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Vehicle effects on in vitro skin permeation of and stratum corneum affinity for model drugs caffeine and testosterone

F.P. Bonina [a], V. Carelli [b], G. Di Colo [b], L. Montenegro [a] and E. Nannipieri [b]

[a] *Institute of Pharmaceutical Chemistry, University of Catania, Catania (Italy) and* [b] *Pharmaceutical Technology Laboratory, Institute of Pharmaceutical Chemistry, University of Pisa, Pisa (Italy)*

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Summary

The effects of Labrasol (LBS) (glycolysed ethoxylated C_8/C_{10} glycerides), Labrafil (LBF) (glycolysed ethoxylated glycerides), Transcutol (TSC) (diethylene glycol monoethyl ether) and DPPG (propylene glycol dipelargonate) on the flux across excised human skin of the lipophilic testosterone (TST) and the hydrophilic caffeine (CAF) and on the affinity of the human stratum corneum for these drugs are compared taking propylene glycol (PG) and liquid petrolatum (LP) as reference vehicles. DPPG and LBF enhance CAF flux relative to PG while LBS and TSC increase the stratum corneum affinity for TST relative to LP. However, the materials tested enhance neither the flux of nor the stratum corneum affinity for both drugs with respect to either reference. On the other hand, a saturated solution of DPPG in PG enhances both properties for both drugs relative to PG. Such effects are ascribed to the ability of DPPG to interact with the lipid bilayers and to that of PG to promote DPPG penetration into stratum corneum and to create interaction sites in such a tissue.

Introduction

Low systemic and cutaneous toxicity are prerequisites of the materials used to enhance the percutaneous penetration of drugs. The low toxicity of Labrasol (LBS) (glycolysed ethoxylated C_8/C_{10} glycerides), Labrafil (LBF) (glycolysed ethoxylated glycerides), Transcutol (TSC) (diethylene glycol monoethyl ether) and DPPG (propylene glycol dipelargonate) has recently prompted investigation of the ability of these materials to promote the percutaneous penetration of drugs. According to literature data, TSC can promote the percutaneous penetration of the lipophilic prostaglandin (Watkinson et al., 1991) and the hydrophilic theophylline (Touitou et al., 1991). In particular, the effect on prostaglandin percutaneous absorption was found to be 3–4-fold as strong as that of Azone. However, TSC is unable to exert any such effect on the lipophilic morphine (Rojas et al., 1991) or the hydrophilic peptide vasopressin (Banerjee and Ritschel, 1989). On the other hand, the flux through skin of

Correspondence to: E. Nannipieri, Pharmaceutical Technology Laboratory, Institute of Pharmaceutical Chemistry, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy.

morphine and vasopressin can be accelerated by DPPG (Rojas et al., 1991) and LBF (Banerjee and Ritschel, 1989), respectively.

Consideration of the above results convinced us of the utility of further evaluating the ability of the above materials to influence the skin characteristics relevant to the therapeutic effect of drugs. These are the skin permeability by the drug and its retention capacity of the drug, which are related to the systemic and/or topical action of the drug. The latter is particularly important in cases where the skin is the target organ (Shah et al., 1992). To this aim we studied the effects of LBS, LBF, TSC and DPPG on the flux across excised human skin of testosterone (TST) and caffeine (CAF), chosen as models of lipophilic and hydrophilic drugs, respectively, and on the affinity of the human stratum corneum for these drugs.

Materials and Methods

Materials

Caffeine (CAF), liquid petrolatum (LP) and propylene glycol (PG) were bought from Farmitalia Carlo Erba (Milano, Italy). Testosterone (TST) was purchased from Fluka Chemie AG (Switzerland). DPPG, Labrafil M 1944 (LBF), Labrasol (LBS) and Transcutol (TSC) were a gift from Gattefosse (Saint-Priest Cedex, France).

Strips of human callus were removed from the plantar surface of volunteers with a scalpel, and stored in a desiccator over $CaCl_2$.

Samples of whole adult human skin (mean age 15–45 years) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 ± 1°C for 2 min (Kligman and Christophers, 1963), after which the stratum corneum plus attached viable epidermis (SCE) was peeled off. The SCE samples were dried at room temperature in a desiccator maintained at approx. 25% RH. The dried samples were wrapped in aluminium foil and stored at 4 ± 1°C.

