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Percutaneous penetration of liposomes using the tape stripping technique

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Abstract

The reservoir capacity of the stratum corneum was studied by topical application of sodium fluorescein encapsulated in vesicles in order to elucidate the mechanism involved in the (trans)dermal transport of drugs when vesicles are applied to the skin. The penetration profile of sodium fluorescein in the different strips was found to be logarithmic with both the constant and the slope of the regression curves, accounting for the superficial non-penetration content and for the penetration rate inside the stratum corneum, respectively. The results show a small but significantly enhanced penetration of these vesicle structures for the release of hydrophilic substances. Moreover, similar values obtained when the same liposomes with a varying encapsulation content were applied could lend support to the penetration mechanism where the vesicle enhancement is mainly due to stratum corneum structural modification.

Keywords: Liposome; Sodium fluorescein; Stratum corneum; Percutaneous penetration; Tape stripping

1. Introduction

The mimetic character of liposomes in relation to the organized lipid structures of the stratum corneum provides a suitable strategy for achieving an accurate vehiculization in the percutaneous absorption of a particular compound.

Considerable work has been done in our laboratories on stability, permeability and solubilization of liposomes with surfactant agents bearing in mind their mimetic properties with biomembranes (de la Maza and Parra, 1994, 1995a,b). Furthermore, lipids from stratum corneum and another keratinized tissue, such as wool, have been analyzed (Coderch et al., 1995a) and stable vesicles have been formed (de la Maza et al., 1995; Coderch et al., 1995b).

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Although liposomes have been investigated for many years as parenteral drug carrier systems, they have only recently been considered for topical drug delivery (Mezei, 1988; Egbaria and Weiner, 1990). Despite numerous studies highlighting the advantages of liposomes as drug carriers in skin and an increase in the use of cosmetics with a liposome base, little is known about the molecular mechanism by which the penetration of liposomal lipophilic and hydrophilic drugs is promoted (Schreier and Bouwstra, 1994).

Evidence that liposomes do not penetrate deeper than the stratum corneum layer have been published (Schreier and Bouwstra, 1994). It seems that intact liposomes are mainly confined to the horny layer and do not penetrate deeper, but enhance penetration of hydrophilic and especially lipophilic drugs (Lasch et al., 1991). Fluidisation of intercellular lipid domains in the stratum corneum (SC) has been suggested as a relevant hypothesis. Therefore, the enhanced penetration of drug transport that is observed when drugs are encapsulated in vesicles may be due to SC structural modification.

The SC, despite being the main barrier through which substances penetrate the skin, also acts as a reservoir for topically applied substances (Rougier and Lotte, 1993). A relationship between the reservoir effect of the horny layer and the percutaneous absorption of molecules has been established (Rougier et al., 1983). A weak reservoir capacity would correspond to a weak penetration and, therefore, a strong barrier. Inversely, a high reservoir capacity would provide a high penetration and, hence, a weak barrier effect. Consequently, barrier and reservoir functions of the horny layer may reflect the same physiological phenomenon (Rougier and Lotte, 1993).

Although the stripping technique is mainly based on the use of radiolabelled compounds (Rougier and Lotte, 1993; Rougier et al., 1983), a number of papers have been published on the use of non-radioactive assays to evaluate the in vivo penetration of compounds such as erythromycin (Van Hoogdalem, 1992), lanolin (Clark, 1992) or amino acids (Coderch et al., 1994) in the upper skin layers in man.

In the light of the foregoing discussion, an analytical methodology based on the stripping of human stratum corneum was développed with sodium fluorescein encapsulated in liposomes. Thus, the study of the reservoir capacity of the stratum corneum by the tape stripping technique could provide a good predictive assessment of the total amount of the hydrophilic drug that penetrates the skin with liposomes. This could improve our understanding of the mechanism involved in the (trans)dermal transport of drugs when applied in vesicles to skin.

2. Materials and methods

Fluorescein sodium salt (NaFl) was purchased from Sigma Chemical (Missouri, USA). Boric acid, potassium chloride, sodium hydroxide, sodium chloride and hydrochloric acid fuming 37% were supplied by Merck (Darmstadt, Germany). All the chemicals were of the purest grade available.

Alkaline Borate Buffer (pH 9.0), which was prepared from boric acid and potassium chloride solution, adding the specified volume of the sodium hydroxide solution (USP, 1995), was used as a buffer solution in this study.

