Topical Delivery of Lycopene Using Microemulsions: Enhanced Skin Penetration and Tissue Antioxidant Activity

LUCIANA B. LOPES,1 HILLARY VANDEWALL,1 HSIN T. LI,1 VIJAY VENUGOPAL,1 HSIN K. LI,1 STAN NAYDIN,1 JACLYN HOSMER,2 MARK LEVENDUSKY,1 HAIAN ZHENG,1 M. VITÓRIA L.B. BENTLEY,2 ROBERT LEVIN,1 MARTHA A. HASS1

1Department of Pharmaceutical Sciences, Albany College of Pharmacy and Health Sciences, 106 New Scotland Ave., Albany, New York 12208
2Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil

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ABSTRACT: Topical delivery of lycopene is a convenient way to supplement cutaneous levels of antioxidants. In this study, lycopene was incorporated (0.05%, w/w) in two microemulsions containing BRIJ-propylene glycol (2:1, w/w, surfactant blend) but different oil phases: mono/diglycerides of capric and caprylic acids (MG) or triglycerides of the same fatty acids (TG). Microemulsions containing MG and TG were isotropic, fluid, and clear, with internal phase diameters of 27 and 52 nm, respectively. Both MG- or TG-containing microemulsions markedly increased lycopene penetration in the stratum corneum (6- and 3.6-fold, respectively) and in viable layers of porcine ear skin (from undetected to 172.6 ± 41.1 and 103.1 ± 7.2 ng/cm², respectively) compared to a control solution. To assure that lycopene delivered to the skin was active, the antioxidant activity of skin treated with MG-containing microemulsion was determined by CUPRAC assay, and found to be 10-fold higher than untreated skin. The cytotoxicity of MG-containing microemulsion in cultured fibroblasts was similar to propylene glycol (considered safe) and significantly less than of sodium lauryl sulfate (a moderate-to-severe irritant) at 1–50 μg/mL. These results demonstrate that the MG-containing microemulsion is an efficient and safe system to increase lycopene delivery to the skin and the antioxidant activity in the tissue. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:1346–1357, 2010

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INTRODUCTION

A prolonged exposure to ultraviolet (UV) radiation leads to several deleterious effects to the human skin, including damage to the DNA, premature skin aging, and skin cancer.1,2 Because naturally occurring cutaneous compounds that counteract these effects are continuously depleted by UV radiation and other harmful environmental factors, topical application of antioxidants can replenish cutaneous stores and reduce/prevent UV-induced skin damage.3,4

Among many available antioxidants, lycopene has aroused great interest because of its strong in vitro antioxidant activity.4 Lycopene is a C₄₀ carotenoid commonly found in red-colored fruits, which is up to 10-fold more efficient at quenching singlet oxygen than β-carotene or α-tocopherol.4,5 Several animal studies support a preventive role...
of lycopene against photodamage due to inhibition of UVB-induced ornithine decarboxylase and myeloperoxidase, reducing inflammatory responses and skin thickness, among other effects. In addition, it was recently demonstrated that lycopene content in the skin was inversely proportional to skin roughness, supporting the hypothesis that lycopene may be able to reduce skin aging, and formation of furrows and wrinkles. Development of topical formulations for lycopene is challenging, in part because of its strong lipophilicity (log $P \sim 15$), which makes it insoluble in water as well as in several oils used in food and cosmetics. Because of the same characteristic, lycopene penetration across the stratum corneum (SC) (epidermis' outermost layer) and into viable skin layers may be a difficult task due to its affinity with SC components and tendency to be retained in this layer. Because microemulsions present penetration-enhancing ability and high drug loading capacity, the use of these systems for topical delivery of lycopene may overcome the aforementioned difficulties.

Since the type and ratio of components play a significant role on the efficacy of microemulsions, we evaluated the influence of two types of oil on microemulsion characteristics and penetration-enhancing ability: mono/diglycerides of capric and caprylic acids (MG) and triglycerides of the same fatty acids (TG). These two oils were chosen since they differ in the number of acyl chains linked to the glycero molecule, and consequently, on the lipophilicity. For the sake of simplicity, microemulsions containing mono- and diglycerides of capric and caprylic acids will be referred here as ME-MG, while microemulsions containing the triglycerides will be referred as ME-TG. The antioxidant activity of skin samples treated with ME-MG containing lycopene and the cytotoxic potential of this formulation were also studied.