In vitro skin permeation

Skin permeation of CAF and TST was measured using Franz cells (Franz, 1975). Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in diffusion cells. The receiving chamber had a volume of 4.5 ml and was filled with saline. The receptor phase was stirred and kept at 30 ± 1°C during the experiment. The surface area for permeation was 0.75 cm^2. Suspensions (100 μl) of CAF or TST in PG, PG saturated with DPPG, LP, LBS, LBF, DPPG and TSC were applied to the skin and the experiment was run for 24 h. Samples of the receiving solution were withdrawn at intervals, analysed for drug concentration and replaced with fresh solution.

The flux, J, through the skin was obtained, using linear regression analysis, by plotting the cumulative amount of drug permeated against time and dividing the slopes of the steady-state portion of the graph by the area of the diffusion cell. Each measurement was made in triplicate.

Solubility determination

An excess of drug was added to 4 ml of the appropriate solvent in a glass-stoppered test tube. This was stirred for 48–72 h in a thermostated (30°C) water bath, then the excess solid was removed by filtering through a 0.2 μm pore size polytetrafluoroethylene filter (SM11807 Sartorius GmbH, Gottingen, Germany) in an atmosphere thermostated at 30°C. The clear filtrate was diluted with ethanol and analysed for drug concentration. The assay of TST concentration in LP needed the following procedure. A 50 ml sample of clear LP solution was added with 10 μl of ethanol and the resulting mixture was vigorously shaken for 5 min; the ethanolic phase was assayed for TST concentration after centrifugation at 4000 rpm for 20 min..

Partition coefficient determination

n-Octanol / water. *n*-Octanol and water were presaturated with each other. 1.5 ml of TST solution in *n*-octanol was added with 14.0 ml of water and the mixture was tumble-mixed for 24 h in an atmosphere thermostated at 30°C. After separating the phases by centrifugation, both the alcoholic and the aqueous solution were diluted and analysed for drug concentration.

Human callus / vehicle. Solutions of CAF or TST in the vehicles under study were prepared so that the drug concentration was about 50% of the saturation concentration. Fragments of dry callus (0.5–1.0 g) were hydrated by exposure to water vapour in a closed system at 37°C until the water content was about 30% (w/w) of the hydrated tissue. The hydrated callus fragments were placed in a screw-cap glass vial, stored overnight at 4°C, then added with a drug solution (5.0 ml) in the proper vehicle. The vial was placed in a thermostated (30°C) shaker water bath, and the equilibrium concentration of the fluid phase was determined after 60 h (30 h for water); missing drug was assumed to have entered the tissue. The assay of TST concentration in LP needed the special procedure described under Solubility determination. The fragments of callus were gently sandwiched between filter paper to remove clinging vehicle and then weighed; this weight was used for calculating the partition coefficient, which was expressed as the ratio of molal to molar concentrations.

For the purposes of the present study, human callus was considered representative of epidermal stratum corneum. Our choice was validated by comparing literature data on CAF and TST partitioning into stratum corneum with some of our data obtained using callus (see Table 1).

Assay methods

The drugs were assayed by high-performance liquid chromatography. An isocratic pump (Perkin Elmer, model 250) equipped with UV/Vis spectrophotometric detector (Perkin Elmer, model 290) was used. A 250 × 4.6 mm Partisil ODS-3 (5 μm) column (Whatman Chemical Separation Inc., U.S.A.) was used for both TST and CAF. The mobile phase was a methanol/water mixture at 7:3 and 3.5:6.5 volume ratio for TST and CAF, respectively. TST and CAF were detected at 250 and 273 nm, respectively.

TABLE 1

CAF and TST partitioning data [a]

System	CAF	TST
Human callus/water [b]	1.56 ± 0.50	44.0 ± 3.5
Human callus/LP [b]		4.9 ± 2.8
Horny layer/water [c]	3.50 ± 0.40	47.4 ± 1.7
Horny layer/petrolatum [c]		5.3 ± 0.7
n-Octanol/water [b]	0.70 ± 0.02 [d]	1679 ± 44

[a] Values are the mean ± SD ($n \geq 3$).
[b] The experiments were carried out at 30°C.
[c] Data from Bronaugh and Franz (1986). The experiments were carried out at room temperature.
[d] Datum from Carelli et al. (1990).

