Acta Pharm. **64** (2014) 257–265
DOI: 10.2478/acph-2014-0017

Short communication

In vivo human skin penetration of (–)-epigallocatechin-3-gallate from topical formulations

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The aim of the study was to examine the effect of topical vehicles on the *in vivo* human stratum corneum penetration of the antioxidant and skin photoprotective agent (–)-epigallocatechin-3-gallate (EGCG). Model oil-in-water (o/w) emulsion and gel formulations containing 1 % (*m/m*) EGCG were prepared and subjected to photodegradation studies in order to select excipients that minimize the light instability of EGCG. The optimized emulsion and gel were applied to human volunteers and the EGCG percutaneous permeation was evaluated *in vivo* by the tape-stripping technique. No significant differences in the percentage of the applied EGCG dose diffused into the stratum corneum were observed between the o/w emulsion (36.1 ± 7.5 %) and gel (35.5 ± 8.1 %) preparations. However, the amount of EGCG permeated into the deeper region of human stratum corneum was significantly larger for the o/w emulsion compared to the gel. Therefore, the emulsion represents a suitable vehicle for topical delivery of EGCG.

Keywords: (–)-epigallocatechin-3-gallate, topical application, *in vivo* human study, tape-stripping, emulsion, gel

Accepted February 7, 2014

(–)-Epigallocatechin-3-gallate (EGCG), the major and the most physiologically active catechin of green tea, has been shown to prevent, *in vitro* and *in vivo*, oxidative damage and depletion of antioxidant enzymes caused by exposure to solar UV radiation (1). Moreover, topical treatment with EGCG has been found to decrease the skin inflammatory response due to sun exposure by inhibiting inflammatory leukocyte infiltration and prostaglandin metabolite production (2, 3). In addition, EGCG has been reported to protect against sunlight-induced suppression of the cutaneous immune system and to prevent photoaging of the skin by reducing the expression of matrix metallo-proteinases triggered by solar UV radiation (4, 5).

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In order to deliver the catechin directly to the skin, EGCG is incorporated in topical formulations (1). Namely, the UV radiation penetrates into the skin and therefore EGCG must diffuse through the stratum corneum and reach the viable epidermis and dermis in order to elicit its activities. However, percutaneous penetration of EGCG is scarce; a possible reason is its large molecular size (6).

Since skin permeation of active ingredients from topical preparations is influenced by the vehicle (7), to optimize the potential therapeutic application of EGCG, we have evaluated the percutaneous permeation of catechin from different model formulations (*i.e.*, oil-in-water emulsion and hydrophilic gel). Previous investigations of cutaneous delivery of EGCG have been performed either *in vitro* on excised animal and human skin (6, 8–11) or *in vivo* in mice and rats (6, 12, 13), and hence they do not reproduce the actual applicative conditions of dermatological products in man. To overcome this drawback, in this study the influence of topical formulations on the percutaneous penetration of EGCG was evaluated *in vivo* on human volunteers, using the non-invasive tape-stripping technique (14). Further, since EGCG is unstable under sunlight (15), prior to skin penetration, the examined topical preparations were subjected to photodegradation studies in order to select formulation ingredients that minimized catechin photolability.

EXPERIMENTAL

Materials

EGCG was obtained from DSM (Switzerland). Excipients for the cream and gel preparations were purchased from Seppic (France) and ACEF (Italy). Methanol, acetonitrile and water were of high-performance liquid chromatography (HPLC)-grade from Sigma (Germany). All other reagents and solvents were of analytical grade (Sigma).

