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Microemulsions for topical delivery of estradiol

S. Peltola [a], P. Saarinen-Savolainen [c], J. Kiesvaara [b], T.M. Suhonen [a], A. Urtti [a,*]

[a] *Department of Pharmaceutics, University of Kuopio, P.O. Box 1627, 70211 Kuopio, Finland*
[b] *Orion Corporation Orion Pharma, P.O. Box 425, 20101 Turku, Finland*
[c] *Orion Corporation Orion Pharma, P.O. Box 1780, 70701 Kuopio, Finland*

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Abstract

Estradiol has been widely used for the treatment of hormonal insufficiencies. Due to its extensive first pass metabolism after oral administration, transdermal administration of estradiol in gels and emulsions has been used to improve its bioavailability, prolong activity and to optimize metabolic profile. The purpose of this study was to investigate microemulsions as delivery systems for estradiol. Various o/w microemulsions were used to deliver estradiol across human abdominal skin in vitro. Trasdermal flux of estradiol was determined using Franz-type diffusion cells and the samples were analyzed by high-performance liquid chromatography (HPLC). The permeation data showed that microemulsion formulations increased estradiol flux 200–700-fold over the control, but permeability coefficients were decreased by 5–18 times. The superior transdermal flux of estradiol was due to 1500-fold improvement in solubilization of estradiol by microemulsions. The results suggest that microemulsions are potential vehicles for improved topical delivery of estradiol.

Keywords: Estradiol; Microemulsion; Transdermal delivery; Human skin; Permeation enhancement

1. Introduction

Microemulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant and cosurfactant at appropriate ratios. Unlike coarse emulsions micronized with external energy microemulsions are based on low interfacial tension. This is achieved by adding a cosurfactant which leads to spontaneous formation of a thermodynamically stable microemulsion. The droplet size in the dispersed phase is very small, usually below 140 nm in diameter, which makes the microemulsions transparent liquids (Tenjarla, 1999). In principle, microemulsions can be used to deliver drugs to the patients via several routes, but the topical application of microemulsions has gained increasing interest.

The three main factors determining the transdermal permeation of drugs are the mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. These factors affect either the thermodynamic activity that drives the drug into the skin or the permeability of drug in the skin, particularly stratum corneum. Microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions (Ktistis and Niopas, 1998; Kreilgaard et al., 2000) and gels (Gasco et al., 1991; Kriwet and Müller-Goymann, 1995). Mobility of drugs in microemulsions is more facile (Kriwet and Müller-Goymann, 1995; Trotta, 1999; Kreilgaard et al., 2000), but the gel former in microemulsion will increase its viscosity and further decrease the permeation in the skin (Gasco et al., 1991).

* Corresponding author. Tel.: +358-17-162480; fax: +358-17-162252.
E-mail address: arto.urtti@uku.fi (A. Urtti).

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The superior transdermal flux from microemulsions has been shown to be mainly due to their high solubilization potential for lipophilic and hydrophilic drugs. This generates an increased thermodynamic activity towards the skin (Trotta et al., 1997; Kreilgaard et al., 2000; Alvarez-Figueroa and Blanco-Méndez, 2001). The drug may also be retained in the droplets of microemulsion formulation that will decrease its permeation in the skin. For example, the increased concentration of surfactant in dispersed systems may decrease drug release and its permeation in the skin (Ktistis and Niopas, 1998).

On the other hand, microemulsions may affect the permeability of drug in the skin. In this case, the components of microemulsions serve as permeation enhancers. Several neat compounds used in microemulsions have been reported to improve the transdermal permeation by altering the structure of the stratum corneum. For example, short chain alkanols are widely used as permeation enhancers (Pershing et al., 1990; Liu et al., 1991; Kim et al., 1992). It is known that oleic acid, a fatty acid with one double bond in the chain structure, perturbs the lipid barrier in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways in the stratum corneum (Pershing et al., 1993; Tanojo et al., 1997; Hadgraft, 2001). Isopropyl myristate (IPM) is used as a permeation enhancer in transdermal formulations, but the mechanism of its action is poorly understood (Goldberg-Cettina et al., 1995). Nonionic surfactants are widely used in topical formulations as solubilizing agents but some recent results indicate that they may affect also the skin barrier function (López et al., 2000; Fang et al., 2001). It is of interest to explore the effects of these components in the organized microemulsion structures.

