release and skin permeability properties.

Heparin sodium salt is a macromolecule (average Mol. Wt 17000) commonly used for topical therapy of vascular permeability diseases, superficial thrombotic and other pathological symptoms (Tauschel et al., 1984). Schaefer et al. (1982) proved that, notwithstanding its high molecular weight, heparin is able to penetrate human skin from gel formulations, although to a low extent. Since the most commonly used topical heparin formulations are aqueous gels, in this study we assessed the effects of different gel bases such as Carbopol, Methocel and Pluronic on both heparin release rate and its penetration through excised human skin. Furthermore, since in a previous paper (Bonina and Montenegro, 1992) we proved that Azone and *N*-methylpyrrolidone increased in vitro heparin flux through human skin from aqueous solutions, we also considered it

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worthwhile to investigate the ability of Azone and *N*-methylpyrrolidone to increase in vitro heparin permeation from aqueous gels.

Materials and Methods

[^{3}H(G)]Heparin sodium salt with a specific activity of 0.49 mCi/mg was obtained from NEN (U.K.). [^{3}H]Water with a specific activity of 5 mCi/ml was purchased from Amersham (U.K.). Azone was a gift from Whitby Research Inc. (Richmond, VA). Propylene glycol (PG) and *N*-methylpyrrolidone (NMP) were bought from Sigma (St. Louis, MO). Carbopol 934 was supplied by Biochim (Italy). Pluronic F127 was a gift from BASF (Germany). Methocel J5MS was supplied by Dow Chemical (U.S.A.). All other reagents were of analytical grade. Cellulose acetate membranes (Spectra/Por CE; Mol. Wt cut off, 100000) were bought from Spectrum (Los Angeles, CA).

Preparation of aqueous gel formulations

The composition of the heparin sodium salt gels used in this study is reported in Table 1. Aqueous Carbopol gels were prepared by dispersing 0.8% w/w Carbopol 934 in water containing 0.1% w/w heparin sodium salt (4 μCi/mg) with constant stirring. The dispersion was then neutralized and made viscous by the addition of triethanolamine. Carbopol gels containing the enhancers to be tested were prepared according to the method reported by Okabe et al. (1990). Ethanol was used in order to solubilize Azone in the vehicle.

Pluronic F127 25% (w/v) gels were prepared in accordance with the cold method reported by Schmolka (1972). A suitable amount of Pluronic F127 was slowly added to cold distilled water (5–10°C) containing 0.1% w/v heparin sodium salt (4 μCi/mg) and constantly agitated with a magnetic stirrer; the dispersion was left overnight in a refrigerator to complete dissolution. The gel was formed when the solution was brought back to room temperature.

Methocel J5MS gels (4%) were obtained by slowly adding the appropriate amount of methylcellulose to water containing 0.1% w/v heparin sodium salt (4 μCi/mg) under constant stirring.

All the gels were stored at room temperature for 24 h under air-tight conditions prior to use.

Viscosity measurements

A Brookfield RVT viscosimeter was used to measure the viscosities (in cps) of the gels prepared. A spindle (no. 7) was rotated at 20 rpm. Samples of the gels were left to settle over 30 min at the assay temperature (37°C) before measurements were taken.

Release studies

Heparin release rates from the gels were measured through cellulose acetate membranes using

TABLE 1

Composition (%w/w) of the gels used in heparin release and percutaneous absorption studies

Constituents	Gel code						
	A	B [a]	C	D	E	F	G
Carbol 934	0.8	–	–	0.8	0.8	0.8	0.8
Pluronic F127	–	25	–	–	–	–	–
Methocel J5MS	–	–	4	–	–	–	–
Ethanol	50	–	–	30	30	50	30
Triethanolamine	0.9	–	–	0.9	0.9	0.9	0.9
PG	–	–	–	20	20	–	20
Azone	–	–	–	–	5	–	–
NMP	–	–	–	–	–	10	10
Heparin [b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water	48.2	74.9	95.9	48.2	43.2	38.2	38.2

[a] Gel composition is expressed as % w/v.

