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Research Article

Microemulsions Containing Medium-Chain Glycerides as Transdermal Delivery Systems for Hydrophilic and Hydrophobic Drugs

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Abstract. We evaluated the ability of microemulsions containing medium-chain glycerides as penetration enhancers to increase the transdermal delivery of lipophilic (progesterone) and hydrophilic (adenosine) model drugs as well as the effects of an increase in surfactant blend concentration on drug transdermal delivery. Microemulsions composed of polysorbate 80, medium-chain glycerides, and propylene glycol (1:1:1, *w*/*w*/*w*) as surfactant blend, myvacet oil as the oily phase, and water were developed. Two microemulsions containing different concentrations of surfactant blend but similar water/oil ratios were chosen; ME-lo contained a smaller concentration of surfactant than ME-hi (47:20:33 and 63:14:23 surfactant/oil/water, *w*/*w*/*w*). Although *in vitro* progesterone and adenosine release from ME-lo and ME-hi was similar, their transdermal delivery was differently affected. ME-lo significantly increased the flux of progesterone and adenosine delivered across porcine ear skin (4-fold or higher, $p<0.05$) compared to progesterone solution in oil (0.05 ± 0.01 μg/cm^2/h) or adenosine in water (no drug was detected in the receptor phase). The transdermal flux of adenosine, but not of progesterone, was further increased (2-fold) by ME-hi, suggesting that increases in surfactant concentration represent an interesting strategy to enhance transdermal delivery of hydrophilic, but not of lipophilic, compounds. The relative safety of the microemulsions was assessed in cultured fibroblasts. The cytotoxicity of ME-lo and ME-hi was significantly smaller than sodium lauryl sulfate (considered moderate-to-severe irritant) at same concentrations (up to 50 μg/mL), but similar to propylene glycol (regarded as safe), suggesting the safety of these formulations.

KEY WORDS: adenosine; medium-chain glycerides; microemulsion; progesterone; transdermal delivery.

INTRODUCTION

An efficient transdermal delivery of compounds is generally difficult to achieve due to the barrier function of the skin, provided mainly by the highly organized structure of the skin's outermost layer, the stratum corneum (SC) (1). This explains why, in spite of the many advantages of topical and transdermal administration of drugs, there are still few products commercially available for these routes. Among many different strategies and formulations studied to overcome the barrier function of the stratum corneum and increase the skin penetration of drugs, the use of microemulsions has generated considerable interest over the past years (2,3). Microemulsions present multiple advantages over other dermatological formulations, including (a) thermodynamic stability, (b) ease of preparation, (c) possibility to incorporate both hydrophilic and lipophilic drugs (at the same time, if desirable), and (d) possible skin penetration-enhancing ability. This last property may be related not only to the structure of the system (small droplet size associated with a high surface area) but also to the possibility of incorporating skin penetration enhancers in the oil phase and/or in the surfactant blend (3,4).

The ability of medium-chain mono- and diglycerides (which will be referred to as MCG throughout the text) to enhance the absorption of drugs through the intestinal mucosa has been demonstrated (5,6). This effect is associated with the ability of MCG to interact with membrane lipids and proteins, increasing membrane permeability (6,7). Although the skin differs from the intestinal mucosa in many aspects, some compounds that increase the intestinal permeability have also been demonstrated to increase the skin penetration of drugs (1,5,6). Hence, the incorporation of MCG in microemulsions has the potential to enhance the efficacy of such formulations as transdermal delivery systems. In this context, the first goal of this study was to evaluate the ability of microemulsions containing MCG as part of the surfactant blend to improve transdermal delivery of lipophilic (progesterone (PGT), log$P$=4.04) and hydrophilic (adenosine (ADN), log$P$=−0.05) model drugs (8,9).

It is well known that the structure and composition of microemulsions play an important role on the transdermal delivery of drugs. Many studies have demonstrated the

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influence of the type of oil, of surfactant/cosurfactant ratio, and of the microemulsion structure (o/w *versus* w/o) on drug release and skin penetration (2,10,11). Less studied, however, is the effect of the concentration of the surfactant blend used. In the face of these facts, the second goal of this study was to evaluate whether and how the concentration of the surfactant blend (and thus, the concentration of MCG) influences the transdermal delivery of the model drugs. Because of the importance of developing systems with low irritation potential, the third goal of this study was to evaluate the relative safety of the microemulsions containing different amounts of the surfactant blend by evaluating their concentration-dependent effects on the viability of cultured fibroblasts in comparison to those of propylene glycol (a commonly used compound in topical formulations) and of sodium lauryl sulfate (considered a moderate-to-severe irritant).

MATERIALS AND METHODS

Materials

Propylene glycol, Polysorbate 80 (P80), PGT, and ADN were obtained from Sigma (St. Louis, MO, USA). Medium-chain mono- and diglycerides (Capmul) were a kind gift from Abitec Corporation (Janesville, WI, USA). Acetonitrile and methanol were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA) and myvacet 9–45 oil (diacetylated monoglycerides from soybean oil; for the sake of simplicity, we will refer to this component solely as oil) was obtained from Quest (Norwich, NY, USA).

