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Research paper

Influence of phloretin on the skin permeation of lidocaine from semisolid preparations

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Abstract

The purpose of the present study was to determine the significance of phloretin on the topical permeation of lidocaine using different semisolid preparations as delivery systems. One hydrophilic and three lipophilic formulations were used. After estimation of the solubility of phloretin and lidocaine in the vehicles and analysis of the viscoelastic properties, standard diffusion experiments with Franz type diffusion cells through porcine skin were performed. Results indicate a general lidocaine enhancement by phloretin in the tested vehicles. The permeation was enhanced 1.39-fold in the hydrophilic formulation and between 1.25- and 1.76-fold in the lipophilic formulations.

Keywords: Lidocaine; Semisolid formulation; Topical delivery; Phloretin; Permeation enhancer; Porcine skin

1. Introduction

Transdermal and topical delivery of drugs provide advantages over conventional oral administration. The benefit of transdermal systems includes convenience, improved patient compliance and elimination of hepatic first pass effect. Nevertheless, most drugs are not applicable to this mode of administration due to the excellent barrier properties of the skin. Molecules must first penetrate the stratum corneum, the outer horny layer of the skin. The molecule then penetrates the viable epidermis before passing into the papillary dermis and through the capillary walls into systemic circulation. It is the stratum corneum, a complex structure of compact keratinized cell layers that presents the greatest barrier to absorption of topical or transdermal administered drugs. The common method to improve drug permeation through the skin is to use penetration enhancers [1,2], i.e. organic solvents like ethanol or *N*-methylpyrrolidone, fatty acids like oleic acid, surfactants like sodium laurate, etyltrimethylammonium bromide and lecithin or cyclodextrins [3]. Penetration enhancers can change the structure of skin lipids and alter the skin barrier function. These compounds often generate massive skin irritation [4]. An additional solution for this problem is the use of supersaturated liquids [5,6]. Other methods have been proposed to increase the permeability of drugs through the skin, i.e. iontophoresis [7] and ultrasound [8], but these methods are not frequently used due to the requirement of qualified staff for their application.

Recently, phloretin, the aglycone of phlorizin, a polyphenolic substance occurring in the root bark of apple trees has been studied as a possible penetration enhancer [9–13]. Phloretin derivatives have been found as antagonists of the pharmacological actions of prostaglandins [14]. Therefore, they have therapeutic potential as anti-inflammatory agents. In addition, in other in vivo studies an anti-irritating effect could be demonstrated [15]. A pre-exposure of skin to phloretin-liposomes, 12 h prior to drug application, showed a significant increase in skin flux of lidocaine [16]. However, this working technique is not applicable in clinical practice because after drug application the effect should occur immediately.

On the basis of these data a more rapid effect could be expected by the combination of this multifunctional penetration enhancer with the local anaesthetic lidocaine. This drug is frequently used on skin in order to suppress pain from burning, itching, surgical operations, injections and

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dermatological diseases [17,18]. It was the aim of the present study to ascertain permeation enhancement after simultaneous cutaneous exposure to phloretin-containing lidocaine preparations. Permeation profiles of such formulations through porcine skin were investigated in order to verify these proposals. Rheological experiments were also performed to examine the viscous and elastic properties of the different formulations.

2. Materials and methods

2.1. Materials

Phloretin (3-[4-hydroxyphenyl]-1-[2,4,6-trihydroxyphenyl]-1-propanone), lidocaine, lidocaine hydrochloride (L-HCL) and Natrosol (HEC) were purchased from Sigma (St. Louis, USA). Polyoxyethylene-5-cetyloleylether (PO5) and polyoxyethylene-10-cetyloleylether (PO10) were gifts from Cognis (Duesseldorf, D). A mixture of alkyl-branched esters of 2-ethylhexanoic acid with 1-hexadecanol and 1-octadecanol (C_{16}–C_{18}) (ABE) was obtained from Symrise (Vienna, Austria).

All other chemicals used were of analytical reagent grade and used as received without any further purification.