2.1. Liposomes

Unilamellar liposomes of a defined size (about 100 nm) were prepared by extrusion of large unilamellar vesicles previously obtained by reverse-phase evaporation (Paternostre et al., 1988; de la Maza and Parra, 1994). For preparation of liposomes, 5% of soya lecithin (containing 93–97% Phosphatidylcholine), water solution of NaCl 0.9%, ethanol and DL-α-tocopherol (as antioxidant) were used.

Liposome solution (13 ml) containing 10 mg/ml of soya lecithin and 1 mg/ml of NaFl were cleared of unencapsulated fluorescent probe by ultracentrifugation, at 55 000 rpm for 24 h, thus separating the pellet from the supernatant. The NaFl content in the pellet fraction was spectrofluorimetrically determined obtaining an encapsulation efficiency of $15.89\% \pm 0.49$. The pellet was then

resuspended in 2 ml water solution of NaCl 0.45% in order to obtain approximately 1 mg/ml of sodium fluorescein just before application to the skin.

2.2. Penetration studies

The penetration behaviour was investigated in vivo in accordance with the stripping method (Rougier et al., 1983). The study was carried out with the same concentrations (1 mg/ml) of NaFl in all the formulations: water solution, liposomes, liposomes free of unencapsulated probe and the supernatant.

Topical application assays and SC strippings were performed in a conditioned room at 20°C with 60% relative humidity. Prior to the test, the subject was allowed to become acclimatized in these conditions for 30 min.

The volume applied with an Exmire microsyringe (Fuji, Japan) was 10 μl and the applied dose of fluorescent probe was 2.5 $\mu g/cm^2$. Preparations were applied onto areas of 4 cm^2, of the central area of the forearm of three caucasian volunteers aged 30–40. Each assay was repeated 3–5 times. The application area was delimited by an adhesive cell. A Dow Corning Medical Adhesive (Brussels, Belgium) was placed around the area in order to prevent lateral diffusion.

After 30 min of contact, 15 successive tape strips of the SC were performed with Scotch Magic™ 810 adhesive tape under defined conditions (pressure: four times with a 1 Kg roller; rapid stripping off; same investigator). The extraction of the NaFl content in the different samples was done four times with borate buffer, pH 9.0 (Vortex and Ultrasounds). The liquid extracted from the 1st, 2nd and 3rd stripping was separately analyzed and the remaining strips were joined in groups of three (4–6, 7–9, 10–12, 13–15) in order to obtain a good analytical detection level.

NaFl fluorescence found in these samples was measured by spectrofluorimetry (Shimadzu RF-540, CRF-1 program) at 20°C (Thermocirculator Heto-Birkerod): λ excitation 493 nm and λ emission 513 nm. The NaFl quantities were determined with the help of the corresponding calibration curves.

The fluorescence values of the control strips were always deducted from those of the corresponding assays to avoid any interference of the autofluorescence in the biological material by spectrofluorimetry.

3. Results and discussion

As discussed above, most investigators agree that the enhancement of drug transport observed when vesicles are applied may be due to a mechanism of stratum corneum structural modification rather than to a vehiculizing mechanism where the liposomes would penetrate deeper in the skin.

In order to shed light on these hypotheses we determined the amount of penetrating NaFl using a small unilamellar vesicle formulation with, in one case, an encapsulation efficiency of approximately 15% and, in the other case, with theoretically 100% of encapsulation obtained by separating the pellet from the supernatant as described in the experimental part. These two experiments were also compared with the study of NaFl penetration in water and in the supernatant obtained from the separation of the pellet from the liposome solution.

The different results of these four experiments could enable us to assess the possible modification of the barrier effect of SC and the importance of the vehiculizing mechanism, which would be reflected in the differences obtained using liposomes with several encapsulation efficiencies.

The fluorescent probe was selected for its solubility, specificity, selectivity and atoxicity (Spanish Health Ministry, 1992) (widely accepted for cosmetic formulation and ophthalmic use).

Calibration curves with different amounts of NaFl in alkaline borate buffer solution were obtained with a λ excitation at 493 nm and λ emission at 513 nm. The linear zone of fluorescent response used in this study is shown in Fig. 1.

Water and liposome formulations (10 μl) were spread on the surface of the tape and extracted with the methodology described in the experimental part to assess the extraction capacity of the borate buffer and the possible interference of the adhesive components in the fluorescence results.