Methods

Pseudoternary Phase Diagram Construction and Sample Preparation

Ternary phase diagrams were constructed using the water titration method at room temperature. Because of its penetration-enhancing potential, MCG was chosen as one of the surfactants of the microemulsions. Since MCG is very lipophilic (hydrophilic lipophilic balance (HLB)=5–6, which allows its use as surfactant and as oil solvent) (6,12), we combined MCG with a more hydrophilic surfactant, polysorbate 80 (HLB=15), in the surfactant blend so that we could increase water incorporation and obtain o/w systems (5). In selected systems, propylene glycol was used as cosurfactant due to its ability to increase water incorporation in P80-based microemulsions (2). As a result, four different surfactant blends were used in the preparation of the microemulsion: P80/MCG (1:1 *w*/*w*), P80/MCG/propylene glycol (3:3:1 *w*/*w*/*w*), P80/MCG/propylene glycol (1:1:1 *w*/*w*/*w*), and P80/MCG/propylene glycol (1:2:1 *w*/*w*/*w*). The oil phase (myvacet oil) was added to the surfactant blend at ratios varying from 1:9 to 9:1 (*w*/*w*, surfactant blend/oil). These mixtures were titrated with water under vortexing, and the systems were first characterized using visual inspection to determine phase separation, fluidity, and transparency. Formulations that were fluid, clear, and did not undergo phase separation were classified as microemulsions.

Two microemulsions were chosen and subjected to further characterization: ME-lo (47:20:33 surfactant/oil/water, *w*/*w*/*w*) contained a smaller concentration of surfactant blend than ME-hi (63:14:23 surfactant/oil/water, *w*/*w*/*w*). These formulations were chosen because they have different concentration of the surfactant blend but similar water/oil ratios. PGT was incorporated in these microemulsions at a final concentration of 1% (*w*/*w*, as this concentration was used in previous transdermal delivery systems), whereas ADN was incorporated at 0.5% (*w*/*w*).

Microemulsion Characterization

Since microemulsions are isotropic systems by definition (i.e., they present same optical properties when probed in all directions), ME-lo and ME-hi were characterized using a polarized light microscope (Axiotop, Zeiss, Thornwood, NY, USA). Light scattering assays were performed to measure the droplet size, and a Zetasizer nano series instrument (Zetasizer Nano Series, Malvern, Westborough, MA, USA) was used for this aim. The mean droplet size for each microemulsion was determined at room temperature. The electrical conductivity of the two selected microemulsions was measured using the same equipment (Zetasizer Nano Series, Malvern, Westborough, MA, USA) and compared to the conductivity of the oil phase or water separately to determine whether the microemulsions are oil continuous or water continuous (4).

In Vitro Skin Permeation Assay

Transdermal delivery of PGT and ADN were studied *in vitro* using porcine ear skin mounted on Franz diffusion cells. Briefly, the skin from the outer surface of a freshly excised porcine ear was carefully removed, stored at −20°C, and used within a month. On the day of the experiment, the skin was thawed and mounted in a Franz diffusion cell (diffusion area of 1 cm^2; Laboratory Glass Apparatus, Berkeley, CA, USA), with the SC facing the donor compartment and the dermis facing the receptor compartment. The test formulation (100 mg) was placed in the donor compartment. The receptor compartment of the cells was filled with phosphate buffer (pH 7.4, 100 mM) maintained at 37±0.5°C with magnetic stirring at 350 rpm throughout the assay. The receptor phase contained 20% of propylene glycol when the delivery of PGT was studied; previous studies have used receptor phases containing up to 50% of propylene glycol without compromising the skin barrier (13). ME-hi, ME-lo, or control formulations (myvacet oil containing 1% PGT or water containing 0.5% ADN) were allowed to interact with the skin for 2, 4, 6, 8, or 12 h, after which the experiment was terminated for sample collection. To determine whether any of the surfactants have an effect of its own on the skin penetration of the model drugs, formulations of MCG in propylene glycol (15%, *w*/*w*) and P80 in propylene glycol (15%, *w*/*w*) were also studied; these formulations contained ADN or PGT at the same concentrations as the others, and they were left in contact with the skin for 8 h.

At the end of the experiment, skin samples were rinsed to remove excess formulation. The tape-stripping technique was used to separate SC from the epidermis (E) and dermis (D); 15 pieces of tape were used, and the pieces were placed in conical tubes containing 5 mL methanol (for PGT) or 5 mL of water/methanol (1:1 *v*/*v* for ADN). The remaining skin

(viable epidermis + dermis, E+D) was cut in small pieces, placed into conical tubes containing 2 mL methanol (for PGT) or water/methanol (1:1 *v*/*v* for ADN), and homogenized using a hand-held tissue homogenizer (Biospec products, Bartlesville, OK, USA). The SC and [E+D] samples were then sonicated for 20 min, filtered through a 0.45-μm pore membrane and assayed for PGT and ADN. Aliquots of the receptor phase were collected, filtered, and assayed for the drugs. The concentrations of drugs in SC and [E+D] are indices of topical delivery, whereas the concentration in the receptor phase is an index of transdermal delivery. The flux of drug across the skin was calculated using linear regression analysis; the amount of drug delivered across the skin was plotted as a function of time, and the slope of the linear portion of the curve was determined.