2.2. Formulations

Four different vehicles were chosen; one hydrophilic and three hydrophobic. As can be seen in Table 1 each formulation contained 1% (w/w) phloretin and 2% (w/w) L-HCl or lidocaine, respectively. The preparations without phloretin were used as controls.Formulation 1: HEC-hydrogel. Phloretin was dispersed in hot water and additional L-HCl was dissolved in it. Then a conventional hydrogel was formed with the aid of HEC.Formulation 2: silicium-dioxide-hydrophobic gel. Lidocaine was dissolved in the mixture of liquid petrolatum and eucalyptus oil. Afterwards silicium dioxide was added and a gel was formed. Both formulations 1 and 2 showed a slight turbid mucilage appearance.

As presented in Table 1, formulations 3 and 4 have a similar composition, with a different oil phase. Formulation 3: hydrophobic semisolid preparation. Formulation 4: hydrophobic semisolid preparation.

In both vehicles PO5 and PO10 were heated to 80 °C (together) with the other lipophilic components. Afterwards the water was poured into this hot mixture and vigorously stirred. In both cases transparent hydrophobic semisolid preparations resulted.

2.3. Rheological experiments

Oscillatory shear experiments were performed on a Haake rheometer Rotovisco RT 20 (Haake, Karlsruhe, Germany, thermo controller Haake F6/8). The rheometer tool was a thermostatically controlled cone/plate with a diameter of 35 mm and 2° angle (C35/2Ti) for formulations 1 and 2 and with a 20-mm diameter plate/plate (PP20/Ti) for formulations 3 and 4. Throughout the experimental period the plate temperature was kept at 32 °C ± 1.5. The sample amount was between 0.5 and 1 g. At this modus the induced response (strain) is measured when a sinusoidal stress is applied to the sample. After the identification of the linear viscoelastic region, samples were investigated over a frequency of 0.1–10 Hz (ν). The parameters obtained are the elastic modulus G' and the viscous modulus G''. The measurements were performed in triplicate.

2.4. Solubility experiments

The solubility of phloretin, L-HCL and lidocaine in the vehicles was analyzed by a microscopic method. For the phloretin solubility a defined amount of phloretin was added to each formulation (1–4). The formulations were then examined under a microscope at room temperature. If no crystals could be detected an additional defined amount of phloretin was added and examined again under the microscope. This was repeated until crystals could be seen. For L-HCl (formulation 1) and lidocaine (formulations 2–4) the same method was used.

2.5. Skin preparation

Porcine abdominal skin from one pig was shaved and then prepared with a dermatome (GB 228R, Aesculap, Germany) set at 1.2 mm. The skin was stored in a freezer at

Table 1
Compositions of the used formulations

Formulation	Phloretin	L-HCL	Lidocaine	HEC	Water	Silicium dioxide	Eucalyptus oil	PO5	PO10	Liquid petrolatum	ABE
1	1	2	–	2	96	–	–	–	–	–	–
2	1	–	2	–	–	4	0.5	–	–	93.5	–
3	1	–	2	–	53	–	–	15	15	15	–
4	1	–	2	–	53	–	–	15	15	–	15

All data are in % (w/w) of the indicated components. L-HCl, lidocaine hydrochloride; HEC, hydroxyethylcellulose; PO5, polyoxyethylene-5-cetyloleylether; PO10, polyoxyethylene-10-cetyloleylether; ABE, alkyl-branched esters of 2-ethylhexanoic acid with 1-hexydecanol and 1-octadecanol.

−20 °C until use but not longer than 3 months. Two hours prior to the experiments the samples were thawed.