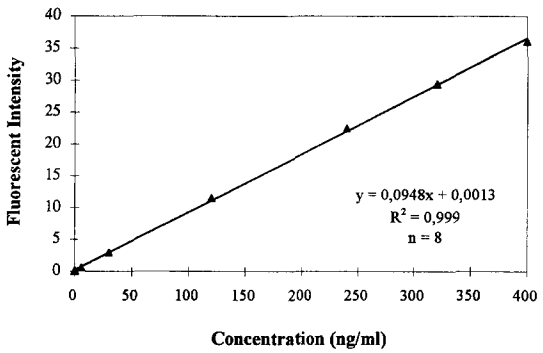

Fig. 1. Calibration curve for Sodium Fluorescein.

The spectrofluorimetric detection of the extractive liquid disclosed that the total amount recovered exceeded 95% in all the cases (Table 1). It can, therefore, be concluded that there is a good recovery of NaFl using this methodology with borate buffer and that there is no interference of tape and liposome components in the NaFl detection.

The potential interference due to the autofluorescence of the biological material was also controlled. This interference of the SC was very low; however, we always deducted, where necessary, the fluorescence values of the control strips from those of the corresponding assays.

The amount of NaFl recovered in every strip or group of strips expressed in percentage with respect to the total dose applied for the different formulations was determined. The experiments were performed five times for water and liposome formulations and three times for supernatant and pellet formulations. The mean values and standard deviations were calculated and the values obtained are indicated in Table 2.

Bearing in mind that the NaFl content in the first strip accounts for the non-penetration content, the higher amount of NaFl content obtained in this first strip, when water and supernatant are applied with respect to liposome and pellet, should be noted. These results lend support to the weakness of the SC barrier effect when liposomes are applied.

Table 1
Total NaFl recovered after extraction (mean ± S.D.)

Formulation	% Recovered	*n*
Water	101.02 ± 1.04	21
Liposome	101.03 ± 4.0	24

The total percentages of NaFl analyzed by adding all the strips are also lower in the case of liposome and pellet penetration. This result reflects a higher penetration in the deeper layers of skin, which also highlights the aforementioned weak barrier effect.

Moreover, the total amount of NaFl in the SC (from the 2nd to the 15th strip) was also calculated to determine the reservoir capacity of this layer. The lowest amount of NaFl in SC when water is applied is consistent with the low penetration and strong barrier effect of this layer discussed above. However, the similar mean values obtained and the standard deviation values in the amount of NaFl in the SC do not allow us to reach more reliable conclusions.

In order to follow the penetration profile of NaFl in the different strips, cumulative percentages were calculated and plotted as a function of the strip numbers (Fig. 2). This figure shows the similar behaviour of the liposome and pellet formulations as well as the water and supernatant formulations with a similar enhancement of liposome formulations with respect to the others. It seems that NaFl contained in liposomes and pellet formulations penetrates the SC more readily than NaFl both in water and in supernatant.

The shape of the curves obtained indicates a logarithmic relationship between the NaFl cumulative values and the different strips. In fact, a regression analysis was performed and the functions $y = a\ \mathrm{Ln}(x) + b$ were obtained, y being the cumulative % of applied dose, x the strip number and a and b the estimated equation coefficients. Parameters a, b and the determination equation coefficient R^2 are given in Table 3. The regression curves and the experimental points are also expressed in Fig. 3 logarithmically for the sake of simplicity.

It should be noted that the curves that most accurately fit with the experimental points, having the highest correlation coefficients, are the ones that correspond to the liposome formulation, the

Table 2
Recovery % of the applied dose in the different strips (mean ± S.D.)

Number of Strip	Water ($n = 5$)	Liposome ($n = 5$)	Supernatant ($n = 3$)	Pellet ($n = 3$)
1st	32.66 ± 3.37	20.12 ± 2.03	26.89 ± 2.56	22.42 ± 5.48
2nd	14.88 ± 4.00	11.94 ± 4.37	19.32 ± 1.44	12.26 ± 6.66
3rd	7.78 ± 1.46	8.65 ± 1.89	12.05 ± 0.95	7.99 ± 0.21
4th–6th	12.43 ± 0.69	14.60 ± 4.73	12.01 ± 2.30	12.98 ± 3.22
7th–9th	5.99 ± 0.47	8.68 ± 2.58	5.39 ± 1.47	7.96 ± 1.71
10th–12th	4.08 ± 0.40	5.34 ± 1.73	4.50 ± 1.66	4.87 ± 1.34
13th–15th	3.17 ± 0.96	3.76 ± 1.16	2.31 ± 0.86	4.02 ± 1.44
Total %	81.01 ± 6.30	73.08 ± 10.26	82.48 ± 4.16	72.51 ± 3.13
Stratum corneum %	48.34 ± 3.67	52.96 ± 10.42	55.59 ± 6.55	50.09 ± 4.53

supernatant case being the least reliable. The values of constant b (independent term), which account for the non-penetration content, are lower in the case of the liposome systems and statistically different from the values for the water systems. The slopes which accounts for the penetration rate inside the stratum corneum are statistically almost identical. Therefore, a similar penetration rate could be concluded regardless of the vehicle.