**MATERIALS AND METHODS**

**Materials**

Propylene glycol (PG), polyoxyethylene (10) oleyl ether (BRIJ 97), neocuproine, ammonium acetate, and copper(II)-neocuproine were obtained from Sigma (St. Louis, MO). Mono/diglycerides of capric and caprylic acids (MG) and triglycerides of the same fatty acids (TG) were kindly supplied by Abitec Corporation (Janesville, WI). Lycopene standard was purchased from Wako Chemicals (Richmond, VA). Acetonitrile, methanol, ethanol, and dichloromethane were purchased from Mallinckrodt Baker (Phillipsburg, NJ).

**Methods**

**Lycopene Extraction and Purification**

Lycopene was extracted from commercial tomato paste as previously described. Because lycopene is easily degraded, samples were protected from light and heat. Briefly, tomato paste (100 g) was dehydrated with 95% ethanol, filtered, and the residue was dissolved in dichloromethane and dried with anhydrous sodium sulfate (~3 g). Solvent was removed in vacuo, and the crude extract was purified by flash column chromatography (silica gel, petroleum ether/dichloromethane 75:25, v/v). Fractions containing pure lycopene were combined and solvent was removed in vacuo. This process yielded 27 mg of lycopene/100 g of tomato paste, which is similar to previous reports.

The identity of the purified lycopene was confirmed and compared to a standard lycopene using thin layer chromatography, HPLC (methodology in “Lycopene analysis”), UV-Vis spectrophotometry (Hitachi, Pleasanton, CA) and electrospray ionization mass spectrometry (positive ionization mode, Waters TQD tandem quadrupole detector, Milford, MA). Purified and standard lycopene presented similar $R_f$ values in silica plates, and similar UV-Vis spectra, with the characteristic absorption peaks at 504, 472, and 446 nm. The standard and purified lycopene exhibited similar retention times (9.4 min) when assayed by HPLC; the peak areas obtained when 1, 2, and 5 $\mu$g/mL solutions of the extracted lycopene were analyzed and were 20–25% smaller than those obtained after analysis of standards, suggesting that some degradation occurred during extraction. The antioxidant activity of the purified lycopene was ~15% less than that of the standard lycopene (methodology in “in vitro antioxidant activity”). When analyzed by mass spectrometry, the standard and purified lycopene presented a molecular ion of $m/z$ 537, which is consistent with the literature. The purified lycopene was used for formulation development and evaluation.

**TOPICAL DELIVERY OF LYCOPENE USING MICROEMULSIONS**

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**Phase Diagram Construction and Sample Preparation**

Ternary phase diagrams were constructed using the water titration method at room temperature. BRIJ was chosen as surfactant because it increases lycopene stability during contact with lipids.\(^{18}\) Propylene glycol was added to avoid formation of liquid crystalline phases by BRIJ, and enlarge the region of existence of microemulsions in phase diagrams. BRIJ and propylene glycol were mixed at 2:1 (w/w), and the oil phase (either the mono/diglycerides or the triglycerides of capric and caprylic acid) was added to the surfactant blend at ratios varying from 1:9 to 9:1 (w/w, surfactant–cosurfactant/oil). These mixtures were titrated with water under vortexing, and the systems were characterized by 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 selected for further characterization: one containing mono- and diglycerides of capric and caprylic acids as oil phase (which will be referred here as ME-MG) and another containing the triglycerides (which will be referred as ME-TG). ME-MG (40:36:24, surfactant/TG/water, w/w/w) and ME-TG (50:36:14, surfactant/TG/water, w/w/w) were chosen because they have the same oil concentration. This concentration was set to maximize topical delivery of lycopene, since MG at 20–50% increases topical (to the skin) delivery of a lipophilic drug while exerting a minor influence on its transdermal delivery.\(^{19}\) Lycopene was incorporated in these microemulsions at a final concentration of 0.05% (w/w).