Results and Discussion

Under the conditions of our permeation experiments, if the stratum corneum is the only rate-determining barrier, then the drug flux, J, is expressed by the well known equation:

$$J = (D/h) \cdot S_m \quad (1)$$

where D is the apparent diffusivity of the drug in the barrier, h denotes the effective barrier thickness, and S_m is the drug solubility in the stratum corneum at equilibrium with the vehicle.

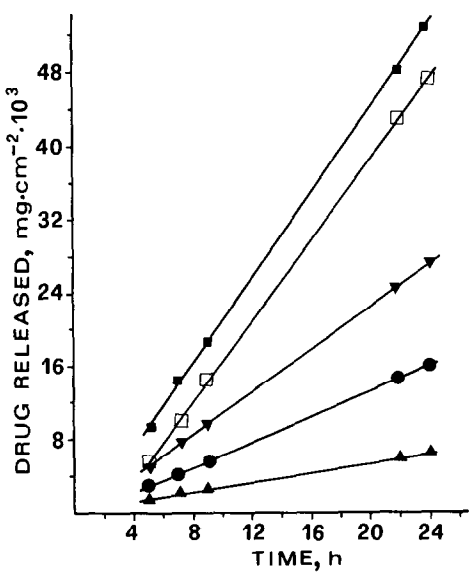

Fig. 1. Typical skin permeation curves of CAF from a drug suspension in LBS (▲), PG (●), LBF (▼), PG-DPPG (□) or DPPG (■). The permeation curve relative to TSC could not be represented because it was superimposed on the PG curve.

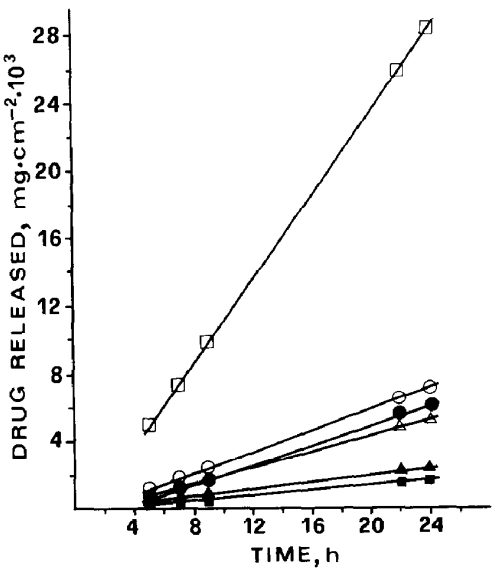

Fig. 2. Typical skin permeation curves of TST from a drug suspension in DPPG (■), LBS (▲), TSC (△), PG (●), LP (○) or PG-DPPG (□). The permeation curve relative to LBF could not be represented because it was superimposed on the PG curve.

Compliance with Eqn 1 implies that the plot of the drug amount permeated vs time should be linear, after a lag time required for attainment of the stationary state. Figs 1 and 2 show that this was in fact the case with all of the drugs and vehicles tested. The J and S_m values for the vehicles under study were expressed in terms of the corresponding values for a reference vehicle. LP and PG were chosen as reference vehicles for their frequent use in topical formulations. For a given vehicle, the relative values, J^* and S_m^*, gauged the modifications by the vehicle of the skin properties relevant to drug flux and of the stratum corneum affinity for the drug, with respect to the reference vehicle. An approximate estimation of S_m^* for each vehicle was achieved from the relevant values of callus/vehicle partition coefficient, K, and drug solubility in vehicle, S_v, listed in Tables 2 and 3, according to the following equation:

$$S_m^* = (K/K_r) \cdot (S_v/S_{vr}) \cdot (d_m/d_{mr}) \qquad (2)$$

where d_m is the density of callus at equilibrium with vehicle and the subscript r identifies the factors for the reference vehicle.

TABLE 2

Solubility (S_v), partitioning (K) and flux (J) data for CAF [a]

Vehicle	S_v (mg ml^{-1})	K [b] (ml g^{-1})	J (μg cm^{-2} h^{-1})
PG	13.9 ± 1.2	2.87 ± 0.5	0.698 ± 0.099
Water	27.9 ± 1.7	1.56 ± 0.5	0.724 ± 0.041
TSC	14.1 ± 0.9	0.44 ± 0.2	0.635 ± 0.197
LBS	12.0 ± 0.8	0.53 ± 0.2	0.277 ± 0.057
LBF	3.3 ± 0.4	1.64 ± 0.4	1.162 ± 0.085
DPPG	1.9 ± 0.05	2.28 ± 0.1	2.278 ± 0.353
PG-DPPG	13.9 ± 0.9	3.27 ± 0.2	2.193 ± 0.174

[a] Values are mean ± SD ($n \geq 3$).
[b] Human callus/vehicle partition coefficient.