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

Franz diffusion cells (Franz, 1975). Shah et al. (1989), studying in vitro release of hydrocortisone through cellulose acetate membranes from creams, reported that the use of Franz diffusion cells provided a reliable and reproducible method for estimating drug release from topical dosage forms. Franz cells with a receiver compartment volume of 4.5 ml and an effective diffusion area of 0.75 cm^2 were used in this study. The receptor phase (normal saline) was continuously stirred and thermostated at 37°C during the experiments. Approx. 300 mg of each gel was placed on the membrane surface previously moistened with the receptor phase. Samples (50 μl) of the receptor phase were periodically withdrawn and replaced with an equal volume of fresh normal saline equilibrated at the experimental temperature. Heparin content was measured by mixing the samples with Insta-Gel scintillation cocktail (Packard, U.S.A.) and counting with a liquid scintillation counter (Beckman LS 9800 series; 50–55% efficiency). Studies were performed in triplicate runs and the mean values were used for the analysis of data.

In vitro skin permeation experiments

Samples of human adult skin (mean age 41 $\pm$ 6 years) were obtained from breast reduction operations. Stratum corneum and epidermis (SCE) were removed from the dermis, stored and assessed for the barrier integrity as described in a previous paper (Bonina and Montenegro, 1992). In vitro heparin skin permeation was evaluated using the same Franz diffusion cells described above. The receptor phase (normal saline) was stirred and thermostated at 37°C during the experiment. 200 mg of aqueous gel formulation was placed on the skin surface in the donor compartment and the latter was covered with Parafilm. Each experiment was run for 24 h. At intervals, samples of the receiving solution were withdrawn and assessed for heparin content as described above.

Calculation of results

Heparin release rates were calculated using the simplified Higuchi diffusion equation (Eqn 1) (Chi and Jun, 1991) which depicts the drug release from one side of a semisolid layer in which the drug is completely dissolved:

$$q = 2C_0(Dt/\pi)^{1/2} \quad (1)$$

where q is the amount of drug released into the receptor phase per unit area of exposure, C_0 denotes the initial drug concentration in the vehicle, D is the apparent diffusion coefficient of drug, and t represents the time elapsed since the start of drug release.

Heparin fluxes through the skin were calculated using linear regression analysis, by plotting the cumulative amount of drug permeated vs time and dividing the slope of the steady-state portion of the graphs by the area of the skin surface through which diffusion took place. The lag time was determined from the x-intercept values of the regression lines. The effectiveness of penetration enhancers (enhancement factor: EF) was determined by comparing heparin flux in the presence and absence of enhancers:

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

Statistical analysis of the data was performed using Student's t-test.

Results and Discussion

The ability of a drug to exert its topical therapeutic action depends primarily on two consecutive physical events. First, the drug must be able to diffuse from the vehicle to the skin surface and second, the drug must be able to penetrate the skin. Both these processes can influence the percutaneous absorption rate, the slowest step being rate-limiting.

In order to evaluate which of these two processes was the rate-limiting step in heparin percutaneous absorption, we determined the rate of heparin release from Pluronic F127, Methocel J5MS and Carbopol 934 gels and its flux through excised human skin. As can be seen in Fig. 1,

TABLE 2

Amount released, diffusion coefficients, fluxes through the skin of heparin and viscosity of the gels

Parameter	Gel code		
	A (Carbopol)	B (Pluronic)	C (Methocel)
Amount released for 9 h ($\mu g/cm^2$)	81.98	72.46	62.77
D (cm^2/h) ($\times 10^4$)	8.42	6.55	4.90
Viscosity (cps) ($\times 10^{-3}$)	60	125	33.6
Flux through the skin [a] ($\mu g/cm^2$ per h)	0.189 ± 0.059	0.229 ± 0.047	0.175 ± 0.039

[a] Values are means ± S.D., $n = 3$.

plotting the amount of heparin released through cellulose acetate membranes from each vehicle as a function of the square root of time, a linear relationship was obtained. The excellent linearities ($r > 0.99$) obtained indicate that the kinetics of heparin release from these gels followed the simplified Higuchi diffusion model shown in Eqn 1.

The amount of heparin released over a period of 9 h, the heparin diffusion coefficients calculated from the slope of the Higuchi plots and the viscosity values for the gels are reported in Table 2.