In Vitro Drug Release from Microemulsions

The release of PGT and ADN from ME-lo and ME-hi was assessed using the Franz diffusion cells and the setup described in "*In Vitro* Skin Permeation Assay" section, except that a cellulose membrane was used instead of the skin. Samples of the receptor phase were collected at 2, 4, 8, and 12 h postapplication, filtered and assayed for PGT and ADN.

Evaluation of Electrical Resistance of Skin

To evaluate the effect of the microemulsions on the barrier function of the skin, the electrical resistance of this tissue was measured before and after application of water (control), ME-lo or ME-hi using a LCR multimeter (Mod 5300, accuracy 0.8%, Sperry Instruments, Milwaukee, WI, USA). Skin samples were mounted in diffusion cells, and the donor and receptor compartments were filled with phosphate buffered saline (PBS); after 20 min of equilibration, the electrodes were inserted in the donor and receptor compartments for measurement of baseline skin resistance (14,15). Immediately thereafter, PBS in the donor compartment was replaced with 100 mg of water (control), ME-lo or ME-hi for 8 h. This time point was chosen since most of the differences between ME-lo and ME-hi skin penetration enhancement were observed after 8 h. By the end of the experiment, skin samples were washed with water and blotted dry. The donor compartment was then refilled with PBS, and electrical resistance was measured. The change in electrical resistance (Δ resistance) was calculated by subtracting the values of resistance measured 8 h after treatment from the baseline resistance values.

Quantification of Drugs

HPLC Methodology. Both drugs were assayed by high performance liquid chromatography (HPLC) using an equipment consisting of a Waters 600 controller, a Waters 717plus autosampler, and a Waters 996 photodiode array detector. The separation was performed by a Prevail C-18 column (5 μm), equipped with a C-18 precolumn. ADN was assayed using a mobile phase composed of water/acetonitrile at 9:1 (*v*/*v*, flow rate of 1 mL/min) and detection wavelength of 260 nm, whereas PGT was assayed using a mobile phase composed of acetonitrile/water at 8:2 (*v*/*v*, flow rate of 1 mL/min) and detection wavelength of 240 nm.

Standard solutions of PGT were prepared in methanol or in propylene glycol/phosphate buffer (1:4, *w*/*w*), whereas solutions of ADN were prepared in methanol/water (1/1, *v*/*v*) or phosphate buffer. Calibration curves of PGT in methanol or ADN in methanol/water were used to assay the amount of drugs in the skin; linearity was observed over 0.03–500 μg/mL (r^2=0.999). Calibration curves of PGT in propylene glycol/phosphate buffer or ADN in phosphate buffer were used to assay the drugs in the receptor phase of the diffusion cell; linearity was achieved over 0.03–100 μg/mL (r^2=0.999). The HPLC method for PGT and ADN were reproducible with within-day and between-day variations of less than 10%.

Recovery of PGT and ADN from Skin Samples. To standardize the recovery of PGT and ADN from skin tissue, skin sections (1.0 cm^2) were spiked with 1, 10, 20, and 50 μg of PGT (in a methanolic solution) or 0.5, 1, and 10 μg of ADN (in a water/methanol solution). Fifteen minutes later, the drugs were extracted from the skin sections as described in the "*in vitro* permeation studies". PGT recovery was linear over the concentration range of 1–50 μg/cm^2 (r^2=0.997) and varied from 88% to 92%. ADN recovery was linear over the range of 0.5–10 μg/cm^2 (r^2=0.988) and varied from 72% to 80%.

Evaluation of Cellular Viability

To evaluate the relative safety of the microemulsions, we compared their cytotoxic effects to those of propylene glycol (a commonly used compound in topical formulations) and sodium lauryl sulfate (considered a moderate-to-severe irritant) (16,17). Murine Swiss 3T3 mouse fibroblasts were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and grown at 37°C and 5% CO_2 atmosphere in Dulbecco's modification of Eagle's medium (ATCC, Manassas, VA, USA) containing 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) and additional penicillin and streptomycin (1%). For the cellular viability assay, cells were plated in 96-well plates (6,000 cells/well) and treated for 24 h with either PBS, propylene glycol, ME-lo, ME-hi, or sodium lauryl sulfate at concentrations ranging from 1 to 500 μg/mL in cell culture medium.

Cell survival was evaluated using a cell proliferation assay reagent (CellTiter 96 Aquous One solution, Promega, Madison, WI, USA) consisting of 3-(4,5-dimethylthiaziazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium salt (MTS) and an electron coupling reagent. The MTS salt is reduced to a colored formazan product, and the amount of this product is directly proportional to the number of living cells. After treatment with PBS, propylene glycol, ME-lo, ME-hi, or sodium lauryl sulfate, cells were washed with PBS, and 100 μL of cell culture medium plus 20 μL of the cell proliferation assay reagent were added to each well. The plates were incubated for 2 h at 37°C in a humidified atmosphere of 5% CO_2 in an incubator, and the absorbance was recorded at 490 nm using a plate reader (SpectraMax, Molecular Devices Corp., Sunnyvale, CA, USA). These experiments were performed in triplicate using cells between passages 2 and 6.