High-performance liquid chromatography

The HPLC apparatus consisted of a modular chromatographic system (Model 1580-PU pump and Model 975-UV detector; Jasco, Tokyo, Japan) linked to an injection valve with a 20 μL sample loop (Model 7725i Rheodyne, USA). The detector was set at 280 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, France). Sample injections were effected with a Model 701 syringe (10 μL; Hamilton, Bonaduz, Switzerland). Separation was performed by a previously developed and validated method (15), at ambient temperature on a 5-μm Luna C18 column (150 × 4.6 mm *i.d.*; Phenomenex, Torrance, CA, USA) fitted with a guard column and eluted isocratically at a flow-rate of 1.0 mL/min. Sodium phosphate buffer (pH 3.0; 0.03 mol L^{-1})/acetonitrile (82:18, *V/V*) was used as the mobile phase. Identity of the EGCG peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of peak areas using the external standardization method.

Formulations

In vivo skin penetration experiments were performed on oil-in-water (o/w) emulsion and hydrogel preparations, containing 1.0 % (*m/m*) EGCG. The composition of the formulations is given in Table I. For the emulsion, the selection of excipients was based on a previous study on the optimization of cream preparations containing EGCG (15). Creams were prepared by mixing the melted lipid phase in the aqueous phase at 70 °C with an Ultra-Turrax at 6500 rpm for 2 min. EGCG (solubilized in propylene glycol) was added into the cooling phase of the emulsion preparation at about 35 °C. Hydrogels were prepared by dispersing, under mechanical stirring, the rheological additive in pre-heated (70 °C) deionized water containing the excipients. EGCG (solubilized in propylene glycol) was added to the rest of the formula at a temperature of 35 °C.

Photodegradation

Aliquots (40 mg) of the emulsion and hydrogel preparations were evenly spread by means of a syringe onto the bottom of beakers and irradiated with a solar simulator (Suntest CPS+, Atlas, Germany) for 1 h, as previously described (15). Solar simulator

Table I. Composition (%, m/m) of EGCG o/w emulsion and gel formulations

Components	Emulsion 1	Emulsion 2	Gel 1	Gel 2	Gel 3	Gel 4
EGCG	1.0	1.0	1.0	1.0	1.0	1.0
cetearyl alcohol	1.5	1.5	–	–	–	–
glyceryl monostearate	1.5	1.5	–	–	–	–
sweet almond oil	5.0	5.0	–	–	–	–
cetearyl isononanoate	5.0	5.0	–	–	–	–
dimethicone	0.5	0.5	–	–	–	–
Phenonip®	0.8	0.8	–	–	–	–
Montanov 82®	5.0	5.0	–	–	–	–
propylene glycol	5.0	5.0	5.0	5.0	5.0	5.0
EDTA	0.1	0.1	0.1	0.1	0.1	0.1
sodium dehydroacetate	0.1	–	0.1	0.1	–	–
benzyl alcohol	–	0.1	–	–	0.1	0.1
xanthan gum	–	–	1.5	–	1.5	–
carboxymethylcellulose	–	–	–	4.0	–	4.0
sodium methylparaben	–	–	0.25	0.25	0.25	0.25
sodium propylparaben	–	–	0.25	0.25	0.25	0.25
citric acid *qs*						
water *qs*						

emission was maintained at 500 W m^{-2}, corresponding to a UV irradiance of 54.9 W m^{-2}, comparable to natural sunlight (15). After the exposure interval, the samples were quantitatively transferred into a 20-mL calibrated flask with methanol (2 × 8 mL), subjected to sonication (10 min) and analyzed by HPLC after dilution to volume (20 mL) and filtration (0.45 μm membrane filters). The degree of photodegradation was evaluated by measuring the percentage of recovered EGCG with respect to non-irradiated samples. The results were the average of at least six experiments.