**Microemulsion Characterization**

To verify whether ME-MG and ME-TG were isotropic systems, they were observed under a polarized light microscope (Axiotop, Zeiss, Thornwood, NY). Internal phase diameter was determined using a Zetasizer nano series instrument (Zetasizer nano series, Malvern, Westborough, MA) at room temperature. To evaluate the internal structure of the systems, electrical conductivity of samples composed of surfactant blend/MG (1:1:1, w/w) or surfactant blend/TG (1:4:1, w/w) and variable amounts of water (1–35%, w/w) was determined at 25 ± 0.5°C using a Jenway 4520 conductivity/TDS meter (Techne, Inc., Burlington, NJ).

**In Vitro Skin Penetration Assays**

**Skin Penetration of Lycopene.** Skin penetration of lycopene was studied using Franz diffusion cells. Briefly, the skin from the outer surface of a freshly excised porcine ear was carefully dissected, 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 cm²; Laboratory Glass Apparatus, Inc., Berkeley, CA), with the SC facing the donor compartment (where the formulation was applied) and the dermis facing the receptor compartment. The latter compartment was filled with 100 mM phosphate buffer (pH 7.4) containing ethanol (10%, v/v), and maintained at 37°C under constant stirring. Lycopene-containing microemulsions (100 mg) were placed in the donor compartment of diffusion cells for 4 or 8 h. These time points were chosen because previous studies from our group employing microemulsions for delivery of another lipophilic drug (progesterone) showed that 4 h was the shortest time-point where significant differences between formulations could be observed, whereas at 8 h, these differences were maximized.\(^{14}\) Solutions of 0.05% (w/w) lycopene in myvacet oil were used as control formulations. At the end of the experiment, skin samples were rinsed to remove excess formulation, and the SC was separated from the epidermis (E) and dermis (D) by tape stripping. Fifteen pieces of tape were used, and the pieces were placed in conical tubes containing 4 mL of acetonitrile/methanol (52:48, v/v). The remaining skin (viable epidermis + dermis, ED) was cut in small pieces, placed into conical tubes containing 2 mL acetonitrile/methanol, and homogenized using a hand-held tissue homogenizer (Biospec products, Bartlesville, OK). The SC and ED samples were then sonicated for 20 min, filtered through a 0.45 μm pore membrane and assayed for lycopene by HPLC. Aliquots of the receptor phase were collected, filtered, and assayed for lycopene. The concentrations of drug in SC and ED are indices of topical delivery, whereas the concentration in the receptor phase is an index of transdermal delivery.

In a second set of experiments (designed to evaluate whether lycopene was extensively degraded during contact with the skin), ascorbic acid (0.5 mg/mL) was added to the aqueous phase of the microemulsions and to the control solution (the oil was mixed with 10% propylene glycol containing ascorbic acid) to protect lycopene.
Formulations were applied to the donor compartment of diffusion cells for 8 h, lycopene was extracted from skin layers, and quantified by HPLC. The skin penetration of lycopene from formulations containing ascorbic acid was compared to drug penetration from formulations without ascorbic acid.

Skin Penetration of FITC. To evaluate whether penetration of compounds from the selected microemulsions is homogenous throughout the surface of the skin or is limited to certain skin structures (such as hair follicles), we studied the skin penetration and distribution of a fluorescent compound. FITC was chosen as a model compound because it is a well-characterized fluorophore, and presents relatively high absorptivity and good fluorescence quantum yield. FITC was incorporated at 0.05% (w/w) in the formulations. A 100 mg of FITC-containing ME-MG, ME-TG, or a solution in propylene glycol (used as control formulation) were applied to the donor compartment of Franz diffusion cells. Skin sections treated with phosphate-buffered saline (PBS) were used as control to determine tissue autofluorescence. After 8 h, the surface of the skin was carefully cleaned, and the diffusion area of skin samples was frozen using isopentane at −30°C, embedded in Tissue-Tek OCT compound (Pelco International, Redding, CA), and sectioned using a cryostat microtome (Leica, Wetzlar, Germany). The skin sections (8 μm) were mounted on glass slides. The slides were visualized without any additional staining or treatment through a 20× objective using a fluorescence microscope (Olympus, Center Valley, PA) equipped with a filter for FITC.