The aim of the present study was to investigate the potential of several microemulsion formulations in transdermal delivery of estradiol.

2. Materials and methods

2.1. Materials

Estradiol was a gift from Orion Pharma (Turku, Finland). Oleic acid was received from Nomeco (Hellery, Denmark) and Epicuron 200 (soybean phospatidylcholine) from Lucas Meyer GmbH & Co. Phosphate-buffered saline (PBS) tablets for preparation of pH 7.4 buffer solution and IPM were purchased from Sigma Chemical Co. (St. Louis, MO). Polysorbate 20 (Tween 20) was received from Fluka AG (Buchs, Switzerland) and polysorbate 80 (Tween 80) and sorbitan monooleate 80 (Span 80), from Oriola Oy (Espoo, Finland). Isopropanol was supplied by Riedel-deHaen (Seelze, Germany) and ethanol (99.6%, v/v) by Primalco Oy (Rajamäki, Finland). Acetonitrile was purchased from Rathburn (Walkerburn, Scotland) and methanol from Labscan Ltd. (Dublin, Ireland). Hydroxypropyl-β-cyclodextrin (HβCD) (Cavasol® W7 HP Pharma) was received from Wacker-Chemie GmbH (Burghausen, Germany) and Carbopol® 940 from Goodrich Co. (Cleveland, OH).

2.2. Preparation of the skin

Excised human cadaver skin from the abdomen was obtained from the Kuopio University Hospital, Kuopio, Finland. The skin was stored at 4 °C and the epidermis was separated not more than 5 days postmortum. The skin was first immersed in purified water at 60 °C for 2 min and the epidermis was then peeled off. Dried skin samples were kept at −20 °C for later use.

2.3. Formulations

2.3.1. Microemulsion formulations

The oil (Epicuron 200, oleic acid and IPM) and water phases were first combined with the surfactant (Tween or Span). Cosurfactant (ethanol or isopropanol) was added gradually with magnetic stirring at room temperature until the system was transparent. Microemulsions were allowed to equilibrate with gentle magnetic stirring for 30 min. The compositions of non-viscous microemulsions are shown in Table 1.

Excess amount of estradiol was added to the microemulsion and allowed to equilibrate in the mixer under constant mixing for 4 days at room temperature. The saturated solution of estradiol was then filtered through Millex® 0.45 μm (Millipore, Bedford, MA).

2.3.2. Microemulsion gel formulation (F)

Microemulsion gel formulation was a combination of microemulsion A and Carbopol 940® (Table 1).

Table 1
Compositions of the microemulsions (%, w/w) and the microemulsion gel (F)

	Microemulsion					
	A	B	C	D	E	F
Oleic acid		38.5	29.4	41.3	22.2	
Isopropyl myristate	25.0		11.8			24.0
Epicuron 200	25.0					24.0
Tween 20				8.3		
Tween 80					18.5	
Span 80		7.7	8.8			
Ethanol		38.5	38.2	34.7	27.8	
Isopropanol	25.0					24.0
PBS, pH 7.4	25.0	15.4	11.8	15.7	31.5	24.0
Carbopol 940						4.0

Epicuron 200, IPM, PBS and Carbopol 940 were mixed by magnetic stirring. Isopropanol was added and mixed to the gel.

Estradiol sample of 43.77 mg was gradually ground to 10.09 g of gel until it was totally dissolved in the formulation.

2.3.3. Ethanol and isopropanol solutions

Saturated solution of estradiol in 30% (v/v) ethanol/PBS and in 25% (v/v) isopropanol/PBS solution.

2.4. Characterization of the microemulsions

Size distribution of microemulsion vesicles was determined by quasi-elastic light scattering using Gaussian distribution analysis (Nicomp Submicron Particle Sizer, Model 370, Santa Barbara, CA). The diameters were determined after microemulsion preparation and during storage at 4–8 °C for 6 months.

The phase systems (o/w or w/o) of the microemulsions were determined by measuring the conductivity (PW 9505 Conductivity Meter, Philips, England) of the microemulsions.

To measure the solubility of estradiol in the microemulsions excess of estradiol was added to each microemulsion and then allowed to equilibrate under continuous mixing for 4 days at room temperature. The saturated microemulsions were then filtered through Millex® 0.45 μm filters. The filtrate was diluted with 50:50 acetonitrile:water (microemulsions B, C, D and E) or methanol (microemulsion A) and analyzed for estradiol.