As may be noted in Table 2, heparin release increased in the order: Methocel < Pluronic < Carbopol and no inverse correlation existed between the apparent diffusion coefficient of heparin and the gel viscosity values: therefore, the Methocel gel showed both the lowest viscosity and the slowest heparin release. This is in contrast with the findings of several authors who reported that the drug diffusion coefficient is inversely related to the viscosity of the continuous phase (Al-Khamis et al., 1986; Hadgraft, 1989). We believe that the low heparin diffusion coefficient from Methocel gel and therefore the lack of an inverse correlation between release rate and viscosity observed in our study could be attributed to a possible interaction between heparin and the polymer matrix of the gel due to a similar polysaccharidic chemical structure. As regards in vitro heparin skin penetration, heparin steady-state flux values (Table 2) were not affected by the different gel base used since no significant difference could be detected ($p > 0.05$). Furthermore, comparing release study results to skin penetration data, it may be noted that heparin flux through the skin was considerably lower than its release rate from the gels examined. From these results it may be derived that skin penetration is the rate-limiting step in the process of percutaneous absorption of heparin.

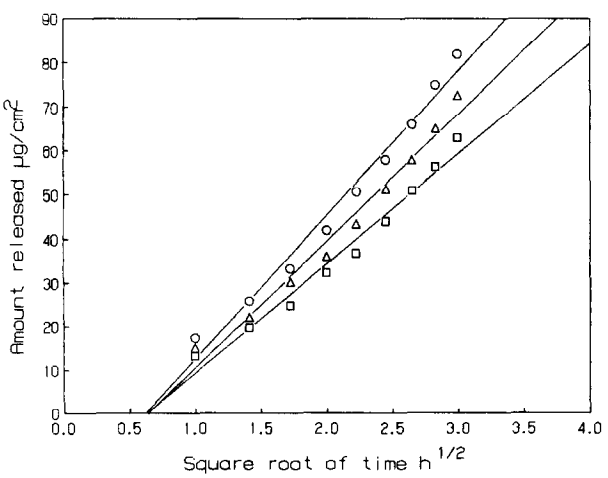

Fig. 1. Amount of heparin released from different gel formulations as a function of the square root of time. (○) Carbopol gel; (△) Pluronic gel; (□) Methocel gel.

Since heparin flux through the skin from all the gels examined was rather low and since we have previously demonstrated (Bonina and Montenegro, 1992) that 5% Azone in the presence of 20% PG and 10% NMP was effective at enhancing the penetration of heparin from aqueous solutions, in the present study we assessed their ability to increase the skin penetration of heparin from gels. Since no change in heparin flux using

TABLE 3

Heparin skin permeation parameters: steady-state flux, lag time and enhancement factor of heparin from Carbopol gels containing Azone, PG and NMP

Gel code	Flux ± S.D. [a] ($\mu g/cm^2$ per h)	Lag time (h)	EF
A	0.189 ± 0.059	0.50	1.00
D	0.304 ± 0.037	0.75	1.61
E	0.748 ± 0.064	0.48	3.96
F	0.856 ± 0.058	0.69	4.53
G	0.329 ± 0.042	0.57	1.74

[a] $n = 3$.

different gels could be detected, Carbopol gels, which are the most commonly used in topical formulations, were chosen to investigate penetration enhancer effectiveness. Heparin steady-state flux values from Carbopol gels, containing the same enhancer concentration as used in the aqueous solution studies reported previously, are listed in Table 3. In Fig. 2 a plot of the cumulative amount of heparin permeated in the presence and absence of enhancers as a function of time is shown.

As shown in Table 3, heparin mean flux values at steady state from control Carbopol gel were found to be 0.189 ± 0.059 and were very similar to those observed by Schaefer et al. (1982) from gels containing 0.1% radiolabelled heparin. Heparin flux from a gel containing 20% PG was not significantly different from that of the control gel ($p > 0.05$). Heparin flux significantly increased ($p < 0.05$) about 4-fold compared to the control gel on the addition of 5% Azone to 20% PG gel or using 10% NMP. Since Priborsky et al. (1987) pointed out that PG was needed to promote the enhancement effect of NMP, we added 20% PG to 10% NMP gels. As can be seen in Table 3, NMP failed to increase heparin flux in the presence of 20% PG. As reported by Hoelgaard et al. (1988), this effect could be due to the slow release of NMP from the PG vehicle, probably caused by a specific enhancer-vehicle interaction.

