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ORIGINAL ARTICLE

In vitro percutaneous absorption of genistein from topical gels through human skin

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Abstract

The objective of this study was to formulate genistein as a topical gel with various penetration enhancers for increased permeation and retention in human skin. The high performance liquid chromatography assay method was validated for precision and reproducibility. The intra-day and inter-day precision as represented by the coefficient of variation (CV) of the peak areas were <0.44% and <0.67%, respectively. Further, the reproducibility was demonstrated by the CV of the assay at different genistein concentrations, which were <1.64%. Genistein was subjected to various stress conditions to obtain basic information on the appropriate pH and aqueous vehicle for formulating topical gels. Genistein was highly stable under neutral and oxidative conditions, but degraded to highly polar and nonpolar compounds under basic and acidic conditions, respectively. Menthol produced a 9- and 22-fold increase in the flux and skin retention of genistein, respectively, after 24 h of gel application as compared with the control (no enhancer). Cineole showed an approximately 7-fold increase in flux, but skin retention did not increase significantly. Transcutol increased the flux and skin retention of genistein by 5- and 7-fold, respectively. When Transcutol was formulated with Lauroglycol, there was a 13- and 9-fold increase in the flux and skin retention, respectively. Incorporation of penetration enhancers into the topical gel increased the skin permeation of genistein, so that the target delivery rate for its therapeutic effects can be achievable based on the *in vitro* human skin data generated in this study.

Keywords: Blood pressure; gels; genistein; human skin; hypertension; penetration enhancers; topical; transdermal

Introduction

According to the American Cancer Society, there were about 500,000 deaths due to cancer in the United States in 2008 along with a total of 1.5 million new cancer cases. The United States is ranked 14th in the world in female breast cancer incidence, whereas Japan ranked 43rd, and China ranked 44th (Jemal et al., 2008). Among the Japanese, the incidence of cancer is less for those living in Japan when compared with those who have immigrated to Hawaii (Persky and Van Horn, 1995). Such a disparity in the occurrence of cancer in Asian and American cultures may be due to fundamental differences in diet. Consumption of soybean products, such as soya milk, soya sauce, tofu, miso, and tempeh, as well as low-fat foods is common in Asian cultures. Specifically, the typical Asian diet contains 10–12 g of isoflavone-rich soy protein per day which is roughly 20–50 times that of the average western diet (Hawrylewicz et al., 1995).

During the last decade, genistein (4′,5,7-trihydroxyisoflavone) has garnered significant attention from the scientific and medical communities for its antioxidative properties. Genistein is the most abundant isoflavone of the soy-derived phytoestrogen compounds (Asbill et al., 2000). A review of the literature revealed genistein to be an effective moiety against skin cancer, but limited data are available regarding its *in vitro* topical and transdermal delivery. Topically applied genistein was shown to reduce the incidence and multiplicity of skin tumors in the dimethylbenz[a]anthracene initiated and 12-*O*-tetradecanoyl phorbol-13-acetate promoted mouse models (Wei et al., 1998; Khan et al., 2008). In the UVB light-induced complete carcinogenesis model,

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topical pretreatment of mice with 10 μmol genistein significantly reduced the formation of H_2O_2 and 8-hydroxy-2-deoxyguanosine (Wei et al., 1998; Khan et al., 2008). Because these are the precursors for free radicals, their attenuation is a significant step for chemoprevention.

Genistein-containing soya complex and supplement preparations are commercially available as tablets and capsules for oral use. Although genistein is well absorbed, it has poor oral bioavailability due to extensive Phase II metabolism in the intestine (Mallis et al., 2003; Rowland et al., 2003; Zubik and Meydani, 2003; Cave et al., 2007; Nielsen and Williamson, 2007). Furthermore, bioavailability of soybean isoflavones depends on gut microflora in women (Xu et al., 1995). Pharmacokinetics of genistein indicate that absorption is relatively rapid and that once peak plasma concentrations are attained the half-life of elimination is on the order of 8 h in most healthy adults (Busby et al., 2002).