2.5. Permeation experiment

The passive permeability of estradiol through human cadaver skin was investigated using Franz diffusion cells with an effective diffusional area of 0.64 cm^2 (Crown Glass Co. Inc., Sommerville, NJ). The skin samples were hydrated in PBS for 15 min before setting them to the chambers. Receiver compartment contained 5 ml of 10% (w/v) HβCD-solution. Solubility of estradiol in PBS is only 3 μg/ml, but cyclodextrin in the receiver phase increased the solubility to 3.3 mg/ml which allowed to maintain sink conditions in the experiments. The skin samples were allowed to equilibrate for about 17 h prior to the experiment. 1 ml of formulation was pipetted into each donor compartment and sealed with Parafilm to prevent evaporation. In the case of microemulsion gel and 25% isopropanol solution donor volume was 0.5 ml. Samples were withdrawn from both the donor (10 μl) and receiver (700 μl) chambers at predefined appropriate time intervals. Then the same volume of pure vehicle was added to the receiver to maintain constant volume. The experiment was carried out long enough to ensure that the steady-state phase was attained (i.e. 3–5 times the lag time).

As a control, saturated solution (3 μg/ml) of estradiol in PBS was used in the donor compartment. In this case the sensitivity of the analytical method was not adequate at normal sample volumes. Therefore, sample volume was increased to 5 ml and the samples were concentrated by evaporation using SC-3 Sample Concentrator, Ori-Block O8-3 (Techne Inc., Priston, NJ) and then diluted back to 700 μl with PBS before analysis.

2.5.1. Effect of ethanol on the permeation of estradiol through the skin

Permeation enhancement by ethanol was studied by using either pretreatment of the skin samples with ethanol or by using 30% solution of ethanol both in the receiver and in donor. In pretreatment experiments 100 μl of ethanol was pipetted on the stratum corneum side of the skin sample or, alternatively, the whole skin samples were immersed in ethanol for 4 h prior to the experiment. Donor concentrations were equal in control chambers and in ethanol treatment chambers. In the receiver chambers either PBS or ethanol solution was used.

2.5.2. Determination of estradiol flux and permeability in the skin

The cumulative amount of estradiol permeating across the skin was plotted against time. Drug flux ($\mu g/h/cm^2$) at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface (0.64 cm^2). The permeabilities, K_p (cm/h), were calculated by dividing the steady-state estradiol fluxes by the initial concentration of the estradiol in the donor phase. Only samples that were taken under sink conditions were included.

2.6. Analytical methods

The samples were analyzed using a high-performance liquid chromatography (HPLC) system consisting of a SystemGold 126 solvent delivery pump and a SystemGold 166 UV spectometric detector (Beckman Instruments Inc., Camino Ramon, San Ramon, CA) with a Marathon® autosampler (Spakholland Instrument, Emmen, The Neatherlands) and a Supelcosil® LC-18-DB column (150 mm length, 4.6 mm diameter, 5 μm particle size; Supelco, Bellefonte, USA). Acetonitrile:water (1:1) was used as the mobile phase at a flow rate of 1.0 ml/min. The injection volume was 100 μl and the retention time of estradiol was 4 min. Estradiol was dissolved in the mobile phase and diluted in appropriate concentrations as standard solutions. The peak area correlated linearly with estradiol concentrations in the range of 0.05–100 μg/ml. The correlation coefficient varied in the range of 0.996–0.999 with an average 0.999.

2.7. Pharmacokinetic calculation

Pharmacokinetics of transdermal estradiol at steady state can be estimated using Eq. (1) (Hansch, 1990; Valjakka-Koskela et al., 2000), where A represents the absorption area on the skin, C_{ss} is the steady-state drug concentration in the plasma, Cl is the total body clearance of estradiol and J_{ss} is the drug flux across the skin. Thus, we calculated the area needed for drug absorption to reach therapeutic levels in plasma, when flux and clearance are known.

$$A = \frac{C_{ss}\mathrm{Cl}}{J_{ss}} \quad (1)$$

2.8. Statistics of the permeation experiments

The significance of the differences between groups was tested using Kruskal–Wallis one-way analysis of variance (ANOVA), and thereafter each group was compared using Mann–Whitney's *U*-test so that alcohol solutions (EtOH and *i*-PrOH) was compared to PBS, microemulsions A and F was compared to *i*-PrOH solution and microemulsions B, C, D and E was compared to EtOH solution.