In vivo *human skin penetration*

The *in vivo* permeation studies were performed by the tape-stripping technique. Ten volunteers of both sexes, aged 23–30 years and without any dermatological disorders, gave a signed informed consent for experimentation. The number of subjects was consistent with that reported in the literature for tape-stripping based studies (16 and references therein). The trial was approved by the local ethics committee (Comitato Etico della Provincia di Ferrara). The subjects were allowed to acclimatize (30 min) before the application of test formulations on the inside of their forearms, which were previously wiped with ethanol, water and dried. Test preparations were applied at a dose of 4 mg cm^{-2} and randomly allocated to a delineated area (2 x 5 cm), in the lower and upper part of each volunteer's forearm, respectively. The formulations were homogeneously distributed using rubber gloves. After an application time of 60 min, the remaining preparation was removed from the treated area with a cotton swab and then the stratum corneum was sequentially stripped 18 times with Scotch transparent adhesive tapes (Scotch Crystal 600). The tapes were applied to the skin at constant pressure with a 500 g roller. The first stripped tape was added to the cotton swab for the assay of unabsorbed EGCG. Successive 17 tape strips were collected separately in 5 groups (group 1: strips 2–4; group 2: strips 5–7; group 3: strips 8–10; group 4: strips 11–14; group 5: strips 15–18). Tape samples were extracted twice with 8 mL of methanol under sonication (10 min). Combined fractions were adjusted to volume (20 mL), filtered (0.45 μm membrane filters) and analyzed for EGCG by HPLC. The results were expressed as penetrated percentage of the applied dose.

Validation of the tape-stripping assay was carried out by spiking adhesive tapes of untreated stratum corneum with 4 and 40 mg of the tested emulsion and gel preparations. The samples were processed as outlined above and percentage recoveries were calculated by comparing the peak areas of EGCG extracted from the tape samples with those obtained by direct HPLC analysis of equivalent amounts of the catechin dissolved in methanol. Precision of the method was calculated by extraction and HPLC assay of individual tapes (n = 6) spiked with 4 mg of the examined emulsion and gel formulations.

Statistical analysis

Statistical data analysis was performed using Student's t-test. p-values < 0.05 were considered significant. All computations were carried out using the statistical software GraphPad Instat (Graphpad Software, San Diego, CA).

RESULTS AND DISCUSSION

EGCG formulations and photostability

For the *in vivo* percutaneous penetration experiments, o/w emulsion and hydrogel preparations were selected as topical vehicles for EGCG, since they comprise the majority of dermatological products (7) and hence simulate real conditions of use. Moreover, in order to minimize the possible interactions between excipients and catechin, the basic emulsion and hydrogel formulations were selected (see Table I). The pH of the formulations was adjusted to 5 to ensure sufficient chemical stability for the catechin (15) and its compatibility with the skin physiological pH (7). Because of the light-instability of EGCG (15), prior to *in vivo* skin penetration experiments, the examined formulations were subjected to photodegradation studies in order to evaluate the influence of the vehicle on the photochemical behavior of EGCG. In fact, adequate stability under solar UV irradiation is a prerequisite for the effectiveness of EGCG as a skin protective agent against damage induced by sunlight.

Two o/w emulsions (composition given in Table I) containing EGCG (1.0 %, *m/m*) were prepared using the same excipients, with the exception of the anti-fungal preservative (sodium dehydroacetate or benzyl alcohol). The other emulsion ingredients were not varied, since it has been reported that they do not affect significantly the photochemical behavior of EGCG (15). In addition, 4 hydrogels containing 1.0 % EGCG were also prepared (composition given in Table I); they differed in the rheological additive (xanthan gum or carboxymethylcellulose) and anti-fungal agent (sodium dehydroacetate or benzyl alcohol). As illustrated in Fig. 1, in the emulsion containing sodium dehydroacetate (emulsion 1, Table I), 72.5 ± 3.8 % of EGCG was degraded following irradiation. On the other hand, the use of benzyl alcohol instead of sodium dehydroacetate (emulsion 2,