Statistical Analyses

The results are reported as means ± standard deviation. As in previous skin penetration studies (16), data were statistically analyzed using nonparametric tests. The Kruskal–Wallis test (followed by Dunn's post hoc test) was used to compare more than two experimental groups. Values were considered significantly different when $p<0.05$.

RESULTS

Microemulsion Development and Characterization

Phase diagrams representing the phase behavior of mixtures containing different amounts of surfactant, oil, and water are depicted in Fig. 1.When P80 and MCG were combined as the surfactant blend, the area of existence of microemulsions (black-shaded area) corresponded to 23% of the phase diagram (Fig. 1a). Addition of propylene glycol increased the amount of incorporated water (Fig. 1b–d). When the ratio between P80/MCG/propylene glycol was 3:3:1 (*w*/*w*/*w*), the area of microemulsion existence was slightly increased, but transparent and highly viscous systems were observed in the phase diagram (gray-shaded area, Fig. 1b). Further increase in propylene glycol (P80/MCG/propylene glycol at 1:1:1, *w*/*w*/*w*) abolished the existence of such systems, and the area of microemulsion was further increased (Fig. 1c). An increase in the amount of MCG in the surfactant blend (P80/MCG/propylene glycol at 1:2:1, *w*/*w*/*w*) decreased the amount of water that could be incorporated and the size of the area of microemulsion existence (Fig. 1d). Based on these results, a surfactant blend containing P80/MCG/propylene glycol at 1:1:1 (*w*/*w*/*w*) was chosen. Addition of either drug did not change water incorporation or the size of the area of microemulsions existence.

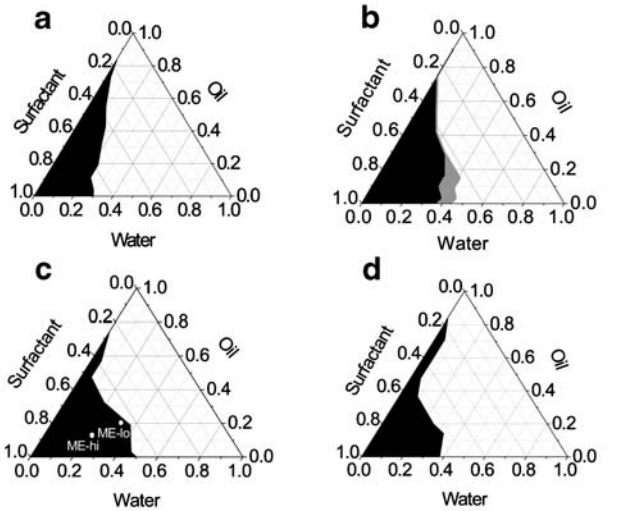

Diagram	Surfactant (w/w/w)	Microemulsion area (%)
A	Polysorbate 80 : MCG (1:1)	23
B	Polysorbate 80 : MCG : Propylene glycol (3:3:1)	28
C	Polysorbate 80 : MCG : Propylene glycol (1:1:1)	32
D	Polysorbate 80 : MCG : Propylene glycol (1:2:1)	26

Fig. 1. Ternary phase diagrams. The *black-shaded areas* in **a–d** represent regions where microemulsions are formed, whereas the *gray-shaded area* in **b** represents a region of clear but viscous systems. Points in **c** represent the composition of ME-lo and ME-hi

The microemulsions chosen for further characterization were ME-lo and ME-hi, which contained ratios of surfactant blend/oil/water equal to 47:20:33 and 63:14:23 (*w*/*w*/*w*), respectively (Fig. 1c; Table I). The physicochemical characteristics of ME-lo and ME-hi are depicted in Table I. The droplet size of the selected microemulsions was 25.2 and 21 nm (for ME-lo and ME-hi, respectively; Table I). Both microemulsions were isotropic when observed under a polarized light microscope, with no specific texture being observed even after drug incorporation. As can be observed in Table I, the conductivity of ME-lo and ME-hi were closer to the conductivity of water than that of oil, suggesting that the systems are oil-in-water microemulsions (18). This is in accordance with the structure formed based on the HLB values of the surfactants used: The calculated HLB for ME-lo and ME-hi is ~10, which generally gives rise to oil-in-water structures (5). The HLB may be even higher since glycols can increase the effective HLB of nonionic surfactants (19).

In Vitro Skin Permeation Assay

Figure 2 shows the skin penetration and transdermal delivery of PGT. Compared to the control formulation (drug solution in the oil), both ME-lo and ME-hi significantly ($p<0.05$) increased topical (SC and [E+D]) and transdermal PGT delivery after periods of application as short as 4 h: enhancements of ~3- and 8-fold on the amount of PGT delivered to the whole skin (sum of SC and [E+D]) and across this tissue, respectively, were observed at this time point. Comparing ME-lo and ME-hi, there was no significant increase in the amount of PGT delivered to the receptor phase or in PGT flux across the skin (Table II) using ME-hi with respect to ME-lo during the studied time period; however, significantly higher drug retention in the SC was observed using ME-hi at 12 h postapplication.