The ratio of densities in Eqn 2 was taken as equal to unity, since in no case did the equilibration with vehicle cause any important weight increase of callus nor was the vehicle density markedly different from that of callus. Determination of S_m^* by Eqn 2 involves the assumptions that callus is representative of epidermal stratum corneum and that Henry's law holds in the phases at equilibrium up to saturation.

Semiquantitative information on the vehicle effect on the kinetic factor, D/h, in Eqn 1 could be obtained, once J^* and S_m^* were assessed, by applying the following equation:

$$J^* = (D/h)^* \cdot S_m^* \qquad (3)$$

TABLE 3

Solubility (S_v), partitioning (K) and flux (J) data for TST [a]

Vehicle	S_v (mg ml^{-1})	K [b] (ml g^{-1})	J (μg cm^{-2} h^{-1})
PG	75.90 ± 0.04	5.20 ± 2.50	0.291 ± 0.054
LP	0.43 ± 0.04	4.90 ± 2.50	0.319 ± 0.084
Water	0.03 ± 0.008	44.00 ± 3.50	0.263 ± 0.040
TSC	104.00 ± 5.00	0.38 ± 0.05	0.231 ± 0.036
LBS	46.10 ± 0.90	0.72 ± 0.04	0.102 ± 0.070
LBF	20.90 ± 1.00	0.05 ± 0.01	0.283 ± 0.029
DPPG	13.20 ± 0.50	0.16 ± 0.06	0.079 ± 0.080
PG-DPPG	74.10 ± 1.10	11.00 ± 2.50	1.226 ± 0.121

[a] Values are mean ± SD ($n \geq 3$).
[b] Human callus/vehicle partition coefficient.

The J^* and S_m^* values for CAF and TST in the different vehicles are listed in Table 4. For CAF only values relative to PG are reported, since we were unable to obtain reproducible values of the callus/LP partition coefficient, perhaps due to excessive sensitivity of this system to the degree of hydration of callus, which could not be controlled with the accuracy needed in this particular case.

Comprehensive consideration of the data in Table 4 shows that in all cases, except that of PG-DPPG, an effect on the affinity parameter, S_m^*, is coupled with an opposite effect on the kinetic parameter, $(D/h)^*$, as calculated from Eqn 3. Nevertheless, it should be recognized that the estimated $(D/h)^*$ values may reflect apparent changes of the kinetic factor instead of real changes. In fact, Eqn 1, from which Eqn 3 was derived, is strictly applicable to the diffusion across a homogeneous plane sheet, therefore, such an equation describes an oversimplified model of the skin barrier, which, in contrast, is a very complex polyphasic membrane. The drug may partly accumulate in zones of the stratum corneum which are excluded from the diffusive pathway (Shah et al., 1992), and the vehicle may influence the drug distribution between the different zones of the tissue. Therefore, variations of drug solubility in the horny layer, ensuing from changes in vehicle composition, may not be reflected by steady-state flux data.

As appears from the relevant J^*(PG) and J^*(LP) values in Table 4, TSC produced no significant change of skin flux of either CAF or TST with respect to PG or of TST with respect to LP. However, the corresponding S_m^* values, S_m^*(PG) and S_m^*(LP), are significantly different from 1, so that a balance between opposite effects on the kinetic factor, $(D/h)^*$, and the thermodynamic factor, S_m^*, in Eqn 3 can be argued. It is worth noting that the TSC effects on S_m^*(PG) were roughly similar for CAF and TST, despite the marked difference in polarity between the two drugs apparent in Table 1 from the respective values of the octanol/water partition coefficient.

Similar considerations can be made for the effects of LBS, except that with this vehicle the J^* values are lower than with TSC.