Topical delivery should help overcome the discrepancies associated with the relative bioavailability of genistein after oral administration, which has been observed in certain studies (Setchell et al., 2001, 2003a,b). These studies have shown genistein to have a nonlinear relationship for dose and absorption, suggesting a saturable mechanism of absorption. Thus, frequent doses throughout the day, rather than a single high dose, would help to achieve the optimum steady-state plasma concentration (Setchell et al., 2003a). Accordingly, topical administration would be a viable route, because this would provide prolonged absorption of this compound at a controlled rate.

A previous study reported the influence of the vehicle on isoflavones (genistein and daidzein) skin permeation from a saturated solution of dry soy extract (Minghetti et al., 2006). Flux was higher for polyethylene glycol 400 (PEG 400) than for propylene glycol, but minimal for Labrasol, Transcutol, oleic acid, and water. However, genistein flux increased significantly when pure genistein was used as a suspension in PEG 400 and resulted in significant skin retention. To further investigate the transdermal delivery of genistein, studies were conducted using pH 6 and pH 10.8 buffers and soybean oil as vehicles (Huang et al., 2008). Of these, the calibrated deposition of genistein into nude mice and pig skin for a saturated solution in pH 6 buffer was higher than the other vehicles. At this pH, genistein is nonionized. Pretreatment of skin for 2 h with either oleic acid or α-terpineol as penetration enhancers did not increase the permeation of genistein at pH 6. It was reported that topical delivery was promising for genistein use against photoaging and photodamage. Most of the published studies were conducted on solution or suspension formulations. This study was designed to formulate genistein as a topical gel. Genistein stability in aqueous vehicles under different influences and pH conditions was evaluated. Topical gels with different penetration enhancers were examined for their ability to increase genistein permeation and retention in human skin, *in vitro*.

Materials and methods

Chemicals

Genistein was obtained from Alexis Biochemicals (Carlsbad, CA). High performance liquid chromatography (HPLC) grade water and methanol were from J.T. Baker (Phillipsburg, NJ) and EMD Chemicals (Gibbstown, NJ), respectively. Methocel® E4M was a gift sample from Dow Chemical Company (Midland, MI). PEG 400, menthol, limonene, cineole, and carvone were from Sigma–Aldrich (St. Louis, MO). Transcutol P®, Labrasol®, Lauroglycol 90®, Capryol PGMC®, and Labrafil M 1944 CS® were gift samples from Gattefosse (Paramus, NJ). All other chemicals were from Fisher Scientific (Suwannee, GA).

HPLC method

An HPLC equipped with a 717plus auto-sampler, 1525 Binary HPLC pumps, and 2998 Photodiode Array detector (Waters Corporation, Milford, MA) was used in this study. The system was interfaced with a computer and Waters Empower2 software. A Waters Symmetry® reverse phase C18 column (150 mm × 3.9 mm; 5 μm particles) was used. The mobile phase was used at a gradient of 0–60% acetonitrile in 0.1% trifluoroacetic acid and was pumped at a flow rate of 1.5 ml/min. The injection volume was 50 μl and the detector was set at 262 nm. All chromatographic procedures were performed at room temperature. The intra-day and inter-day variability of the method was determined by injecting a standard solution of 5 μg/ml genistein at three different times (6 h apart) in a day and on 5 different days, respectively. The coefficient of variation (CV) of the peak areas was determined and represented the precision of the HPLC assay. Known concentrations of genistein (1.0–25.0 μg/ml) were assayed to determine the accuracy of the method.