2.6. Diffusion cell preparation

The permeation of lidocaine, following topical application of preparations containing lidocaine and L-HCl, was investigated using Franz-type diffusion cells, having a permeation area of about 1 cm^2. The receptor compartment was filled with 2 ml phosphate buffer (0.06 M, pH 6.0). Excised skin was mounted in the cell, stratum corneum uppermost, with the dermal side facing the receptor compartment. The diffusion cells were thermostated to achieve a skin surface temperature of 32 °C. At defined time intervals the samples were removed for analysis and replaced with fresh receptor medium for 50 h. Approximately 1 g of all formulations was applied. The cumulative amount of drug released through the porcine skin, Q/t, at any time, was determined from the following formula: $Q = (CxV)/A$ where C is the lidocaine concentration in the receiver compartment in mg ml^{-1} for the corresponding sample time t. V is the volume of fluid in the receptor phase and A is the diffusion area of the cell. For each formulation and all controls three parallel experiments were performed.

2.7. Analysis of lidocaine and L-HCL

The content was analyzed using an HPLC (Perkin Elmer, US) at a flow rate of 1 ml/min and a UV detector (Perkin Elmer, LC 235 diode array) at a detection wavelength of 220 nm. The stationary phase was a C-18 Nucleosil 5-μm column (240 × 4.6 mm). A mobile phase of acetonitrile/0.05 M phosphate buffer/thriethylamine (20:80:1, by vol.) adjusted to pH 4.0 was used; 20 μl was injected using an autosampler ISS-101 (Perkin Elmer).

Calibration curves were calculated on the basis of peak area measurements. They were generated with a correlation coefficient of 0.9988 for L-HCl and a correlation coefficient of 0.9999 for lidocaine.

2.8. Statistical data analysis

Results are expressed as the means of at least three experiments ± S.D. Statistical data analysis was performed using the non-parametric Mann–Whitney test with $P < 0.05$ as a minimal level of significance.

3. Results

3.1. Rheological investigations

In contrast to conventional flow curves viscosity measurement by oscillation is a gentle non-destructive method. It provides information about the elastic properties G′ (elastic modulus) and the viscous properties G″ (viscous modulus) of a preparation. Data for formulations 1–4 are presented in Fig. 1. G′ is a measure for the recoverable energy stored elastically in the system, whereas G″ is a measure for the energy dissipated as viscous flow representing the real and imaginary parts of the complex dynamic shear modulus, respectively.

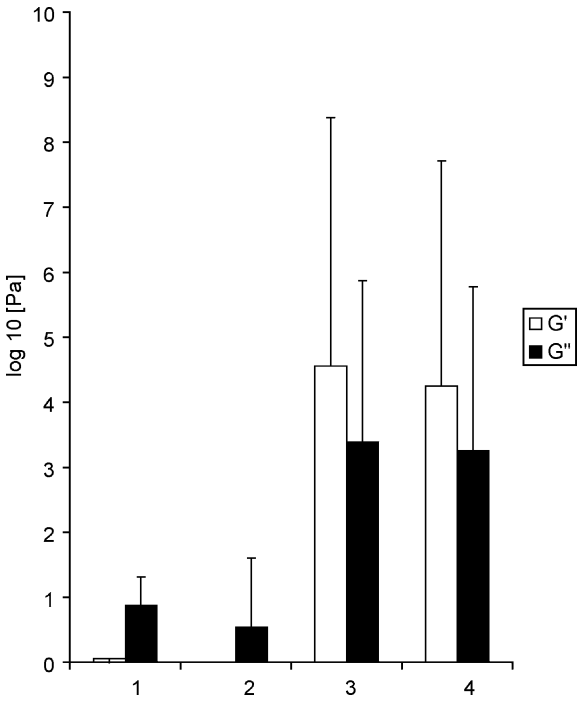

Fig. 1. Comparison of the elastic modulus (G′) and viscous modulus (G″) in log 10 Pa of formulations 1–4 (axis of abscissa) at 1 Hz. Indicated values are means (± S.D.) of three experiments.

All results presented in Fig. 1 are significantly different between the preparations except G″ of formulations 3 and 4.