Microemulsion Effect on the Electrical Resistance of Skin

To evaluate the effect of the microemulsions on the barrier function of the skin, the electrical resistance of the tissue was measured before and after application of water (control), ME-MG and ME-TG using a LCR multimeter (Mod. 179, accuracy 0.8%, Fluke, Everett, WA). Skin samples were mounted in diffusion cells, and the donor and receptor compartments were filled with PBS; after 20 min of equilibration, the electrodes were inserted in the donor and receptor compartments for measurement of baseline skin resistance.

Immediately thereafter, PBS in the donor compartment was replaced with 100 mg of water (control), or the microemulsions for 4 or 8 h. By the end of the experiment, skin samples were rinsed with water and carefully blotted dry. The donor compartment was then refilled with PBS, and electrical resistance was measured.

In Vitro Antioxidant Capacity

To evaluate whether lycopene that penetrated in the skin was active as an antioxidant, we determined the antioxidant capacity of skin samples treated with a selected microemulsion (ME-MG) containing lycopene for 8 h using the CUPRAC (copper reducing antioxidant capacity) assay.

In this assay, the copper(II)-neocuproine reagent was used as the chromogenic oxidizing agent. Standard solutions of lycopene were prepared in 2% methyl-β-cyclodextrin in water/acetone (1:9, v/v) at concentrations ranging from 1 to 20 μg/mL as previously described. Lycopene standard solutions (0.5 mL) or skin samples were combined with copper chloride (0.5 mL, 10 mM), neocuproine (7.5 mM), and ammonium acetate (1 M, pH 7). The samples were vortex-mixed for 1 min, incubated for 30 min at room temperature, and filtered through 0.45 μm pore membranes. Absorbance of the filtrates was determined at 450 nm.

Evaluation of Cellular Viability

The microemulsion that resulted in the highest amount of lycopene delivered to the skin (ME-MG) was further evaluated as to its relative safety. We compared the cytotoxic effects of ME-MG (not containing lycopene) to those of PBS, propylene glycol (a commonly used compound in topical formulations) and sodium lauryl sulfate (considered a moderate-to-severe irritant). Murine Swiss 3T3 mouse fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, VA), and grown at 37°C and 5% CO₂ atmosphere in Dulbecco’s modification of Eagle’s medium (ATCC) containing 10% fetal bovine serum (Gibco, Carlsbad, CA) and additional penicillin and streptomycin (1%). For the cellular viability assay, cells were plated in 96-well plates (6000 cells/well), and treated for 24 h with either PBS, propylene glycol, ME-MG, or sodium lauryl sulfate at concentrations ranging from 1 to 100 μg/mL in cell culture medium.

Cell survival was evaluated using a cell proliferation assay reagent (CellTiter 96 Aqueous One solution, Promega, Madison, WI) consisting of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent (Promega, Madison, WI) dissolved in water.
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, 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% CO2 in an incubator, and the absorbance was recorded at 490 nm using a plate reader (SpectraMax, Molecular Devices Corp., Sunnyvale, CA).

**Lycopene Analysis**

Lycopene was assayed using a Shimadzu HPLC (Prominence series, LC-20AB dual pump, SIL-20A autosampler, SPD-M20A photodiode array detector set at 470 nm and Class-VP software). Separation was performed using a Prevail C18 column (150 × 4.6 mm, Alltech, Deerfield, IL), equipped with a C18 precolumn (7.5 × 4.6 mm, Alltech), and isocratic mobile phase consisted of acetonitrile/methanol (52:48, v/v), at 1.5 mL/min.25

**Statistical Analyses**

The results are reported as means ± SD. Data were statistically analyzed using the ANOVA test followed by Tukey post hoc test. Values were considered significantly different when p < 0.05.