On the other hand, the effects of DPPG and LBF concerning CAF are not quite similar to those for TST. Indeed, for the former drug, the S_m^*(PG) values are much the same as those obtained with TSC or LBS as the vehicle, so the enhancement of flux by LBF and, especially, DPPG with respect to PG, as quantitated by the relevant J^*(PG) values, was mainly determined by a strong enhancement of the $(D/h)^*$ parameter. With TST as the drug, the two vehicles were unable to increase the drug flux with respect to PG, due to exceedingly low S_m^*(PG) values.

Taken together, the results discussed so far have demonstrated the ability of LBF and DPPG

TABLE 4

Relative values of flux (J^*) and drug solubility in stratum corneum (S_m^*)

Vehicle	CAF		TST			
	J^* (PG) [a]	S_m^* (PG) [a]	J^* (PG) [a]	S_m^* (PG) [a]	J^* (LP) [b]	S_m^* (LP) [b]
PG	1	1	1	1	0.91	187.0
LP	–	–	1.10	0.0054	1	1
Water	1.04	1.10	0.90	0.0033	0.82	0.6
TSC	0.91	0.15	0.79	0.100	0.72	18.7
LBS	0.40	0.16	0.35	0.084	0.32	15.7
LBF	1.66	0.14	0.98	0.0027	0.89	0.5
DPPG	3.26	0.11	0.27	0.0054	0.25	1.0
PG-DPPG	3.14	1.14	4.22	2.091	3.84	391.0

[a] Values relative to PG.
[b] Values relative to LP.

to facilitate the flux and the diffusivity of CAF across the stratum corneum with respect to PG, and the inability of any of the materials tested to increase the stratum corneum affinity for either drug over that produced by PG. Also, PG exerted a strong enhancing effect on the affinity of the stratum corneum for TST with respect to LP, as shown by the relevant S_m^*(LP) value in Table 4. These considerations motivated us to examine a saturated solution of DPPG in PG, where the two components could exert their maximum effects on skin. The J^* and S_m^* values in Table 4 for PG-DPPG as the vehicle are indeed the highest of all vehicles. However, even though the affinity parameter was equal or doubled with respect to PG alone, the effect on the kinetic parameter, as extimated through Eqn 3, was much weaker than that assessed for DPPG alone with either drug and reference vehicle. If the effect of PG-DPPG on S_m were assumed to consist in creating sites or zones in the skin interacting with the drug, without varying the thermodynamic activity in its actual diffusive pathway, then the steady-state flux might remain unaltered, while the apparent diffusivity would undergo a change opposite to that of S_m, even though the true diffusivity in such a pathway would be left unchanged. If the data in Table 4 are viewed in the light of the above hypothesis, an enhancement of the true kinetic factor can only taken for certain where both the experimental J^* value and the $(D/h)^*$ value estimated through Eqn 3 are greater than 1. Inspection of Table 4 leads one to conclude that the certainty of an enhancement of the true kinetic factor for both drugs can only be stated for DPPG in the PG-DPPG mixture, with reference to PG. This property of DPPG may be linked to the comparatively low polarity of this material enabling it to penetrate into the stratum corneum and interact with the lipid bilayers, thus increasing their fluidity or forming, as proposed by Walker and Hadgraft (1991) for oleic acid, fluid-like channels. When DPPG is used in combination with PG, the latter appears to promote DPPG penetration into stratum corneum and to create interaction sites in such a tissue. The fact that both CAF and TST fluxes were apparently affected by such an action of DPPG is in accord with the view that the lipid bilayers are an important rate-determining factor for the transcutaneous penetration of polar as well as nonpolar drugs (Barry, 1991). With LP as the reference, no vehicle can be assumed to increase the kinetic factor for TST penetration. Indeed, LP is so highly occlusive as to result in extensive hydration of the stratum corneum, which is known to increase drug diffusivity in the lipid pathway (Barry, 1987).

Conclusions

The potential of a vehicle to increase the affinity between stratum corneum and drug should be considered with regard not only to transdermal but also to topical drug delivery. Indeed, where drug delivery to the skin as target organ is concerned, drug retention in the skin is the main consideration as opposed to drug flux across the skin (Shah et al., 1992). In this respect, the PG-DPPG mixture may be regarded as an interesting vehicle for nonpolar drugs. The present results have shown that the enhancing effects of the materials studied on the flux of either the polar CAF or the nonpolar TST across excised human skin were determined by the kinetic factor, D/h, rather than the thermodynamic factor, S_m, in Eqn 1, even in those cases where the latter factor was increased to a substantial degree.

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