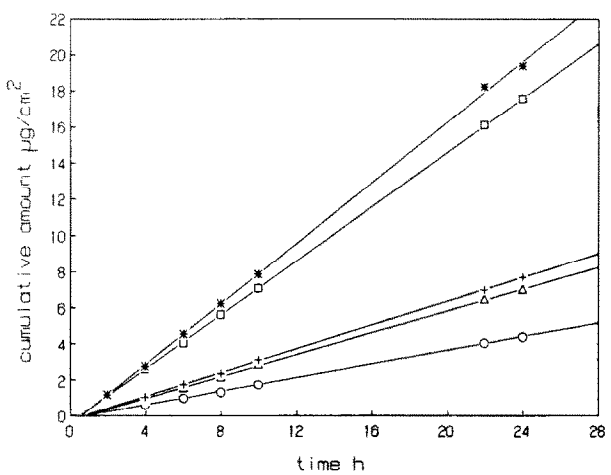

Fig. 2. Effect of different penetration enhancers on heparin percutaneous absorption from gels. (○) Control; (△) PG; (□) 20% PG-5% Azone; (*) 10% NMP; (+) 20% PG-10% NMP.

Lag time values with different enhancers were not significantly different from those obtained from the control gel. This levelling off of lag times has already been observed in studies using heparin aqueous solutions and we attributed it to the skin penetration characteristics of heparin: this drug is known to penetrate the skin and reach a steady-state rapidly, showing rather short lag time values (Schaefer et al., 1982; Stuttgen et al., 1990), thus minimizing the effect of the enhancers on lag time reduction.

Penetration enhancers are thought to act by changing drug partition coefficients (stratum corneum/vehicle), thus increasing drug solubility in the stratum corneum and/or enhancing lipid bilayer fluidity thereby improving penetrant diffusion coefficients. Azone and NMP are considered to act by increasing either the drug diffusion coefficients through the skin or drug partitioning into the skin (Barry, 1988; Beastall et al., 1988; Okamoto et al., 1988).

Previously, we found that both Azone and NMP increased heparin skin permeation from aqueous solutions by improving its SC/vehicle partitioning without affecting its diffusion coefficient (Bonina and Montenegro, 1992). Since in the present study a pattern similar to that obtained using aqueous solutions was observed, we may assume the same mechanism for Azone and NMP from gel formulations.

In Fig. 3 the enhancement factors for PG-Azone and NMP gels are summarized together with the corresponding values obtained from aqueous solutions. Comparing the EF obtained

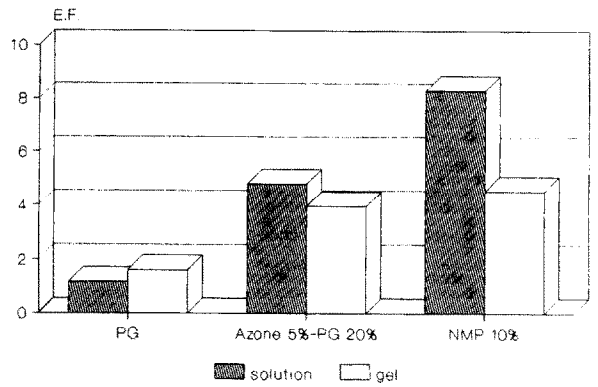

Fig. 3. Comparison of the enhancement factors (EF) obtained for heparin aqueous solutions and aqueous gels containing 20% PG, 20% PG-5% Azone and 10% NMP.

from gels to those from solutions, it can be noted that values from gels are lower than those from solutions. The lower efficacy of Azone and NMP from gels than from aqueous solutions could be due to slower release of penetration enhancer from gels and hence to a smaller amount of enhancer within the skin which produces a decrease in heparin partitioning into the skin.

On the basis of the results obtained in this study we can conclude that:

(1) Among the different gel bases examined, Carbopol gels showed the highest heparin release rate and no inverse correlation was found between gel viscosity and heparin release rate.
(2) Comparing heparin release rates with heparin permeation fluxes, penetration through the skin can be regarded as the rate-limiting step during the process of percutaneous absorption of heparin.
(3) Confirmation of the ability of NMP and Azone-PG to increase heparin percutaneous absorption was also obtained from gel vehicles even though their enhancement factors proved lower than those in the case of aqueous solutions.

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