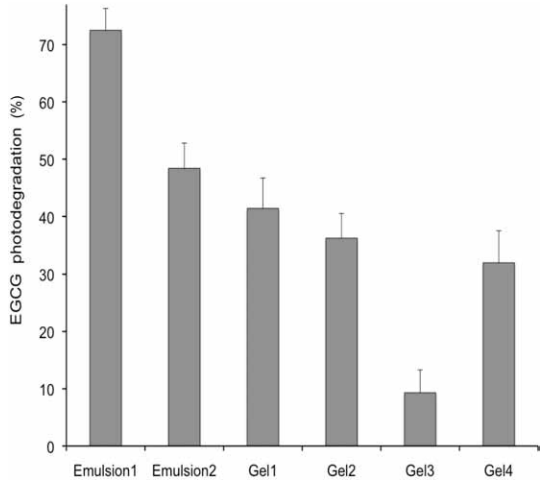

Fig. 1. EGCG photodegradation (%) in its cream and gel formulations (see Table I for composition), after 1 h irradiation with a solar simulator. Values are means ± SD of at least 6 experiments.

Table I), significantly ($p < 0.05$) decreased the light-induced degradation of the catechin in the emulsion vehicle to 48.4 ± 4.4 % (Fig. 1). This effect can be traced to the reactivity of sodium dehydroacetate with reducing agents such as EGCG. These results are in good agreement with those reported in a previous study (15) and indicate that the anti-fungal component of the formulation influenced the photoreactivity of EGCG.

The extent of EGCG degradation in gel preparations was 9.3–41.4 % (Fig. 1). Hence, catechin photodecomposition in the gel was lower than in the emulsion (Fig. 1). This effect can be probably ascribed to limited light-induced reactions between EGCG and the gel vehicle, due to the smaller number of excipients compared to the emulsion formulation (Table I). Because of its simpler composition, there is a lower probability of instability interactions occurring in the gel. Moreover, marked differences in EGCG photolability were also observed among the examined gel preparations (Fig. 1); the lowest catechin degradation (9.3 ± 4.0 %) was attained by the formulation (gel 3, Table I) prepared with xanthan gum and benzyl alcohol as rheological and antifungal additives, respectively. This result can be explained in terms of the inherent higher stability of xanthan gum and benzyl alcohol, as compared to carboxymethylcellulose and sodium dehydroacetate, respectively.

On the basis of the above results, the emulsion (emulsion 2; Table I) and gel (gel 3; Table I) preparations that exhibited the lowest degree of EGCG photodecomposition were selected for the *in vivo* skin penetration experiments.

In vivo *skin penetration*

Previous studies on percutaneous absorption of EGCG have been performed *in vitro* (6, 8–11), while *in vivo* investigations have been restricted to animals (6, 12, 13). However, *in vitro* testing may produce responses different from the *in vivo* situation due to modifications of the barrier organization of excised skin (17). Further, animal skin used in *in vivo* experiments exhibits different permeability compared to human skin (7, 17). Therefore, in order to attain a more realistic assessment of the degree of EGCG skin uptake in typical "in-use" conditions, in the present work the skin permeation of EGCG was measured *in vivo* in human volunteers using the tape-stripping technique. This method is based on the progressive removal of the stratum corneum outer layers by sequential stripping with adhesive tapes (14). Following topical application of the tested formulations, the amount of EGCG transferred to individual tape strips is quantified to provide the catechin's *in vivo* stratum corneum penetration profile, which is predictive of skin absorption (18). Average recoveries of EGCG from adhesive tapes spiked with known catechin levels were higher than 87.4 %. The precision of the method gave a relative standard deviation value of 7.2 %.

The emulsion and gel preparations that provided the best performance in the photodegradation studies (emulsion 2 and gel 3, Table I) were applied to the volunteers' volar forearms and the amount of EGCG in the collected tapes was determined by HPLC after solvent extraction. The total EGCG recovery (sum of catechin levels unabsorbed and diffused in the horny layers removed by the strips) was > 73.9 % (Table II), which is rather low but acceptable considering that the stratum corneum was stripped only 18 times, which corresponds to the removal of approximately 50 % of its thickness (14).