3. Results and discussion

3.1. Microemulsion formulations

The droplet size of microemulsion vesicles was small with all the formulations having a mean vesicle size between 8 and 22 nm. The droplet size did not change during storage of 6 months at 4 °C. However, microemulsion E aggregated in some experiments which resulted in 10-fold increase in droplet size. During storage the homogenity of microemulsions B, C, D and E was broken down, but it was easily recovered by shaking.

According to conductivity results all formulations were oil-in-water microemulsions.

3.2. Estradiol permeation across the skin

The results of transdermal transport experiments reveal that the steady-state flux of estradiol from the microemulsions was 200–700-fold higher than from the control (Fig. 1, Table 2). Also, the lag times of permeation were shorter (about 1 h) for microemulsions than for the control (10–20 h). However, the permeability coefficients for estradiol from the microemulsions were actually 5–18 times lower than in PBS, ethanol or isopropanol. Estradiol solubility in the microemulsions was 1400–4500 times greater than in PBS leading to a greater concentration gradient in the skin, and subsequently, to improved maximum flux of estradiol. The increased solubility by three orders of magnitude easily overcomes the impaired permeability.

Ethanol and isopropanol had substantial effects on estradiol flux across the skin and again this effect seems to be based on improved solubility (Table 2). Comparison of results from ethanol solution to ethanol

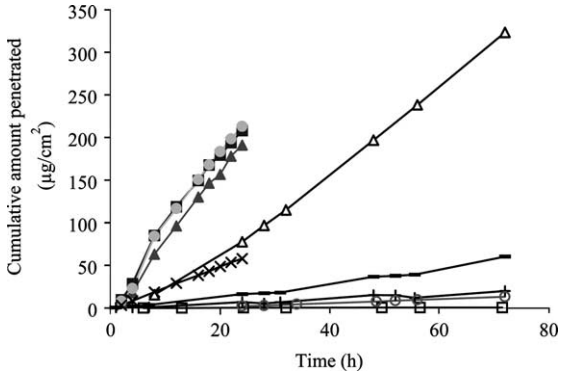

Fig. 1. Estradiol permeation through human skin from microemulsion A–F (△, ■, ▲, ●, ×, –), from 25% isopropanol (+), 30% ethanol (○) and PBS (□) (mean ± S.D.). Each data point represents an average of 3–25 determinations. The standard deviation varied between 30 and 76% of the mean.

containing microemulsions B, C, D and E shows that estradiol solubility and flux improved 130–170 and 10–30-fold, respectively. Similarly, solubility and flux of estradiol from the isopropanol based microemulsion A increased 25 and 18-fold, respectively, compared to isopropanol solution.

From all microemulsions (A–F) the flux of estradiol was clearly higher than from buffer, 30% ethanol or 25% isopropanol. Microemulsions B, C and D have quite similar compositions and no remarkable differences in fluxes, permeabilities or solubilities were seen (Fig. 2a and b, Table 2). Microemulsion E has same components as B, C and D, but it contains lower amounts of oleic acid and ethanol, and considerably higher amounts of water phase and non-ionic surfactant. Despite of the different composition estradiol solubility still remains equally high, but the flux and permeability were remarkably lower than in the other microemulsions, probably because of lower amounts of possible permeation enhancing components.

Formulation F was otherwise similar to the composition A except for the viscosity inducing Carbopol polymer. Fluxes of A and F should not be compared since, unlike formulation A, the microemulsion gel formulation F was not saturated with estradiol (Table 2). Permeabilities can however be compared. It appears that the permeability of estradiol from gel microemulsion is less than from A. The rate of drug release may decrease, when the microemulsion is transformed to lamellar structure or emulsion having a highly ordered microstructure and increased viscosity (Trotta, 1999).