As can be seen in Fig. 1, there are two groups of formulations. Group 1 contains a hydrophilic gel as well as a hydrophobic gel (formulations 1 and 2). Their elastic properties can be neglected. It is 1.15 Pa for formulation 1 and 0.25 Pa for formulation 2 and also the viscosity is rather small, namely 7.4 Pa for formulation 1 and 3.4 Pa for formulation 2. In contrast to this, the elasticity and the viscosity of formulations 3 and 4 show quite high G′ values of 36×10^3 Pa for formulation 3 and 18×10^3 Pa for formulation 4 and for G″ 25×10^2 Pa for formulation 3 and 18×10^2 Pa for formulation 4.

3.2. Solubility

According to Fick's first law the solubility of a drug in the vehicle plays an important role in terms of skin diffusion. Therefore the saturation solubility of phloretin as well as of L-HCl/lidocaine in the vehicles was analyzed. The method was a microscopic approximation method but suitable for valuation assessment. According to Table 2, 1% (w/w) phloretin is completely dissolved in formulations 3 and 4 and only partly dissolved in formulations 1 and 2

Table 2
Approximated saturation solubility of phloretin in % (w/w) and of L-HCl (formulation 1)/lidocaine (formulations 2–4) in % (w/w) in the used vehicles

	Pholretin solubility ± S.D.	L-HCl/lidocaine solubility ± S.D.
Formulation 1	0.021% ± 0.0028	30.05% ± 6.09
Formulation 2	0.035% ± 0.015	2.23% ± 0.67
Formulation 3	1.570% ± 0.16	16.68% ± 5.33
Formulation 4	2.970% ± 0.37	16.90% ± 0.47

whereas in all preparations the 2% (w/w) of L-HCl/lidocaine are completely dissolved (Table 2).

3.3. Skin permeation

The diffusion experiments in the present study were carried out with porcine skin, with a similar lipid composition as human skin [19].

Formulations 1, 2 and 3 showed a significant difference between the formulations with phloretin and the control from the 18th hour for formulation 1, from the 24th hour for formulation 2 and from the 28th hour for formulation 3. In contrast to this, formulation 4 exhibited a significant difference between the phloretin containing formulation and the control from the 6th hour of the diffusion experiment.

The results of formulation 1, a hydrogel preparation based on HEC, are depicted in Fig. 2 and show a significant 1.39-fold increase of L-HCl permeation by phloretin compared to the control after 50 h of diffusion. Phloretin increased the lidocaine diffusion significantly also from the hydrophobic siliciumdioxide gel (formulation 2) up to 1.76-fold (Fig. 3) after 50 h of diffusion. Although the lidocaine skin diffusion of formulations 3 and 4 was high, an

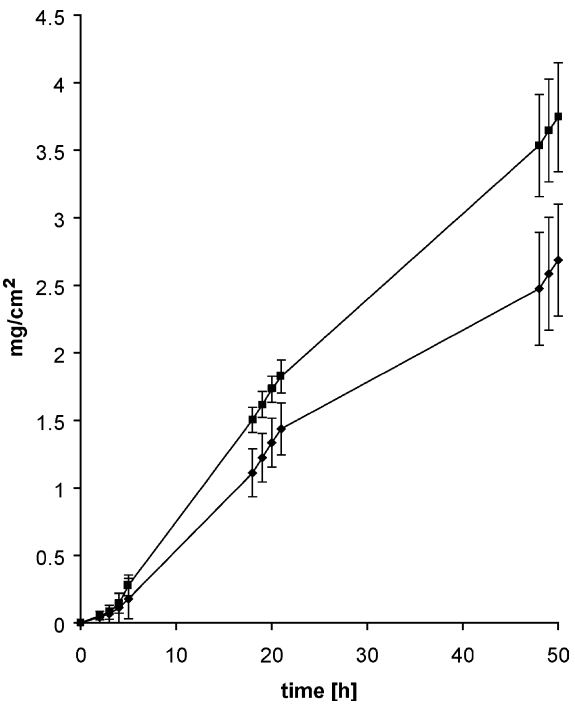

Fig. 2. Skin diffusion profile of L-HCl of formulation 1 through porcine skin. Formulation 1 (■) and control (◆). Indicated values are means (± S.D.) of three experiments.