Both microemulsions also increased ADN delivery into and across the skin compared to the control solution. However, unlike PGT, ADN penetration into [E+D] and across the skin was higher using ME-hi than ME-lo at 8 h postapplication and up. After 8 h, ADN delivery to [E+D] was increased from undetected (using the control solution, Fig. 3b) to 0.19±0.03 and 0.35±0.04 μg/cm^2 using ME-lo and ME-hi (Fig. 3e–f), respectively. Similarly, the transdermal delivery of ADN was increased from undetected (using control solution, Fig. 3j) to 0.11±0.07 and 0.30±0.08 μg/cm^2 using ME-lo and ME-hi, respectively (Fig. 3k–l). ADN flux across the skin was two times higher using ME-hi than ME-lo (Table II).

In order to determine whether the surfactants affected the skin penetration of model drugs, solutions of MCG or P80

Table I. Characteristics of the Microemulsions

Formulation	Composition (surfactant/oil/water, *w*/*w*/*w*)	Diameter (nm)/polydispersity	Conductivity (μS/cm)
ME-lo	47:20:33	25.2/0.27	75.4
ME-hi	63:14:23	21/0.30	96.2
Water	–	–	80.2
Oil	–	–	13.3

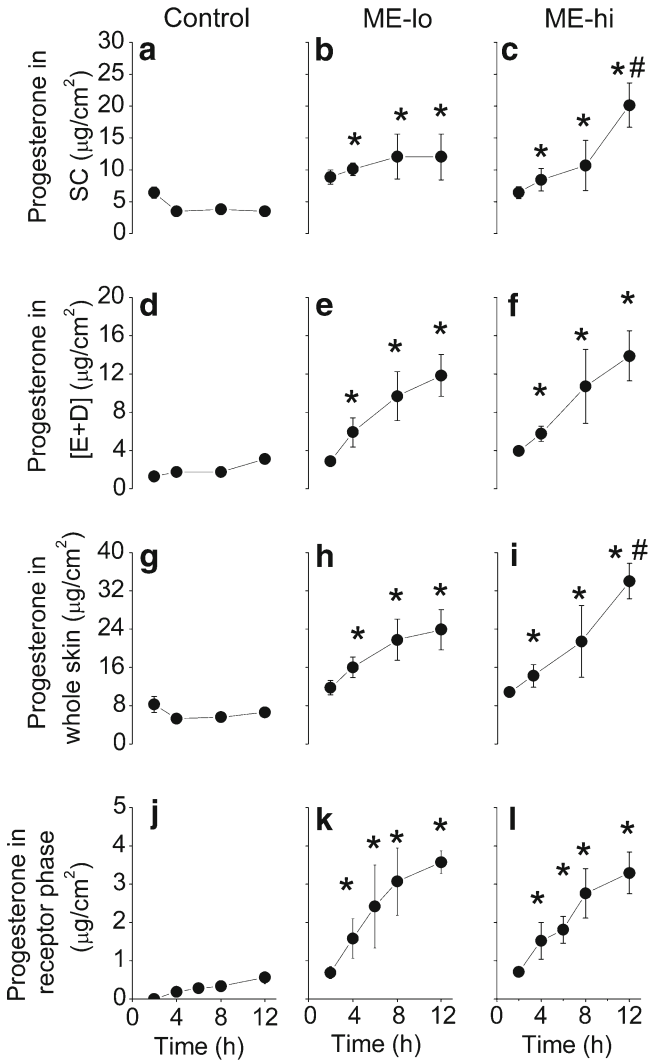

Fig. 2. PGT delivery to the skin (topical delivery) and across this tissue (transdermal delivery) using the two different microemulsions compared to the control formulation (drug solution in oil) as a function of time. **a–c** PGT penetration in the SC; **d–f** PGT penetration in the viable skin layers (E+D); **g–i** PGT penetration in the whole skin (SC + [E+D]); **j–l** transdermal delivery. *$p<0.05$ compared to the control formulation, #$p<0.05$ compared to ME-lo

in propylene glycol were prepared and their effect on the topical and transdermal delivery of PGT and ADN was evaluated (Fig. 4). MCG, but not P80, significantly increased the delivery of PGT and ADN to the SC (3- and 1.5-fold, respectively), [E+D] (3- and 1.5-fold, respectively), and to the receptor phase (5- and 2-fold, respectively). This enhancement was smaller than that caused by the microemulsions, suggesting that MCG may not be the only factor responsible for the penetration-enhancing effect of the microemulsion.