3.3. Estradiol solubility

Our intention was to investigate whether the high estradiol solubility in the formulations can be explained by the solubility of estradiol in individual

Table 2
The steady-state flux (J_{ss}) and permeability (K_p) of estradiol through human skin (mean ± S.D.), estradiol solubility (C_{sat}) into the formulations used in the study (mean) and calculated surface area of the skin (A) that provide an adequate amount of estradiol to reach the level of the therapeutic plasma concentration

Delivery system	n	J_{ss} (μg/h/cm^2)	K_p (×10^3 cm/h)	C_{sat} (μg/ml)	A[a] (cm^2)
PBS	12	0.01 (0.007)	3.8 (2.6)	3.5	402
30% EtOH-PBS	7	0.3 (0.2)***	2.9 (2.1)	93	13
25% *i*-PrOH-PBS	5	0.3 (0.1)**	1.2 (0.3)	248	13
Microemulsion					
A	8	5.1 (2.7)**	0.7 (0.4)	6378	0.8
B	16	8.0 (2.9)***	0.6 (0.2)	13648	0.5
C	15	8.0 (2.0)***	0.5 (0.1)	16044	0.5
D	25	7.2 (4.4)***	0.6 (0.3)	13171	0.6
E	19	2.4 (1.2)***	0.2 (0.1)	12046	1.7
F[b]	4	0.9 (0.6)*	0.2 (0.1)	4296[c]	4.5

[a] $A = C_{ss}\mathrm{Cl}/J_{ss}$, when the therapeutic plasma concentration (C_{ss}) is 60 pg/ml and the total body clearance (Cl) is 67 l/h.
[b] The donor volume 0.5 ml.
[c] Not saturated donor concentration.
* Significantly different from the control ($P < 0.05$).
** Significantly different from the control ($P < 0.01$).
*** Significantly different from the control ($P < 0.001$).

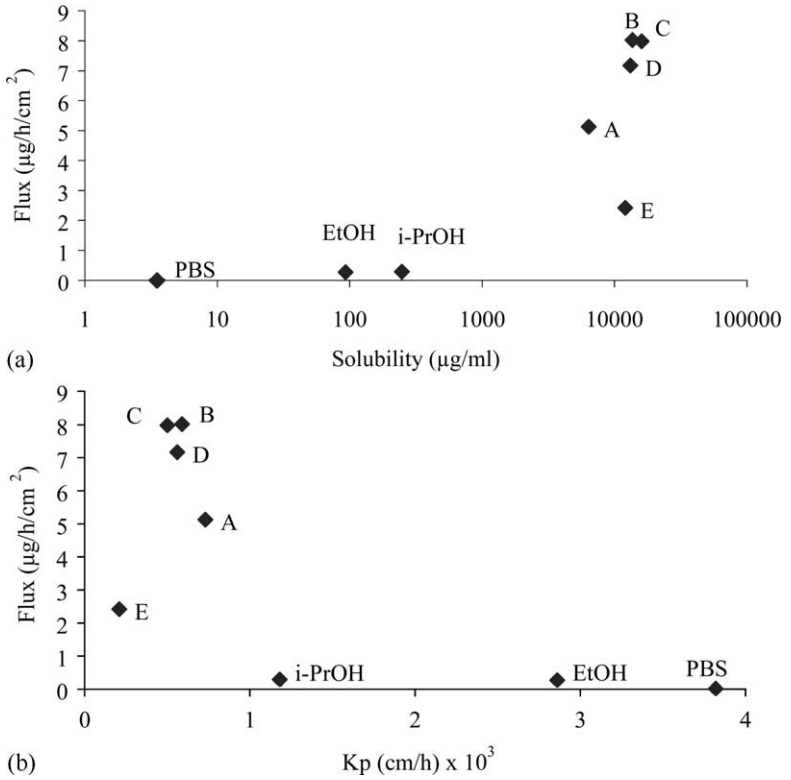

Fig. 2. Estradiol flux from the microemulsions (A–E), 30% ethanol (EtOH), 25% isopropanol (*i*-PrOH), and buffer (PBS) as a function of solubility (a) and permeability (b).

components. Estradiol solubility in oleic acid, IPM, Span 80, Tween 80 and Tween 20 was 0.6, 1.2, 2.5, 23.4 and 31.1 mg/ml, respectively. Fig. 3 shows estradiol solubility in the components of microemulsions and in the microemulsions. The solubility of estradiol in water is negligible and has a negligible effect on total solubility. Significant differences in estradiol solubility in the individual components and in the solubilities in the microemulsions were seen. Therefore, it seems that the microemulsion structure does not contribute to solubilization.