Forced degradation of genistein

Genistein was subjected to forced degradation in acid, alkaline, neutral, and oxidative (hydrogen peroxide) conditions in the solution state. An initial stock solution of 500 μg/ml of genistein was prepared in methanol. This was diluted (1:9) separately with aqueous solutions of NaOH, HCl, or H_2O_2 to obtain a final concentration of 50 μg/ml of genistein in 1N NaOH, 1N HCl, or 1.5% v/v H_2O_2, respectively. These were stored in glass containers, protected from light, at 60°C. Samples were periodically collected and stored frozen (–20°C) until analyzed by HPLC to determine the extent of degradation. For HPLC analyses, 1.0 ml aliquots of the solutions were transferred to 10 ml volumetric flasks, neutralized as needed, and diluted to volume with methanol to obtain a final maximum concentration of 5 μg/ml.

Determination of partition coefficient

The partition coefficient of genistein was determined using an *n*-octanol/water system. Equal volumes (30 ml) of *n*-octanol and water were placed in a screw-capped test tube, shaken vigorously and then stored for 24 h.

After equilibration, the aqueous phase saturated with *n*-octanol and the oil phase saturated with water were separated. In 20 ml of this *n*-octanol, 20 mg of genistein was dissolved and then mixed with 20 ml of the aqueous phase in a separatory funnel. The funnel was vigorously shaken for 1 h and then stored for 24 h at 25°C. The concentration of genistein in each phase was then determined through UV absorbance using a Jasco V-630 Spectrophotometer (Tokyo, Japan). The partition coefficient was calculated as:

$$\log P_{(\text{octanol/water})} = \log([\text{Conc}]_{\text{octanol}} / [\text{Conc}]_{\text{water}}).$$

Formulation of gels

Topical gels containing terpenes were prepared with 0.5% w/w Methocel; 0.5% w/w genistein; 50% w/w ethanol; 0.4% w/w of terpenes such as menthol, limonene, cineole, and carvone; and 48.6% w/w water. Methocel, terpenes, and genistein were added to ethanol and mixed well. Addition of water to this mixture under stirring led to the swelling of Methocel and formation of a gel and simultaneous precipitation of the drug as a fine particle dispersion.

Topical gels containing glycol were prepared with 0.5% w/w Methocel; 0.5% w/w genistein; 0.5% w/w ethanol; 25% w/v glycols such as Transcutol, PEG 400, or Transcutol plus Lauroglycol (3:1); and 73.5% water. Methocel, ethanol, and genistein were mixed well with glycols. Addition of water to this mixture under stirring led to the swelling of Methocel and formation of a gel and simultaneous precipitation of the drug as a fine particle dispersion.

The solubility of genistein in each gel formulation was determined after equilibration at 25°C for 2 days. The formulation was centrifuged at 14,000*g* for 90 min, and the supernatant was filtered through a 0.45-μm syringe filter. The amount of genistein in the filtrate was determined using HPLC after appropriate dilution.

Permeation studies

Dermatomed human skin of 0.35 mm thickness was obtained from a tissue bank (International Institute of Advancement of Medicine, Exton, PA). The skin was collected from a single donor within 8 h of death and frozen at −70°C until use. Before skin permeation experiments, the frozen skin was thawed by storing at ambient temperature for about 20 min. All experiments were performed at least three times.

The Franz diffusion cell apparatus used in this study (PermeGear, Bethlehem, PA) holds up to six diffusion cells in series. The skin was mounted horizontally between the donor and receptor halves of the diffusion cell. The surface area of the skin exposed to the formulation was 0.64 cm^2. The receptor cell was filled with 5 ml of 0.01 M phosphate buffered saline pH 7.4 and ethanol (8:2). The receptor cell was stirred with a magnetic bead at 600*g*. A water circulation jacket (37.0°C) surrounded the receptor cell to maintain the skin temperature at a physiologic level.

The skin was mounted on the cells approximately 30 min before application of the formulations. The formulation (200 mg) was applied over the surface of the epidermis gravimetrically using a syringe. The donor chamber was occluded with Parafilm to prevent evaporation of water from the formulations. Samples (0.4 ml) were taken from the receptor cell to measure the amount of drug transported across the skin at 0, 1, 2, 4, 8, 12, and 24 h. The samples were replaced by fresh buffer solution and a correction factor was applied to account for drug removed during sampling.