Similar values were obtained when liposomes with varying amounts of encapsulated probe were applied. These findings lend further support to the hypothesis that the liposome penetration mechanism is not related to the amount of encapsulated probe, which would have suggested a vehiculizing mechanism of the vesicles. The lipophilic components of liposomes may co-diffuse into the SC and thus produce a change in the skin barrier properties by possibly making the stratum corneum environment more favorable to the fluorescent probe.

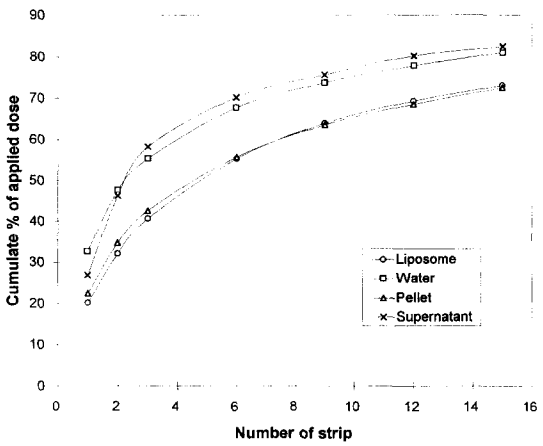

Fig. 2. Penetration of Sodium Fluorescein into stratum corneum after 30 min.

These results indicate a small but significantly enhanced penetration of these vesicle structures for the release of hydrophilic substances. The low non-penetration content in the stratum corneum and the high reservoir capacity of the stratum corneum correspond to a high penetration and therefore a weak barrier effect (Rougier and Lotte, 1993).

The results obtained from this percutaneous penetration study using the tape stripping technique lends additional support to other works on liposome penetration based on visualization by microscopy concerning the mechanism of liposome penetration into human skin (Schreier and Bouwstra, 1994; Lasch et al., 1991).

Since the liposome enhancement penetration using a hydrophilic probe (sodium fluorescein) is small, further work is being done with a hydrophobic probe such as acid fluorescein to clarify

Table 3
Mathematical equation parameters and determination coefficients in accordance with different formulations

Formulation	a	b	R^2
Liposome	20.052	19.215	0.9988
Water	17.684	34.548	0.9943
Pellet	18.659	22.181	0.9998
Supernatant	20.078	31.377	0.9741

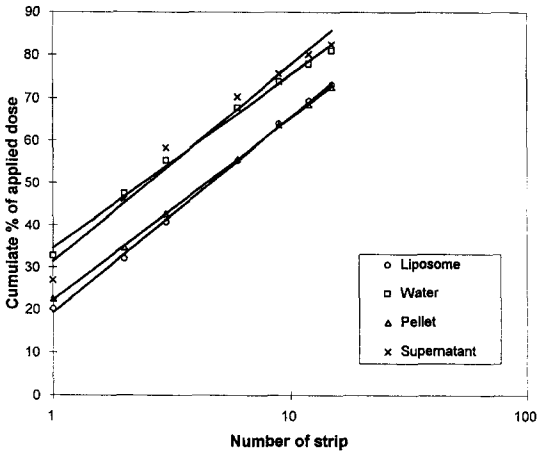

Fig. 3. Relationship between the cumulative percentage of NaFl in the stratum corneum and the logarithm of the strip number.

the delivery mechanism of this vehicle through skin in order to improve our understanding of the transport path and interaction with the skin.

4. Conclusion

Tape stripping associated with spectrofluorimetry is applied to the study of in vivo skin penetration behaviour of a hydrophilic probe in the stratum corneum in accordance with the vehicle structure, in this case, liposomes.

The penetration profile of sodium fluorescein in the different strips was found to be logarithmic with the constant and the slope of the regression curves accounting for the superficial non-penetration content and for the penetration rate inside the stratum corneum, respectively.

Our results suggest a small but significantly enhanced penetration of these vesicle structures for release of hydrophilic substances. The low non-penetration content in the stratum corneum and the high reservoir capacity of the stratum corneum correspond to a high penetration and, therefore, a weak barrier effect.

Moreover, similar values obtained when the same liposomes with a varying encapsulation content were applied could lend support to the penetration mechanism where the vesicle enhancement is mainly due to SC structural modification.

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