**RESULTS**

**Microemulsion Characterization**

Phase diagrams representing the phase behavior of mixtures containing different amounts of surfactant, oil, and water are depicted in Figure 1. When MG was used as the oil phase, the area of existence of microemulsions (black-shaded area) corresponded to 50.3% of the phase diagram (Fig. 1A). Replacement of MG for TG decreased the area of microemulsion existence to about 21.5% (black-shaded area, Fig. 1B). This is consistent with other reports showing that the use of triglycerides as oil phase decreases water incorporation.26 Based on these diagrams, two microemulsions were chosen and subjected to further characterization: ME-MG (40:36:24 surfactant/MG/water, w/w/w) and ME-TG (50:36:14 surfactant/TG/water, w/w/w); these formulations were chosen because they have the same oil phase concentration, set to maximize topical delivery of lycopene.19 The droplet size of ME-MG and ME-TG was 26.8 ± 0.8 and 51.8 ± 4.7 nm, respectively, and both microemulsions were isotropic when observed under a polarized light microscope. No significant changes were observed after incorporation of lycopene at 0.05% (w/w). This concentration was chosen because no drug precipitation in the formulations was observed under light microscope.

Electrical conductivity measurements were performed to investigate the internal structure of the microemulsions. The ratio between surfactant blend and oil was kept constant in the formulations while water was added at small increments along a dilution line (dashed black and white lines shown in the diagrams in Fig. 1A and B). Water was added until the clear, fluid, and isotropic microemulsions were transformed into turbid systems (borderline of the microemulsion region). Conductivity was measured after each addition. ME-MG and ME-TG are within the dilution lines investigated.
Mixtures containing MG and TG behaved differently. Multiple regions were observed after water addition in the microemulsion containing MG. A region with very low conductivity existed with water content below 5%. Addition of water at 5–12% promoted a sharp increase in the conductivity (Fig. 1C). This phenomenon has been observed in water-in-oil systems, and is caused by increasing aqueous droplets’ interlinking process, resulting in the formation of water microchannels that produces a sharp increase in the conductivity. Between 15% and 21% of water (between points 1 and 2, Fig. 1C), the curve exhibited a change in the slope, and conductivity tended towards more constant values, suggesting a change in the structure of the system possibly to bicontinuous structures. Further addition of water increased the conductivity until the limit of stability was reached, when the system became turbid and more viscous. This increase in conductivity was associated with an increase in the slope of the curve above point 2, suggesting that the water phase became continuous and the system was transformed into oil-in-water structure. Since ME-MG contains 24% of water (being located above point 2), these results suggest that ME-MG is an oil-in-water microemulsion.

Addition of water at 2–18% caused a linear increase in the conductivity of the system containing TG (even though the values were still low), until the system lost stability and the microemulsion no longer existed (Fig. 1D). This is consistent with the behavior of water-in-oil systems, suggesting that ME-TG is a water-in-oil microemulsion.

Skin Penetration Assays

Skin Penetration of Lycopene

Skin penetration of lycopene from ME-TG and ME-MG is shown in Figure 2. Lycopene failed to penetrate into viable layers of the skin or to permeate across the tissue when the control solution was used, whereas 123.8 ± 34.6 and 150.6 ± 19.6 ng/cm² of the drug was retained in the SC after 4 and 8 h, respectively. Lycopene delivery to the SC increased 6- and 3.6-fold using ME-MG and ME-TG, respectively, whereas delivery to viable layers (ED) increased from undetected (using the control solution) to 172.6 ± 41.1 and 103.1 ± 17.2 ng/cm² using ME-MG and ME-TG, respectively after 8 h. No lycopene could be quantified in the receptor phase even when the microemulsions were used (quantification limit of the method was 25 ng/mL). These results demonstrate that even though both microemulsions produced a significant increase in skin penetration of lycopene, the effect of ME-MG was superior.

Since lycopene may be susceptible to degradation/oxidation by skin lipids and other components, we next evaluated whether lycopene levels quantified in the skin could be increased by adding another antioxidant to the formulation to protect the drug. Ascorbic acid was added to microemulsions, and the skin penetration of lycopene from these microemulsions was compared to the penetration from the systems free of ascorbic acid. Addition of ascorbic acid produced an average increase of 28.0 ± 2.1% and 34.6 ± 1.3% in the amount of lycopene quantified in the SC and viable layers of the skin, respectively (Fig. 3), but the difference of lycopene quantified in the skin comparing ascorbic acid-containing microemulsions with ascorbic acid-free systems was significant (p < 0.05) only for ME-MG. These results suggest that some lycopene may have been degraded in the skin, but this process is not extensive. Additionally, the same penetration-enhancing ability was observed comparing the formulations with and without ascorbic acid, that is, both ME-MG and ME-TG increased lycopene delivery to the skin, but the former was more effective.