At the end of the experiment (24 h), the residual drug formulation on the surface of the skin was removed using cotton swabs by swabbing (wet) and dabbing (dry) the surface with 200 μl of a solution of ethanol and water (1:1) six alternate times. The active diffusion area of the skin was then collected using a biopsy punch for determining the drug content in the skin. The skin was weighed, cut into small pieces, placed in glass vials and 1 ml of a solution of ethanol and water (1:1) was added. The samples were sonicated for 15 min and allowed to stand overnight. The vials were sonicated again for 15 min and the supernatant was filtered using 0.22-μm syringe filters into HPLC vials.

The extraction method was validated by determining percent recovery of genistein from the skin. Blank skin samples that had not previously been in contact with genistein were used in the recovery determination. Six skin samples (0.64 cm^2, representing the active diffusion area) were cut with a biopsy punch and placed in individual glass vials. The skin was minced and 1 ml of genistein in ethanol:water solution (1:1) at a concentration of 50 μg/ ml was added to each vial. The samples were sonicated for 15 min and allowed to equilibrate for 24 h. The samples were again sonicated for 15 min and the supernatant was filtered through a 0.22-μm nylon filter and analyzed by HPLC. The percent recovery of drug from skin was calculated as the ratio of the amount of genistein extracted from the spiked skin to the amount of genistein extracted from glass vials in the absence of skin, but processed by the same procedure (Padula et al., 2008).

Data analysis

The cumulative amount of genistein permeated through the skin was plotted as a function of time. The slope of the linear portion of the plot was calculated as flux (μg/ cm^2/h). The permeability coefficient was calculated by dividing the flux by the solubility of genistein in the formulation. Data were subjected to one-way analysis of variance followed by Dunnett's test to determine the level of significance between various groups. The data were considered significant at $P < 0.05$.

Results and discussion

The HPLC method used was validated and demonstrated a high precision or reproducibility. The intra-day and

inter-day precision and accuracy of the HPLC assay are summarized in Table 1. Precision is an expression of method reproducibility and is represented by the CV of the peak areas, which were <0.44% (intra-day) and <0.67% (inter-day). Further, the reproducibility was demonstrated by the coefficients of variation of the assay at different concentrations, which were <1.64%. The detection limit of the HPLC assay for genistein was 0.1 μg/ml. Over the concentration range of 1–25 μg/ml, the HPLC method demonstrated good linearity with an $r^2 > 0.9998$.

Stress testing can provide insight into the stability of a drug in aqueous formulations. Drug decomposition often results in loss of potency and possible adverse effects due to the formation of degradation products. The tests were performed on 50 μg/ml solutions of genistein using various stresses such as acidic, basic, and oxidative conditions (Table 2). Under neutral conditions, the samples were stable at 60°C for 30 days and the testing revealed 100% of the active drug in the stability samples (50 μg/ml). On the other hand, there was rapid degradation of genistein under basic conditions resulting in only 20% of the active drug in the stability sample (10 μg/ml) in 24 h. This decomposition resulted in more polar products as identified by shorter retention times on the HPLC chromatogram. Although genistein also decomposed under acidic conditions, it was not as rapid as with the basic conditions. It took approximately 5 days for 80% degradation of the drug in 1N HCl, resulting in 20% of the active drug in the stability samples (10 μg/ml). Genistein was also subjected to forced degradation under oxidative conditions using H_2O_2. The solution was stable at 60°C even after 30 days and the testing revealed 100% of the active drug in the stability samples (50 μg/ml). This study provided some basic information on the appropriate pH and aqueous vehicle for formulating topical gels of genistein.