Table II. Flux of PGT and ADN Across the Skin

Formulation	PGT flux (μg/cm²/h)	ADN flux (μg/cm²/h)
Control	0.058±0.008	–
ME-lo	0.276±0.051	0.031±0.002
ME-hi	0.262±0.057	0.061±0.001

PGT progesterone, *ADN* adenosine

In Vitro Drug Release from Microemulsions

Having demonstrated that skin penetration into and across the skin can be influenced differently by ME-lo and ME-hi (especially the delivery of ADN), we next evaluated whether these results are related to differences in drug release from these formulations. Figure 5 shows the release profiles of PGT and ADN from the two microemulsions studied. The cumulative amount of PGT or ADN released from either ME-lo or ME-hi was plotted as function of time, and a linear relationship was observed for both drugs and both systems. No difference was observed on the release profile of either PGT or ADN when ME-lo and ME-hi were used, suggesting that the observed differences in delivery of

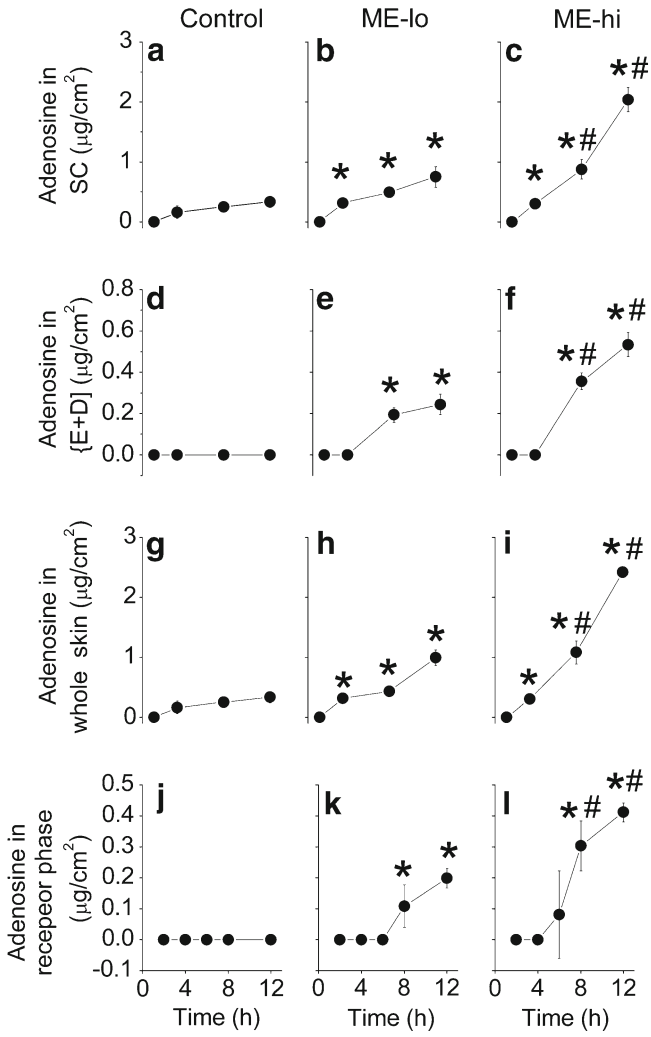

Fig. 3. ADN delivery to the skin (topical delivery) and across this tissue (transdermal delivery) using the two different microemulsions compared to the control formulation (drug solution in oil) as a function of time. **a–c** ADN penetration in the SC; **d–f** ADN penetration in the viable skin layers (E+D); **g–i** ADN penetration in the whole skin (SC + [E+D]); **j–l** transdermal delivery. *$p<0.05$ compared to the control formulation, #$p<0.05$ compared to ME-lo

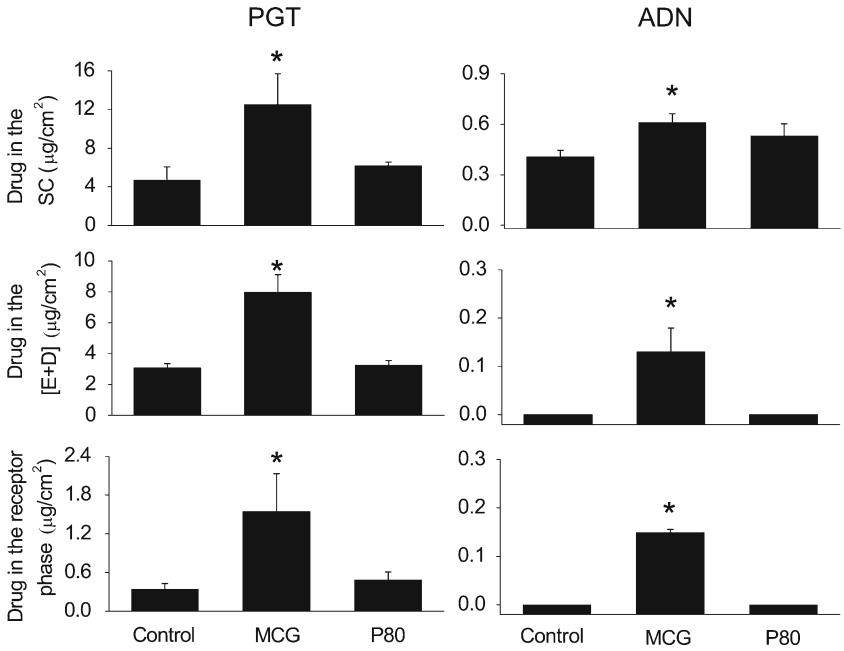

Fig. 4. Delivery of PGT or ADN to the stratum corneum (*SC*), viable skin layers (*E+D*), and across the skin using 15% MCG or polysorbate 80 (*P80*) in propylene glycol after 8 h. *Control*: solution of PGT (1%, *w/w*) or ADN (0.5%, *w/w*) in propylene glycol. *$p<0.05$ compared to the control formulation

the drugs to and across the skin do not result from different drug release from ME-lo compared to ME-hi.