Figure 2. Penetration of lycopene in the stratum corneum (SC) and viable layers of the skin (ED) after 4 or 8 h comparing the control solution (lycopene in myvacet oil, 0.05%, w/w), ME-MG and ME-TG. *p < 0.05 compared to the control formulation, #p < 0.05 compared to ME-TG. Each point represents mean ± SD of 5–6 replicates.
Skin Penetration of FITC

The skin penetration of FITC incorporated in the microemulsions or in a propylene glycol solution was visualized by fluorescence microscopy. Untreated skin presented a very weak autofluorescence (especially the SC; Fig. 4A and B). When FITC was incorporated in the propylene glycol solution, fluorescence was only present in the SC (Fig. 4C). On the other hand, FITC incorporation in either microemulsion resulted in a strong fluorescent staining of SC and viable epidermis; Figure 4D and E clearly demonstrated the enhanced distribution of FITC (greater quantities of FITC in deeper regions) when delivered from the microemulsions. The fluorescence seemed fairly homogenously dispersed into the skin and not concentrated around specific skin structures. Consistent with the results of lycopene penetration in the skin, the fluorescent staining was more intense after treatment with ME-MG than ME-TG.

Microemulsion Effect on Skin Electrical Resistance

Some delivery systems that contain penetration enhancers and/or surfactants can increase the...
skin penetration of drugs by reversibly decreasing the skin barrier function, and consequently, its electrical resistance. Treatment with water (control) produced a mild decrease in the electrical resistance of the skin as function of time (Fig. 5). This effect may be a result of tissue manipulation: prior to resistance measurement, skin sections were rinsed with water and carefully blotted dry. This procedure may have slightly affected the barrier function of the tissues. Compared to water, ME-MG significantly decreased skin resistance after 4 h ($p < 0.05$, 30% decrease, Fig. 5), while the effect of ME-TG was mild (and not significant) at this time-point. After 8 h, both microemulsions had a significant effect: 51.5 ± 5.2% and 67.2 ± 3.0% of the initial resistance remained after treatment with ME-MG and ME-TG, respectively. Again, the effect of ME-MG was more intense, suggesting that its ability to induce barrier disruption was stronger.

Antioxidant Capacity of the Microemulsion-Treated Skin

To evaluate whether lycopene that penetrated in the skin was active as an antioxidant, we determined the antioxidant activity of skin samples treated with lycopene-containing ME-MG compared to the untreated skin. ME-MG was chosen because it delivered the largest amount of lycopene to the skin. The antioxidant activity of ME-MG-treated skin was about 10 times higher than the untreated skin (Fig. 6), suggesting that the drug that penetrated in the skin was active.

Cytotoxic Potential of the ME-MG

Because of the importance of developing topical formulations with low toxicity, the cytotoxic potential of ME-MG was assessed based on the concentration-dependent effects of the microemulsion on the viability of cultured 3T3 fibroblasts in comparison to that of propylene glycol and sodium lauryl sulfate. ME-MG was chosen because it delivered the largest amount of lycopene to the skin. Whereas propylene glycol is considered safe and is widely used in topical formulations, sodium lauryl sulfate is considered a moderate-to-severe irritant.23,24 Fibroblasts viability was not affected by the addition of increasing amounts of PBS (1–100 μg/mL) to the culture medium (Fig. 7). Compared to PBS, a significant decrease in cell viability was observed using sodium lauryl sulfate at 1 μg/mL, whereas only at a concentration of 50 μg/mL or higher, propylene glycol and ME-MG significantly ($p < 0.05$) reduced cell viability.

DISCUSSION

Incorporation of lycopene into topical formulations is challenging due to its low aqueous solubility, stability, and bioavailability.9,34 Because of the structural organization of microemulsions
as dispersed nanodroplets, these systems present multiple regions to solubilize drugs compared to solutions. In addition to the oil phase, lycopene can be solubilized in the oil/water interface among surfactant molecules. This insertion may, however, affect the size of the internal phase. Garti et al. reported decreases in the droplet size and changes in the droplet shape after addition of 4.5 mg lycopene/g oil. We did not observe significant changes in the size of the droplets, probably because the amount of lycopene incorporated in our formulations was lower (1.4 mg/g oil).