Genistein was found to have a log $P_{(\text{octanol/water})}$ of 2.94, which makes it a favorable candidate for transdermal drug delivery. Ethanol, besides being a good solubilizer, is also an excellent permeation enhancer for several drugs (Liu et al., 2006). It has been effectively used at up to 60% v/v in different formulations as a penetration enhancer (Sinha and Kaur, 2000). Therefore, when formulating the Methocel base gel, genistein (0.5% w/w) was first dissolved in ethanol (50% w/w). Then, Methocel (0.5% w/w) was dispersed in the ethanolic solution of drug. Addition of water to this dispersion led to swelling of Methocel along with precipitation of the drug, thus forming a gel with a fine particle dispersion. The precipitated genistein in the dispersion can then replace the absorbed drug to maintain a constant concentration gradient and thermodynamic activity of the drug. Methocel was used because it provides reliable viscosity and forms temperature stable gels. Methocel at a concentration of 0.5% w/v in water provides a viscosity around 1000 cps. To possibly increase the permeation and skin retention of genistein, various penetration enhancers were incorporated into the base Methocel formulation. Terpenes and glycol derivates were investigated as permeation enhancers in this study. Terpenes have been classified as "generally recognized as safe" by the Food and Drug Administration and are known to be nontoxic and nonirritants. Although some terpenes are mild irritants, they do not cause any lasting erythema or do not cause any irritation at low concentrations in a formulation (Okabe et al., 1990; Asbill et al., 2000). Various terpenes have been used in different proportions for their penetration enhancement effect. Based on the literature (Sinha and Kaur, 2000; Aqil et al., 2007), menthol, limonene, cineole, or carvone was added to the gel base at a concentration of 4% w/w. Terpenes reportedly have a synergistic effect when combined with ethanol (Aqil et al., 2007). Thus, the formulations with terpenes maintained the 50% w/w ethanol of the base formulation, which was also necessary to temporarily solubilize genistein for the subsequent production of a fine particle dispersion. The formulations were assayed for the drug content and found to be very close to the theoretical drug content.

The effect of the terpene class of enhancers on the skin permeation and retention of genistein is shown in Figure 1. Menthol, cineole, and carvone showed a significant increase in genistein permeation compared with the Methocel base gel with no enhancer. For skin retention,

Table 1. Validation of HPLC method for genistein.

A: Precision (fixed concentrations, 5 μg/ml)		
Injection Sets	Intra-day variation conc. (μg/ml) Mean ± SD, $N = 4$	% CV
Set 1	5.001 ± 0.039	0.78
Set 2	5.000 ± 0.049	0.99
Set 3	5.001 ± 0.017	0.33
Injection Sets	Inter-day variation conc. (μg/ml) Mean ± SD, $N = 4$	% CV
Day 1	5.001 ± 0.030	0.60
Day 2	5.002 ± 0.062	1.23
Day 3	5.001 ± 0.049	0.98
Day 4	5.001 ± 0.054	1.08
Day 5	5.00 ± 0.013	0.26
B: Reproducibility (variable concentrations)		
Conc (μg/ml)	Inter-day variation conc. (μg/ml) Mean ± SD, $N = 5$*	% CV
1	1.070 + 0.01	0.97
2.5	2.530 + 0.02	0.81
5	5.010 + 0.02	0.57
10	9.770 + 0.16	1.64
25	25.72 + 0.32	1.25

*Injections were made on different days.

Table 2. Forced degradation of genistein at 60°C.

Stress condition	Time	Amount drug remaining in the samples (assay) (μg/ml)
Neutral	30 Days	50 (100%)
Base (1N NaOH)	1 Day	8.7 (17.4%)
Acid (1N HCl)	5 Days	10 (18.0%)
Oxidizing agent (1.5% H_2O_2)	30 days	49.8 (99.6%)