Table II. In vivo *permeation data for EGCG after application of emulsion 2 and gel 3 preparations: cumulative recovery in strips 2–18 of human stratum corneum*

Sample	Percent of the applied EGCG dose[a]		
	not permeated	recovered in strips 2–18	total recovery
Emulsion 2	39.1± 6.0	36.1 ± 7.5	75.2 ± 8.2
Gel 3	38.4 ± 7.2	35.5 ± 8.1	73.9 ± 8.6

[a] Mean ± SD, n = 10.

Percentages of the applied EGCG doses permeated into the stratum corneum from the emulsion and gel preparations were 36.1 and 35.5 %, respectively (Table II), the difference being statistically non-significant. Comparison with earlier investigations is difficult because the skin penetration of EGCG has been previously assessed only *in vitro* in humans.

Distinct differences between the two formulations were observed in the stratum corneum penetration profile of EGCG (Fig. 2), obtained by plotting the catechin levels measured in combined adhesive tape groups, compared to the strip number (related to depth). Specifically, application of the hydrogel yielded a significantly ($p < 0.05$) higher EGCG concentration in the superficial portion of the skin (strips 2–4) compared to the o/w emulsion (Fig. 2). Conversely, the latter formulation produced an increase in the catechin levels permeated into the deepest region (strips 5–18) of the stratum corneum (Fig. 2), the differences being statistically significant ($p < 0.05$) for the tape strip groups 11–14 and 15–18.

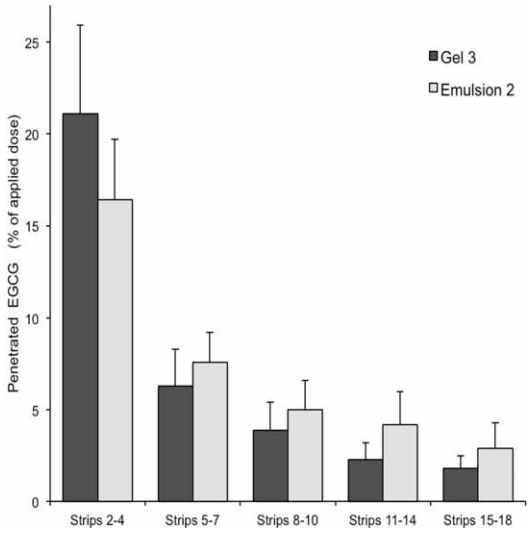

Fig. 2. *In vivo* concentration profiles of EGCG in human stratum corneum after application of its formulations. EGCG amounts (% of applied dose) in strips 2–4, 5–7, 8–10, 11–14 and 15–18 are shown (mean ± SD, n = 10).

Thus, the obtained results indicated that although there was no difference in the total amount of EGCG accumulated in the stratum corneum following topical application of the gel and emulsion formulations (Table II), the emulsion triggered distribution of the catechin to deeper layers of the stratum corneum (Fig. 2). This effect will increase the EGCG fraction that can partition/diffuse into the underlying viable epidermis, where the catechin should act (8). The enhanced *in vivo* penetration of EGCG into the lower region of human stratum corneum, provided by the o/w emulsion, could be traced to the presence of formulation excipients that can alter the intercorneocyte lipid matrix organization (*i.e.*, surfactants used as emulsifiers) or fuse with the stratum corneum lipids (*i.e.*, components of the oil phase of the emulsion), thereby facilitating catechin diffusion.

CONCLUSIONS

The study describes the first *in vivo* investigation of human skin penetration of EGCG. The obtained data indicate that compared to the gel formulation, the emulsion vehicle enhanced the *in vivo* permeation of EGCG into the deeper stratum corneum region. However, in order to obtain more conclusive results, a larger number of subjects should be investigated. On the other hand, EGCG photo-instability was higher in the emulsion than in the gel. However, this disadvantage can be overcome by introducing a photostabilizing agent in the formulation (15). Hence, o/w emulsions represent a promising vehicle for efficient topical delivery of EGCG.

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