Evaluation of Electrical Resistance of Skin

Because penetration enhancers (and delivery systems containing such compounds) can reversibly decrease the skin barrier function and its electrical resistance as function of their concentration, we studied the influence of ME-lo and ME-hi treatment in the electrical resistance of the skin (20). The results are depicted in Fig. 6. Baseline measurements of skin resistance varied from 11.0 to 15.8 kΩ/cm^2, which is consistent with previous reports (14). Compared to treatment with water (control), ME-lo and ME-hi promoted a 2.5 and 4.2-fold ($p<0.05$) decrease in skin electrical resistance, respectively, suggesting that microemulsion treatment decreased the barrier function of the skin. Additionally, there was a significant difference ($p<0.05$) between ME-lo and ME-hi, suggesting that the ME-hi-induced barrier disruption was stronger.

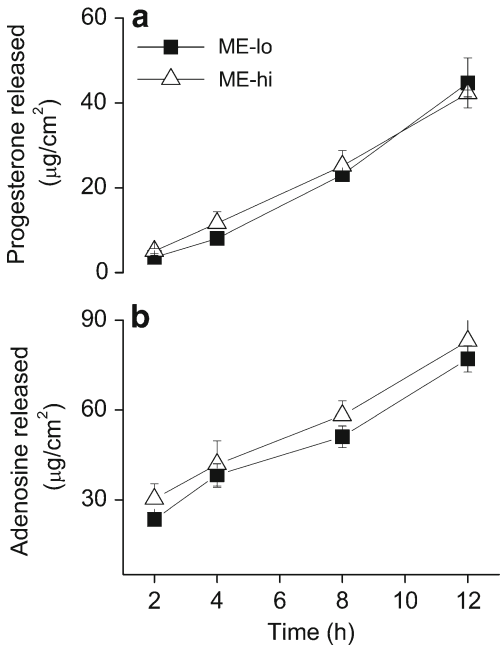

Fig. 5. Cumulative *in vitro* release of **a** progesterone and **b** adenosine from ME-lo and ME-hi as function of time

Evaluation of Cellular Viability

Because of the importance of developing topical formulations with low toxicity, the cytotoxic potential of ME-lo and ME-hi was assessed in comparison to that of propylene glycol and sodium lauryl sulfate. Whereas propylene glycol is considered safe and is widely used in topical formulations, sodium lauryl sulfate is considered a moderate-to-severe irritant (16,17). As expected, fibroblasts viability (expressed as percent of control, *i.e.,* untreated cells) was not affected when increasing amounts of PBS (up to 500 μg/mL) were added to the culture medium (Fig. 6). Compared to PBS, a significant decrease in cell viability was observed when sodium lauryl sulfate was used at a concentration as small as 1 μg/mL, whereas the same concentration of either the microemulsions or propylene glycol produced no significant effect on cell viability. At a concentration of 50 μg/mL or higher, both microemulsions significantly ($p<0.05$) reduced cell viability, as did propylene glycol. However, the cellular viability after treatment with ME-hi was significantly smaller than after treatment with either ME-lo or propylene glycol,

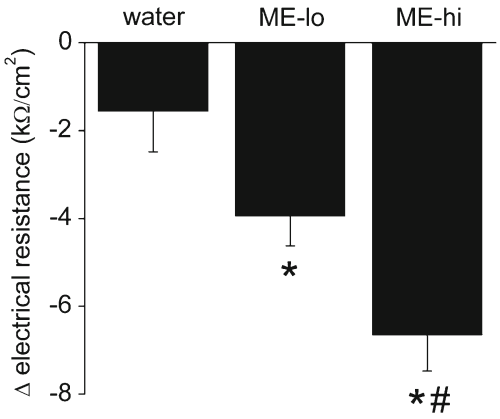

Fig. 6. Effect of treatment with water, ME-lo, and ME-hi for 8 h on skin electrical resistance. Δ electrical resistance = resistance values after 8 h − baseline resistance values; *$p<0.05$ compared to water; #$p<0.05$ compared to ME-lo

but still significantly higher compared to sodium lauryl sulfate (Fig. 7).

DISCUSSION

One of the requirements for microemulsion formation is the existence of a low surface tension at the oil–water interface, achieved by the use of surfactant blends and cosurfactants. Addition of propylene glycol to P80 and MCG blends increased the area of existence of microemulsions in a concentration-dependent manner. This is in accordance with other studies showing that combination of P80 and propylene glycol increases the amount of water incorporated in microemulsions (2). The microemulsions ME-lo and ME-hi were selected as delivery systems for the model drugs since they have different concentrations of the surfactant blend but similar water/oil ratios. Both systems were clear, fluid, and isotropic with a droplet size in order of nanometers.