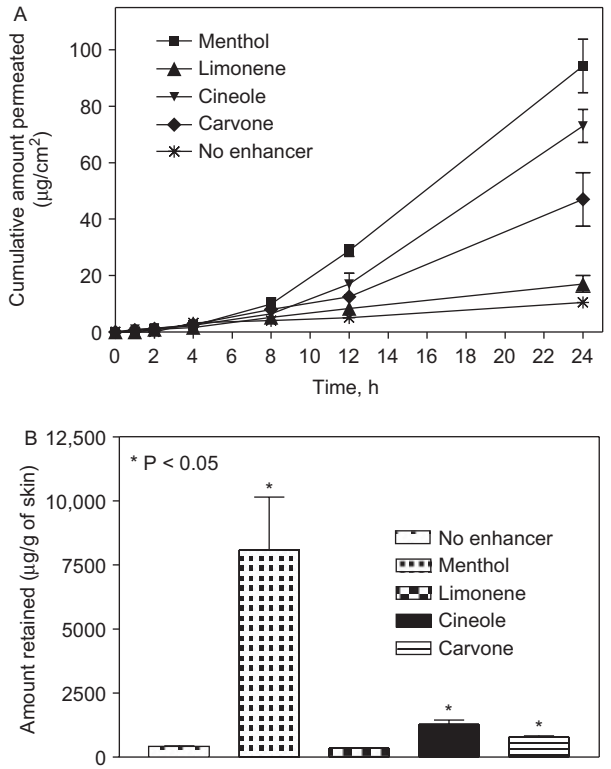

Figure 1. Effect of various terpene enhancers on (A) skin permeation and (B) skin retention of genistein from Methocel gel formulations.

we followed an extraction method (Padula et al., 2008) to determine the amount of genistein retained in the skin after the permeation study. The percent recovery of genistein from the blank skin samples (94.02 ± 1.4%) indicates that there is no significant loss of genistein to the skin after extraction. Of the terpene enhancers, only limonene did not significantly increase the amount of genistein retained in the skin. The amount retained in skin followed the same order as the amount permeated, that is, menthol > cineole > carvone > limonene. Menthol is said to be the most effective permeation enhancer in its class (Al-Khalili et al., 2003; Aqil et al., 2007; Chang et al., 2007), and it has been successfully formulated as an enhancer with imipramine hydrochloride (Jain et al., 2002), propanolol hydrochloride (Amnuaikit et al., 2005), caffeine, triamcinolone, and hydrocortisone (Godwin and Michniak, 1999). Menthol may enhance the permeation by a dual mechanism. Menthol alters the barrier properties of the stratum corneum and may also form a eutectic compound with some drugs, thereby increasing their solubility (Sinha and Kaur, 2000).

Menthol showed a 9-fold increase in the flux (Table 3) and a 22-fold increase in the skin retention of genistein after 24 h of gel application as compared with the control (no enhancer). Menthol does not significantly affect the solubility of genistein in the gel, thus the much higher permeability constant (Table 3) is not due to an increase in the concentration of dissolved drug. This study used terpenes at 0.4% w/w concentration in ethanol 50% w/w. Terpene enhancers in their neat form can accumulate

Table 3. Effect of various terpene penetration enhancers on the skin permeation of genistein from Methocel gel formulations.

Methocel gel with terpenes	Flux (µg/cm²/h)	Mean solubility (µg/ml)	Permeability Constant×10⁻⁴ (cm/h)
None	0.41 ± 0.05	820.1 ± 124.7	5.0 ± 0.7
Menthol	3.93 ± 0.68*	839.5 ± 143.6	46.8 ± 7.6*
Limonene	0.71 ± 0.21*	752.5 ± 102.1	9.4 ± 1.4*
Cineole	3.04 ± 0.42*	831.1 ± 140.1	36.6 ± 7.0*
Carvone	1.96 ± 0.68*	1173.4 ± 181.3*	16.7 ± 2.70*

*$P < 0.05$ versus control (no enhancer).

in stratum corneum lipid bilayers in large quantities (Cornwell et al., 1996). It was suggested that terpenes disrupt the lipid bilayers of the stratum corneum and preferentially distribute into the intercellular spaces (Sinha and Kaur, 2000; Krishnaiah et al., 2002, Narishetty and Panchagnula, 2005; Liu et al., 2006; Aqil et al., 2007). Terpenes are also reported to show a synergestic effect when used with ethanol (El-Kattan et al., 2000; Sapra et al., 2008; Krishnaiah et al., 2008). The increase in the skin permeation and retention of genistein by terpenes in this study may be attributed to their skin accumulation and interactions with stratum corneum lipids.