3.4. Role of microemulsion components in permeation enhancement

Ethanol has been shown to extract stratum corneum lipids and to perturb barrier function improving particularly the permeation of more hydrophilic drugs through skin (Gao and Singh, 1998). In our experiments we did not see any improvement in estradiol permeation when equal concentrations of estradiol were used in ethanol and PBS groups (data not shown). Flux and permeability through skin samples in ethanol group were even lower than in the PBS controls. This is in line with other observations (Pershing et al., 1990; Kim et al., 1992; Gao and Singh, 1998). Lipophilic estradiol may prefer stratum corneum lipid pathway and thereby lipid extraction by ethanol could inhibit its permeation. However, 30% ethanol solution can dissolve more estradiol than PBS. This increases the concentration gradient and flux of drug through the skin. Estradiol flux has been shown to be improved with up to 70% (v/v) of ethanol, but at higher volume fractions of ethanol drug permeation was decreased (Kim et al., 1996). Solubility of estradiol increased from 3 µg/ml to 10 mg/ml at 70% ethanol, but only modest further changes in solubility are seen at higher ethanol concentrations (Kim et al., 1996). It seems that the enhanced solubility of drug is the main factor in the transdermal flux enhancement for lipophilic

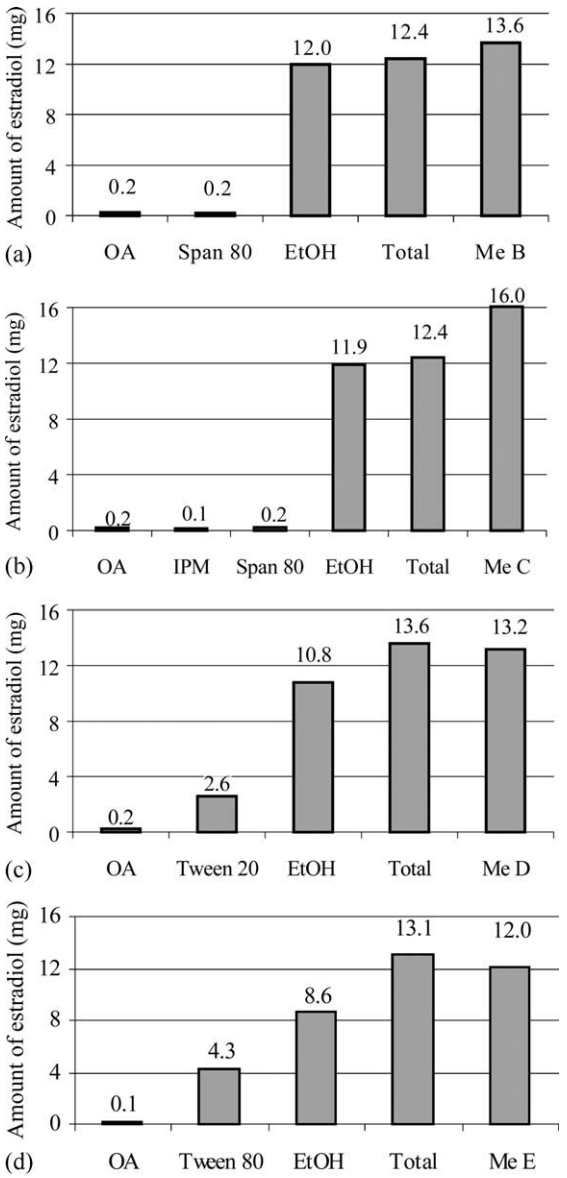

Fig. 3. Solubility of estradiol in microemulsions B (a), C (b), D (c) and E (d), and in each individual microemulsion component.

drugs. Also the other compounds included in the present microemulsions, oleic acid, IPM, Span 20 and Tweens, have been shown to promote drug permeation in the skin (El Maghraby et al., 2000; Kim et al., 1996; Gao and Singh, 1998; Kirjavainen et al., 1999; López-Castellano et al., 2000; López et al., 2000).

In our o/w microemulsions the oil components are in the inner phase, buffer solution as the outer phase and surfactant between the phases. Cosurfactant may be present in all phases. According to the solubility results most of estradiol is dissolved in ethanol in the microemulsions.