ME-lo (lower surfactant concentration) and ME-hi (higher surfactant concentration) increased the skin penetration and transdermal delivery of PGT and ADN compared to control solutions of the drugs. This may be attributed to the possibility of larger drug transfer from the system to the skin due to the larger surface area of microemulsions (which is associated with the low interfacial tension and the small droplet size) (2,3). Our results also demonstrated that the penetration-enhancing effect of ME-hi was significantly higher than ME-lo at 8–12 h postapplication, but affected PGT and ADN delivery differently. A possible explanation for the superiority of ME-hi may rely on differences in the internal structure of the two microemulsions, which leads to different drug release from the formulations and, consequently, different skin penetration (2,11,21). This possibility, however, is not supported by our observation that both microemulsions provided similar release profiles of PGT and ADN.

Another possible explanation relates to the presence of penetration enhancer, its concentration difference between ME-lo and ME-hi and its effect on skin barrier (22,23). MCG, but not P80, significantly increased the skin penetration and transdermal delivery of the model drugs. Therefore, it is reasonable to suggest that MCG plays a role on the penetration-enhancing effect of the microemulsions. The reduction of skin electrical resistance after treatment with ME-hi for 8 h was higher than after treatment with ME-lo, suggestion that the ME-hi-induced barrier disruption was stronger. This is consistent with increases in the skin penetration and transdermal delivery of ADN using ME-hi, but not with PGT retention within superficial skin layers, suggesting that other factors (beside skin permeability) influence penetration of PGT. We have previously demonstrated that MCG concentration plays a major role in topical *versus* transdermal delivery of a lipophilic drug (7). Compared to a solution of PGT in propylene glycol, MCG at 10% enhanced the topical and transdermal delivery of PGT by 2.5- and 7-fold, respectively. MCG concentrations higher than 10% further increased PGT retention in the skin but not its transdermal delivery. This effect was also observed with other lipophilic drugs and monoglycerides and attributed to the affinity between them (24,25). Therefore, increased PGT retention in the skin using ME-hi may be a result of its affinity with MCG present at a higher concentration. The reason why the difference between ME-lo and ME-hi became significant only at later time points is not clear, but it was observed in other studies investigating microemulsions as transdermal delivery systems (2,18). This observation might suggest that the effects of the microemulsions (and their components) are cumulative and, as such, are more easily detected at later time points.

Cell cultures have been widely used to evaluate irritation potential of formulations and their components (26,27). A good correlation between *in vitro* cytotoxicity assays and *in vivo* skin irritation has been demonstrated for surfactants of different irritation potential, and since then, cytotoxicity assays became largely used to predict the irritation potential of substances (28). Although this method does not determine the exact concentration of a substance that may be toxic to the skin (since it does not mimic the complex structure of the skin), it allows comparing the cytotoxic potential of new formulations to that of compounds considered safe or irritant (26). Similar levels of cellular viability were observed after treatment with propylene glycol or the tested microemulsions at 1 μg/mL. However, when the concentration of formulations was increased to 50 μg/mL, the cellular viability after

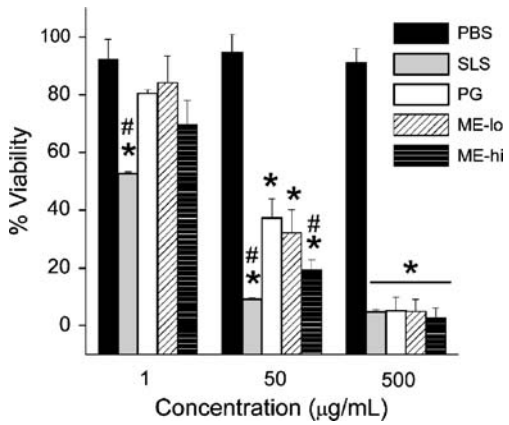

Fig. 7. Concentration-dependent effect of PBS, propylene glycol, sodium lauryl sulfate (*SLS*), ME-hi, and ME-lo on the viability of fibroblasts. Each point represents means ± standard deviation of three replicates. *$p<0.05$ compared to PBS, #$p<0.05$ compared to propylene glycol

treatment with ME-hi was significantly lower than after treatment with either ME-lo or propylene glycol, but still significantly higher compared to sodium lauryl sulfate. Based on these cytotoxicity results, three conclusions can be drawn. First, both microemulsions studied are safer compared to sodium lauryl sulfate. Second, enhancement of surfactant concentration in the microemulsion was associated with an increased cytotoxicity. However, it should be pointed out that, for the hydrophilic compound studied, the enhancement of surfactant concentration is also associated with a significant improvement of transdermal drug delivery. Third, the similarity between the irritant potential of the microemulsions (especially ME-lo) and propylene glycol suggest that selected microemulsions may be safe transdermal delivery systems.

CONCLUSION

In conclusion, microemulsions containing MCG can be considered effective transdermal delivery systems for hydrophilic and lipophilic drugs. The concentration of the surfactant blend affected differently the permeation of the studied hydrophilic and lipophilic drugs across the skin: The transdermal delivery of the hydrophilic drug, but not of the lipophilic one, was significantly enhanced when the concentration of the surfactant blend was increased.

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