The effect of the glycol class of enhancers on the skin permeation and retention of genistein are shown in Figure 2. Various glycol derivatives are known to enhance skin permeation. These also act as solubilizers and can increase the solubility of the drug in the vehicle. All the glycols used in this study are good solubilizers for genistein (Table 4). For this reason, the concentration of ethanol in these formulations had to be decreased to 0.5% w/w to precipitate genistein on addition of water and form fine particles dispersion. This new control also allowed the evaluation of 50% w/w ethanol alone as a permeation enhancer.

When compared with the control formulation with 0.5% w/w ethanol, all glycol derivatives were capable of increasing both flux and skin retention of genistein significantly. Transcutol increased the flux and skin retention of genistein by 5- and 7-fold, respectively. Genistein solubility in the Transcutol gel was around 3-fold higher than that in the control formulation, along with a 1.5-fold higher permeability constant. This suggests a dual mechanism for permeation enhancement of genistein by Transcutol by modifying the stratum corneum and solubilizing the drug. The increased skin accumulation of genistein may be due to the formation of an "intracutaneous depot" by Transcutol. This depot effect is created by a swelling of stratum corneum intercellular lipids without alteration of their multiple bilayer structure (Ritschel et al., 1991). This results in an increased skin accumulation of drug with a simultaneous decrease in transdermal permeation (Ritschel et al., 1991). Transcutol containing liposomal vesicles demonstrated better skin retention of minoxidil without transdermal absorption (Mura et al., 2010). Other studies report that Transcutol was an effective skin permeation enhancer for

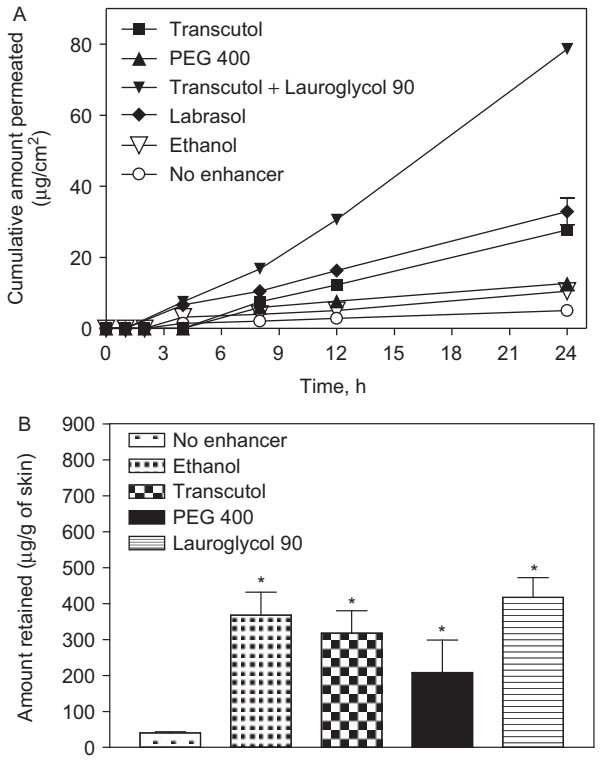

Figure 2. Effect of various glycol enhancers on (A) skin permeation (B) skin retention of genistein from Methocel gel formulations.

finastride (Javadzadeh et al., 2009), clorazepam and lorazepam (Puglia et al., 2001), and bromocriptine (Degim et al., 2003).

When Transcutol was formulated with Lauroglycol, there was a 13- and 9-fold increase in the flux and skin retention, respectively. Lauroglycol 90 is a known solubilizer and with Transcutol it shows a 12-fold increase in the solubility of genistein compared with the control formulation and a 4-fold increase compared with Transcutol alone. A combination of glycols provided a synergistic effect on drug permeation (Minghetti et al., 2003; Baboota et al., 2007; Shakeel et al., 2007; Ritschel et al., 2008). For this reason, Transcutol was also formulated with Lauroglycol.