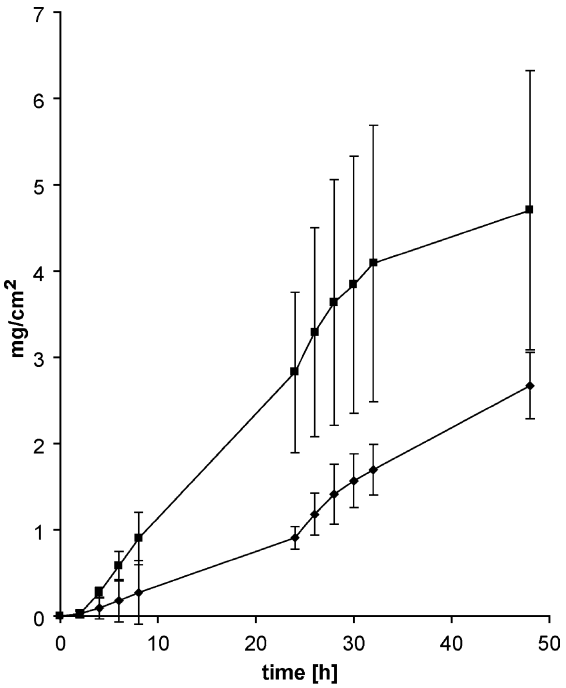

Fig. 3. Skin diffusion profile of lidocaine of formulation 2 through porcine skin. Formulation 2 (■) and control (◆). Indicated values are means (± S.D.) of three experiments.

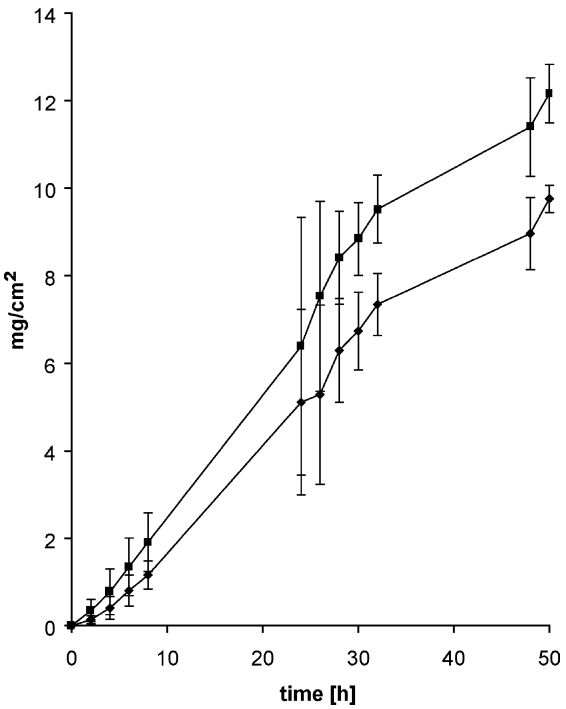

Fig. 4. Skin diffusion profile of lidocaine of formulation 3 through porcine skin. Formulation 3 (■) and control (◆). Indicated values are means (± S.D.) of three experiments.