3.5. Mechanism of action of microemulsions in estradiol flux enhancement

Microemulsions could act as drug reservoirs where drug is released from the inner phase to the outer phase and then further onto the skin. This cannot be the main mechanism of estradiol permeation into the skin from our microemulsions. Outer phase of the formulation may contain either PBS or 30% ethanol/PBS solution if ethanol is evenly spread over the whole microemulsion. Flux from these vehicles was shown to be minimal (Table 2). Even if all ethanol in the formulation would be in the outer phase (70%), the flux should be only about 0.35 μg/h/cm^2 (Liu et al., 1991), but in our microemulsions it was from 2 to 8 μg/h/cm^2.

Another possibility is that the microemulsion droplet breaks down on the surface of the stratum corneum and, subsequently, releases its contents onto the skin. Estradiol may penetrate together with the microemulsion components that may act as permeation enhancers. However, the permeability coefficients of estradiol decreased in the microemulsion formulations (Table 2). Therefore, this mechanism may be excluded.

Third and the most likely mechanism may be permeation of estradiol directly from the droplets to the stratum corneum without microemulsion fusion to the stratum corneum and subsequent permeation enhancement. Due to the small droplet size and large amount of inner oil phase in microemulsions the density of droplets and their surface area are assumed to be high. Therefore, droplets settle down to close contact with the skin providing high concentration gradient and improved estradiol permeation.

3.6. Stability of microemulsions

Non-ionic surfactants have been observed to be sensitive to the changes in the temperature. For example, an increase in the temperature of an o/w microemulsion system prepared with nonionic surfactant can lead to a transition to a w/o system via a bicontinuous structure (Tenjarla, 1999). In our study

only the microemulsions with non-ionic surfactant (B–E) were separated to two phases during storage at +4 to +8 °C, but they were readily recovered after shaking at room temperature.

Phase separation was noticed after 8–24 h during diffusion experiments in Franz chambers. However, the flux versus plots were linear for 24 h in microemulsions B, C and D and for 50 h in microemulsion E (data not shown). The permeation profile from microemulsion A was linear for 70 h and no phase separation was observed in that case. Phase separation is probably due to the permeation of some microemulsion components into the skin and evaporation of ethanol. This leads to changes in the composition and subsequent destabilization. We assume that in the clinical situation the phase separation does not hinder the administration of estradiol microemulsions because the duration of drug absorption from the semi-solid formulation into the skin in vivo is short. This kind of destabilization of formulation is probable in other semi solid topical preparations.

3.7. Pharmacokinetic evaluation

Estradiol has abundant first pass metabolism in liver to estrone and, therefore, transdermal administration of estradiol may improve the systemic bioavailability and maintain favorable estradiol/estrone ratio in blood. Transdermal route has been used particularly for the treatment of postmenopausal symptoms. Estradiol has been administered in emulsions, gels and patches. We investigated the feasibility of microemulsions to deliver estradiol transdermally. In order to estimate the feasibility of delivery we performed pharmacokinetic calculations. The average therapeutic estradiol concentration in plasma (C_{ss}) in the menopause should be about 60 pg/ml (Dollery, 1991). The total body clearance (Cl) of estradiol is 67 l/h (Hansch, 1990). Based on the kinetic parameters and the experimentally determined maximum flux of estradiol across the skin in vitro (J_{ss}; μg/h/cm^2) we can calculate the exposed skin area ($A = C_{ss}\mathrm{Cl}/J_{ss}$) that is needed to maintain C_{ss} at 60 pg/ml (Table 2).

According to the kinetic calculations all microemulsions of this study seem to be promising candidates for transdermal delivery of estradiol. Therefore, it would be possible to incorporate even lower concentrations of estradiol in the microemulsions and still provide adequate total estradiol flux across the skin of reasonable surface area.

4. Conclusions

The utility of microemulsions as vehicles for topical delivery of estradiol was studied. The microemulsions were shown to increase the transdermal delivery of estradiol 200–700-fold over the control. The superior flux appears to be mainly due to the large solubilizing power of the microemulsions, which leads to larger concentration gradients towards the skin. Flux of estradiol in the skin is expected to be at adequate level for therapeutic effects.

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References

Alvarez-Figueroa, M.J., Blanco-Méndez, J., 2001. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int. J. Pharm. 215, 57–65.

El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2000. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int. J. Pharm. 196, 63–74.

Fang, J.-Y., Yu, S.-Y., Wu, P.-C., Huang, Y.-B., Tsai, Y.-H., 2001. In vitro skin permeation of estradiol from various proniosome formulations. Int. J. Pharm. 215, 91–99.