Similarly, PEG 400 showed a 2- and 5-fold increase in skin permeation and retention, respectively, of genistein as compared with the control formulation, although there was no significant increase in the permeability constant (Table 4). Thus, the increase in flux was mainly due to genistein solubilization. Minghetti et al. (2006) reported the skin permeation of genistein from the application of dry soy extract using various glycols as solvents. These included PEG 400, Transcutol, Labrasol, and propylene glycol. Among these vehicles, PEG 400 was the best vehicle for skin permeation of genistein from the dry soy extract in terms of amount permeated, flux, and permeability coefficient. They also found an *ex vivo* human skin flux of 7.1 µg/cm²/h for pure genistein from a saturated solution in PEG 400. This is a 2-fold or higher flux than from the gel formulations of this study. The solubility of genistein in our gels and, perhaps, the gel viscosity and

Table 4. Effect of various alcohol/glycol enhancers on the skin permeation of genistein from Methocel gel formulation.

Methocel gel with alcohol/ glycols	Flux (µg/cm²/h)	Mean solubility (µg/ml)	Permeability constant×10⁻⁴ (cm/h)
None	0.24 ± 0.03	45.7 ± 0.7	51.8 ± 7.0
Ethanol	0.41 ± 0.05 *	820.1 ± 24.7 *	5.0 ± 0.7*
Transcutol	1.15 ± 0.07 *	130.1 ± 35.1 *	88.4 ± 21.6*
Transcutol + Lauroglycol 90	3.27 ± 0.08 *	537.8 ± 68.0 *	61.0 ± 7.4
PEG 400	0.52 ± 0.14 *	122.1 ± 12.9 *	59.4 ± 6.7

*$P < 0.05$ versus control (no enhancer).

formulation components contributed to the lower flux compared with that from PEG 400 alone. In our study, all the glycol derivatives yielded a higher genistein solubility than the 0.5% w/w ethanol control, but there was not a significant increase in the permeability constants except for Transcutol. This suggests that glycol derivatives have the ability to solubilze the drug and partition the drug from the vehicle into the skin. On the other hand, the 50% w/w ethanol-based gel system had an 18-fold increase in the solubility of genistein, while the permeability constant decreased 10-fold. Thus, ethanol solubilizes genistein very highly but is unable to partition the drug into the skin for permeation, as compared with the control formulation.

Formulations containing menthol, cineole, and Transcutol with Lauroglycol provided flux values >3 µg/ cm²/h. Using a published (Huang et al., 2008) clearance (Cl_T) of genistein of 21.85 l/h and assuming the area of application of the gel (A) to be 10 × 10 cm, the steady-state plasma concentration (C_{ss}) obtained from transdermal application can be calculated from the equation:

$$C_{ss} = AK_o/Cl_T$$

where K_o is the zero order delivery rate, in µg/cm²/h. A flux of >3µg/cm²/h can provide genistein steady-state plasma concentrations >14 ng/ml.

The pharmacokinetics of genistein from a slow release (microencapsulated) capsule formulation containing 22.3 mg of genistein was reported (Setchell et al., 2003a). The plasma concentrations obtained from this oral formulation over 24 h yielded a C_{max} and C_{min} of 40 and 10 ng/ml, respectively. The calculated C_{ss} for genistein from three of our formulations range from 14 to 18 ng/ ml and these values are within the C_{max} and C_{min} range reported by Setchell et al. (2005). The C_{ss} of genistein can be further increased by simply increasing the skin application area (A).

The skin retention of genistein from the various formulations, and especially the menthol formulation, was significant. This can provide a possible application of these gels as skin protection agents for UV-induced damage and chemoprevention of melanoma. Future research is directed toward studying the chemopreventive effects of genistein formulations in a mouse model of melanoma.

Declaration of interest

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