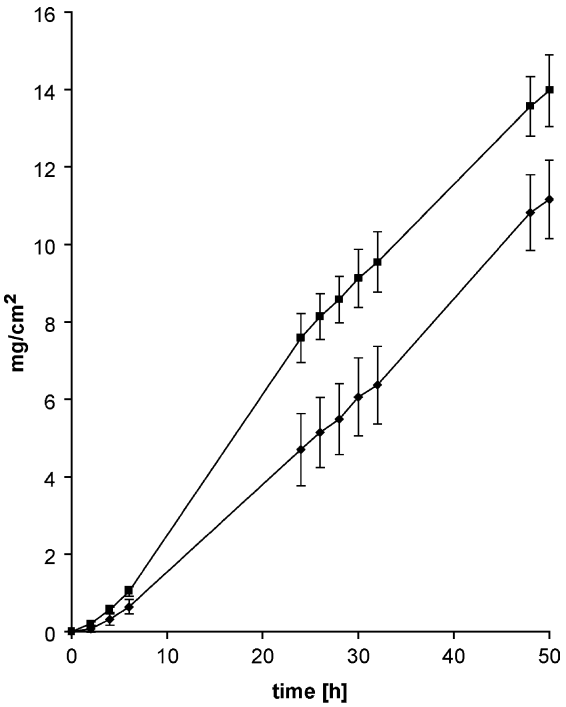

Fig. 5. Skin diffusion profile of lidocaine of formulation 4 through porcine skin. Formulation 4 (■) and control (◆). Indicated values are means (±S.D.) of three experiments.

additional significant 1.25-fold enhancement of lidocaine by phloretin in both formulations could be achieved compared to the controls after 50 h of diffusion. Results for formulation 3 are shown in Fig. 4 and for formulation 4 in Fig. 5.

In Table 3 the cumulative amounts within 50 h of permeated drug in % (w/w) of the applied lidocaine are compared.

4. Discussion

The aim of the present study was to investigate the effect of phloretin and lidocaine when simultaneously applied in a suitable vehicle.

As demonstrated, phloretin could enhance the lidocaine permeation by at least 1.25-fold compared to the control, independent of the used vehicle (hydrophilic, lipophilic, mucilaginous-liquid or semisolid). The benefit is an optimized delivery system without the complicated liposomal pre-exposure prior to drug application.

If the drug is dissolved in a homogenous isotropic phase the Stokes–Einstein equation is valid [20]. According to this equation the diffusion coefficient decreases with increasing viscosity. In contrast to this, the investigated formulations show a contradictory behavior (Table 3), possibly because all chosen vehicles are very complex systems. Formulation 1 is a bioadhesive hydrogel, formulations 2 is a hydrophobic gel and formulations 3 and 4 probably contain surfactant aggregates with self-assembled units. These complex structure will be one of the reasons for the higher drug permeation of formulations 3 and 4, despite their high elasticity and viscosity values.

Furthermore, the high concentration of the non-ionic polyoxyethylene surfactants PO5 and PO10 in formulations 3 and 4 could additionally intensify the skin permeation [4]. Polyoxyethylene alkyl ethers have been shown to enhance the percutaneous absorption of naloxone [21], griseofulvin, proquazone [22], diflorasone diacetate [23], flufenamic acid [24], nicotinic acid [25] and methyl nicotinate [26]. All data for non-ionic surfactants suggest that their mode of action on skin is related to their ability to disperse into the intercellular lipid phase of the stratum corneum. This increased fluidity in this region, which presumably reduced diffusional resistance [4]. Phloretin also shifted the lipid phase transition temperature of model membranes to lower values [27,28]. The data were interpreted in terms of fluidization of the oriented intercellular lipid bilayers, too. In conjunction with this the vehicle is supposed to increase on the one hand the phloretin solubility in the outermost layers of skin and on the other hand the solubility in the formulation itself. Consequently, phloretin is assumed to be targeted more rapidly to the skin lipids where the lipid interactions could be induced in order to facilitate lidocaine permeation.

Other investigations [29,30] assumed that phloretin decreases the membrane dipole potential and therefore support the membrane passage of cationic substances. In our case the cationic lidocaine-skin permeation is promoted.

The permeability of lidocaine through skin has been previously measured and reported in the literature [31,32]. By taking into account several possible sources of experimental errors, including the variability of the skin samples and animal types, the differences in experimental temperatures and skin preparation techniques, results for the phloretin free formulations obtained in the present study are of the same orders of magnitude. The additional use of phloretin can be considered as a suitable enhancer for lidocaine.