Gao, S., Singh, J., 1998. Effect of oleic acid/ethanol and oleic acid/propylene glycol on the in vitro percutaneous absorption of 5-fluorouracil and tamoxifen and the macroscopic barrier property of porcine epidermis. Int. J. Pharm. 165, 45–55.

Gasco, M.R., Gallarate, M., Pattarino, F., 1991. In vitro permeation of azelaic acid from viscosized microemulsions. Int. J. Pharm. 69, 193–196.

Goldberg-Cettina, M., Liu, P., Nightingale, J., Kurihara-Bergstrom, T., 1995. Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropyl myristate and short-chain alkanols. Int. J. Pharm. 114, 237–245.

Dollery, C., 1991. Therapeutic Drugs, vol. 2. Churchill Livingstone, London.

Hadgraft, J., 2001. Skin, the final frontier. Int. J. Pharm. 224, 1–18.
Hansch, C., 1990. Biopharmaceutics. In: Comprehensive Medicinal Chemistry, vol. 5. Pergamon Press, Oxford, p. 647.
Kim, Y.-H., Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., 1992. Short chain alkanols as transport enhancers for lipophilic and polar/ionic permeants in hairless mouse skin: mechanism(s) of action. Int. J. Pharm. 80, 17–31.
Kim, D.-D., Kim, J.L., Chien, Y.W., 1996. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid. J. Pharm. Sci. 85, 1191–1195.
Kirjavainen, M., Urtti, A., Valjakka-Koskela, R., Kiesvaara, J., Mönkkönen, J., 1999. Liposome–skin interactions and their effects on the skin permeation of drugs. Eur. J. Pharm. Sci. 7, 279–286.
Kreilgaard, M., Pedersen, E.J., Jaroszewski, J.W., 2000. NMR characterization and transdermal drug delivery potential of microemulsion systems. J. Control. Release 69, 421–433.
Kriwet, K., Müller-Goymann, C.C., 1995. Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum. Int. J. Pharm. 125, 231–242.
Ktistis, G., Niopas, I., 1998. A study on the in-vitro percutaneous absorption of propranolol from disperse systems. J. Pharm. Pharmacol. 50, 413–418.
Liu, P., Kurihara-Bergstrom, T., Good, W.R., 1991. Cotransport of estradiol and ethanol through human skin in vitro: understanding the permeant/enhancer flux relationship. Pharm. Res. 8, 938–944.
López, A., Linares, F., Cortell, C., Herráez, M., 2000. Comparative enhancer effects of Span®20 with Tween®20 and Azone® on the in vitro percutaneous penetration of compounds with different lipophilicities. Int. J. Pharm. 202, 133–140.
López-Castellano, A., Cortell-Ivars, C., López-Carballo, G., Herráez-Domínguez, M., 2000. The influence of Span®20 on stratum corneum lipids in Langmuir monolayers: comparison with Azone®. Int. J. Pharm. 202, 245–253.
Pershing, L.K., Lambert, L.D., Knutson, K., 1990. Mechanism of ethanol-enhaced estradiol permeation across human skin in vivo. Pharm. Res. 7, 170–175.
Pershing, L.K., Parry, G.E., Lambert, L.D., 1993. Disparity of in vitro and in vivo oleic acid-enhanced β-estradiol percutaneous absorption across human skin. Pharm. Res. 10, 1745–1750.
Tanojo, H., Junginger, H.E., Boddé, H.E., 1997. In vivo human skin permeability enhancement by oleic acid: transepidermal water loss and Fourier-transform infrared spectroscopy studies. J. Control. Release 47, 31–39.
Tenjarla, S., 1999. Microemulsions: an overview and pharmaceutical applications. Crit. Rev. Ther. Drug Carrier Syst. 16, 461–521.
Trotta, M., 1999. Influence of phase transformation on indomethacin release from microemulsions. J. Control. Release 60, 399–405.
Trotta, M., Morel, S., Gasco, M.R., 1997. Effect of oil phase composition on the skin permeation of felodipine from o/w microemulsions. Pharmazie 52, 50–53.
Valjakka-Koskela, R., Hirvonen, J., Mönkkönen, J., Kiesvaara, J., Antila, S., Lehtonen, L., Urtti, A., 2000. Transdermal delivery of levosimendan. Eur. J. Pharm. Sci. 11, 343–350.