Both ME-MG and ME-TG increased the penetration of lycopene into viable layers of the skin compared to a control solution, but ME-MG had a superior effect. The reasons for these observed differences may be related to the oil phase of these microemulsions. By replacing MG with TG, a decrease in the water incorporation capacity of the systems and on the area of existence of microemulsions was observed. Moreover, the internal structure of the systems was affected: water-in-oil systems were obtained in mixtures containing TG independently of the amount of water added (until system destabilization), whereas transformation from water-in-oil to oil-in-water occurred in mixtures containing MG upon water addition.

Therefore, while ME-TG is most likely a water-in-oil microemulsion, ME-MG is an oil-in-water microemulsion. Given the fact that release of lipophilic drugs from water-in-oil systems is generally smaller than from oil-in-water (due to the strong affinity between the drug and the formulation), it is reasonable to suggest that lycopene delivery to the skin using ME-TG may be inferior in part because of the smaller amount of drug released.

Differences in the penetration-enhancing ability of the lipids might also have affected the efficacy of the systems. The penetration-enhancing effect of microemulsions can be optimized by the addition of penetration enhancers as cosurfactants or oil phase; such compounds can interfere with the barrier function of the skin or improve drug partitioning into the tissue.

The reduction of skin electrical resistance after treatment with ME-MG was higher than after treatment with ME-TG, suggesting that ME-MG-induced barrier disruption was stronger. This is consistent with the penetration-enhancing effect of the oil MG, and with a higher delivery of lycopene and FITC (which is also a lipophilic compound with an estimated log $P_{lipid}$) to the skin using ME-MG compared to ME-TG. Other monoglycerides seem to share the penetration-enhancing ability of MG. Previous studies demonstrated that incorporation of monoglycerides containing longer acyl chains (such as glyceryl monooleate and glyceryl monoestearate) in topical formulations resulted in increased delivery of hydrophilic and lipophilic drugs to the skin.

It should be noted that ME-TG contains ~10% more surfactant than ME-MG. Therefore, even though the concentration of surfactants is generally an important parameter in the penetration-enhancing effect of microemulsions, our results suggest that the choice of oil phase had a stronger influence on the ability of the systems to disrupt skin barrier and to deliver lycopene to the skin than the surfactant concentration.

The superiority of ME-MG was maintained when ascorbic acid was included in the formulations. There was a clear trend of the formulations containing ascorbic acid to increase the concentration of lycopene quantified in the skin in comparison to those free of ascorbic acid, even though a significant difference was observed only for ME-MG. This effect was most likely because of lycopene protection. Although to date no studies have evaluated the benefits of combining lycopene and other antioxidants for topical application, this
association may be beneficial to improve lycopene stability and increase its concentration in the skin.

Because of the importance of developing safe, nonirritating topical delivery systems, the effect of ME-MG on the viability of cultured fibroblasts was evaluated. This formulation was chosen because it provided the highest delivery of lycopene to viable layers of skin. Cell cultures have been widely used to evaluate the irritation potential of formulations since a good correlation between in vitro cytotoxicity assays and in vivo skin irritation has been demonstrated. Although the exact concentration of a substance that may be toxic to the skin cannot be determined using this method (since it does not mimic the complex structure of the tissue), a comparison between the cytotoxic potential of new formulations and compounds considered safe or irritant can be made. The minimal cytotoxic concentration of ME-MG was 50 times higher than the concentration of sodium lauryl sulfate that produced a significant decrease on cell viability. Additionally, similar cellular viability was observed after treatment with ME-MG and propylene glycol; since the last is generally regarded as safe, this similarity suggests that ME-MG may be a safe delivery system for lycopene.

In conclusion, BRIJ-based microemulsions containing medium chain glycerides (especially mono- and diglycerides) are promising delivery systems to promote the cutaneous delivery of lycopene and improve the antioxidant activity in the skin. By using mono/diglycerides instead of triglycerides in microemulsions, the structure of the system was affected and its ability to modulate the barrier function of the skin and to deliver lipophilic compounds into the skin was increased, demonstrating that the choice of oil phase is an important parameter to optimize the skin penetration of lycopene and other lipophilic compounds.

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