Table 3
Cumulative amount in % (w/w) of permeated lidocaine after 50 h of diffusion

	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Mean ± S.D.	18.72 ± 2.02	24.53 ± 8.695	60.81 ± 3.33	69.87 ± 4.64
Control ± S.D.	13.44 ± 2.065	13.91 ± 1.505	48.79 ± 1.56	55.81 ± 5.075

5. Conclusion

From the data obtained it can be concluded that phloretin increased the lidocaine skin permeation no matter which vehicle was used. Formulations 3 and 4 should be preferred because of their high lidocaine permeation. This permeation can be further improved by phloretin with probably additional anti-inflammatory and anti-irritative properties. The resulting multifunctional, semisolid and transparent products, should be evaluated in extended studies on human skin.

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References

[1] K. Sugibayashi, S. Nakayama, T. Seki, K. Hosoya, Y. Morimoto, Mechanism of skin penetration-enhancing effect by laurocapram, J. Pharm. Sci. 81 (1992) 58–64.
[2] A.C. Williams, B.W. Barry, Skin absorption enhancers, Crit. Rev. Ther. Drug Carrier Syst. 9 (1992) 305–353.
[3] S.C. Chattaraj, R.B. Walker, Penetration enhancer classification, in: E.W. Smith, H.I. Maibach (Eds.), Percutaneous Penetration Enhancers, CRC Press, Inc, Boca Raton, 1995, pp. 5–20.
[4] K.A. Walters, Penetration enhancers and their use in transdermal therapeutic systems, in: J. Hadgraft, R.H. Guy (Eds.), Transdermal Drug Delivery, Dekker, New York, 1989, pp. 197–246.
[5] K. Moser, K. Kriwet, A. Naik, Y.N. Kalia, R.H. Guy, Passive skin penetration enhancement and its quantification in vitro, Eur. J. Pharm. Biopharm. 52 (2001) 103–112.
[6] S.L. Raghavan, B. Kiepfer, A.F. Davis, S.G. Kazarian, J. Hadgraft, Membrane transport of hydrocortisone acetate from supersaturated solutions; the role of polymers, Int. J. Pharm. 221 (2001) 95–105.
[7] P. Singh, H.I. Maibach, Iontophoresis in drug delivery: basic principles and applications, Crit. Rev. Ther. Drug Carrier Syst. 11 (1994) 161–213.
[8] A. Boucaud, L. Machet, B. Arbeille, M.C. Machet, M. Sournac, A. Mavon, F. Patat, L. Vaillant, In vitro study of low-frequency ultrasound-enhanced transdermal transport of fentanyl and caffeine across human and hairless rat skin, Int. J. Pharm. 228 (2001) 69–77.
[9] T. Akimoto, K. Kawahara, Y. Nagase, T. Aoyagi, Polymeric transdermal drug penetration enhancer. The enhancing effect of oligodimethylsiloxane containing a glucopyranosyl end group, J. Controlled Release 77 (2001) 49–57.
[10] B.G. Auner, C. Valenta, J. Hadgraft, Influence of phloretin and 6-ketocholestanol on the skin permeation of sodium-fluorescein, J. Controlled Release 89 (2003) 321–328.
[11] B.G. Auner, C. Valenta, J. Hadgraft, Influence of lipophilic counter-ions in combination with phloretin and 6-ketocholestanol on the skin permeation of 5-aminolevulinic acid, Int. J. Pharm. 255 (2003) 109–116.
[12] C. Valenta, M. Nowak, J. Hadgraft, Influence of phloretin and 6-ketocholestanol on the permeation of progesterone through porcine skin, Int. J. Pharm. 217 (2001) 79–86.
[13] C. Valenta, C. Kuril, J. Hadgraft, Influence of phloretin and 6-ketocholestanol on the penetration of 5-aminolevulinic acid through porcine skin. Stratum Corneum, Martin Dunitz, Basel, 2001.
[14] P.B. Curtis-Prior, A.R. Oblin, A. Bennet, N.A. Parkinson, A.M. Orloff, Polyphloretin phosphate (PPP) antagonists of prostaglandin action also inhibit prostaglandin biosynthesis in-vitro, J. Pharm. Pharmacol. 42 (1990) 660–662.
[15] G. Blazso, M. Gabor, Effects of prostaglandin antagonist phloretin derivatives on mouse ear edema induced with different skin irritants, Prostaglandins 50 (1995) 161–168.
[16] C. Valenta, J. Cladera, P. O'Shea, J. Hadgraft, Effect of phloretin on the percutaneous absorption of lignocaine across human skin, J. Pharm. Sci. 90 (2001) 485–492.
[17] H. Altunkaya, Y. Ozer, E. Kargi, O. Babuccu, Comparison of local anaesthetic effects of tramadol with prilocaine for minor surgical procedures, Br. J. Anaesth. 90 (2003) 320–322.
[18] A.R. Gammaitoni, N.A. Alvarez, B.S. Galer, Safety and tolerability of the lidocaine patch 5%, a targeted peripheral analgesic: a review of the literature, J. Clin. Pharmacol. 43 (2003) 111–117.
[19] R.C. Wester, H.I. Maibach, Animal models for transdermal delivery, in: A.F. Kydonieus, B. Berner (Eds.), Transdermal Delivery of Drugs, CRC Press, Boca Raton, FL, 1987, pp. 61–70.
[20] B.C. Lippold, Biopharmazie, 2nd ed., Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1984, p. 163.
[21] B.J. Aungst, N.J. Fogers, E. Shefter, Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides, Int. J. Pharm. 33 (1986) 225–234.
[22] J.M. Franz, A. Gaillard, H.I. Maibach, A. Schweitzer, Percutaneous absorption of griseofulvin and proquazone in the rat and in isolated human skin, Arch. Dermatol. Res. 271 (1981) 275–282.
[23] J.S. Turi, D. Danielson, J.W. Woltersom, Effects of polyoxypropylene 15 stearyl ehter and propylene glycol on percutaneous penetration rate of diflorasone diacetate, J. Pharm. Sci. 68 (1979) 275–280.
[24] C.-C. Hwang, A.G. Danti, Percutaneous absorption of flufenamic acid in rabbits: Effect of dimethyl sulfoxide and various nonionic surface-active agents, J. Pharm. Sci. 72 (1983) 857–860.
[25] K.A. Walters, O. Olenjnik, S. Harris, Influence of nonionic surfactant on the permeation of ionized molecules through hairless mouse skin, J. Pharm. Pharmacol. 36 (1984) 78.
[26] K.A. Walters, O. Olenjnik, Effects of nonionic surfactants on the hairless mouse skin penetration of methyl nicotinate, J. Pharm. Pharmacol. 35 (1983) 79.
[27] R. Cseh, M. Hetzer, K. Wolf, J. Kraus, G. Bringmann, R. Benz, Interaction of phloretin with membranes: on the mode of action of phloretin at the water-lipid interface, Eur. Biophys. J. 29 (2000) 172–183.
[28] C. Valenta, A. Steininger, B.G. Auner, Phloretin and 6-ketocholestanol—membrane interactions studied by a phospholipid/polydiacetylene colorimetric assay and differential scanning calorimetry, Eur. J. Pharm. Biopharm. (2003) (submitted).
[29] J.C. Franklin, D.S. Cafiso, Internal electrostatic potentials in bilayers: measuring and controlling dipole potentials in lipid vesicles, Biophys. J. 65 (1993) 289–299.
[30] B. Bechinger, J. Seelig, Interaction of electric dipoles with phospholipid head groups. A 2H and 31P NMR study of phloretin and phloretin analogues in phosphatidylcholine membranes, Biochemistry 30 (1991) 3923–3929.
[31] G.P. Kushla, J.L. Zatz, Influence of pH on lidocaine penetration through human and hairless mouse skin in vitro, Int. J. Pharm. 71 (1991) 167–173.
[32] C. Padula, G. Colombo, S. Nicoli, P.L. Catellani, G. Massimo, P. Santi, Bioadhesive film for the transdermal delivery of lidocaine: in vitro and in vivo behavior, J. Controlled Release 88 (2003) 277–285.