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Transdermal delivery of (−)-epigallocatechin-3-gallate, a green tea polyphenol, in mice

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Abstract

Epigallocatechin-3-gallate (EGCG) is the most studied catechin in green tea (*Camellia sinensis*). EGCG and green tea are cancer preventive in many animal models, and numerous mechanisms have been proposed in cell lines. EGCG is poorly bioavailable in man and rodents. We hypothesized that transdermal delivery of EGCG could result in improved bioavailability. Following application of EGCG transdermal gel (50 mg kg^{-1}, t.d.) to SKH-1 mice, EGCG was observed in the epidermis (1365.7–121.0 ng g^{-1}) and dermis (411.2–42.6 ng g^{-1}). The maximum plasma concentration (C_{max}) of EGCG was 44.5 ng mL^{-1}. The t½ (94.4 h) and $AUC_{0\rightarrow 24h}$ (881.5 ng mL^{-1} h) of EGCG were greater than values previously reported for oral EGCG. The t½ and area under the concentration–time curve up to 24 h ($AUC_{0\rightarrow 24h}$) in the liver, small intestine and colon were 21.3–74.6 h and 715–2802 ng g^{-1} h, respectively. Stability studies showed that the transdermal formulation was stable at 4°C and had a half-life (t½) of 47.1 and 20.2 h at 25°C and 37°C, respectively. These data indicate that transdermal EGCG is useful for delivering prolonged levels of EGCG to plasma and tissues, and may provide an alternative to tea consumption as a dosage form of EGCG.

Introduction

Epigallocatechin-3-gallate (EGCG, Figure 1) is the most abundant catechin in green tea (*Camellia sinensis*, (L.) O. Kuntze). EGCG and green tea have been shown to have cancer preventive activity in animal models of oral, oesophageal, intestinal, lung, prostate and other cancers (Higdon & Frei 2003; Lambert & Yang 2003a). In some of these models, EGCG has been shown to inhibit cell proliferation and induce apoptosis. Numerous mechanisms of action for this activity of EGCG have been proposed based on human cancer cell studies (Gupta et al 2001; Liao et al 2004). Due to the limited bioavailability of EGCG following consumption of green tea, it is unclear which of these mechanisms are relevant in-vivo (Hou et al 2004).

We have previously reported that the absolute bioavailability of EGCG in the mouse and the rat is 26.5 and 1.6%, respectively (Chen et al 1997; Lambert et al 2003). Oral administration of 75 mg kg^{-1} EGCG to mice resulted in a maximum plasma concentration (C_{max}) of 140.0 ng mL^{-1} and half-life (t½) of 82.8 min (Chen et al 1997; Lambert et al 2003). EGCG has been shown to undergo methylation, glucuronidation and sulfation in both rodents and man (Lambert & Yang 2003a; Lu et al 2003; Crespy et al 2004). The major metabolites produced by both the liver and small intestine include 4′,4′-di-*O*-methyl EGCG and EGCG-4″-*O*-glucuronide. The intestine appears to be a major factor in limiting the bioavailability of orally administered green tea polyphenols in rats (Cai et al 2002). We have found that the mouse intestine has the greatest catalytic efficiency for the glucuronidation of EGCG and that human UDP-glucuronosyltransferase (UGT)1A8, an intestine-specific isoform, has high catalytic efficiency (Lu et al 2003). EGCG is also subject to microbial degradation in the colon, resulting in the formation of valerolactone ring fission products (Li et al 2000; Wang et al 2001). We have shown that EGCG is subject to efflux by multidrug-resistance-related proteins (Mrp)1 and 2 in-vitro (Hong et al 2003). Mrp2, which is highly expressed in the

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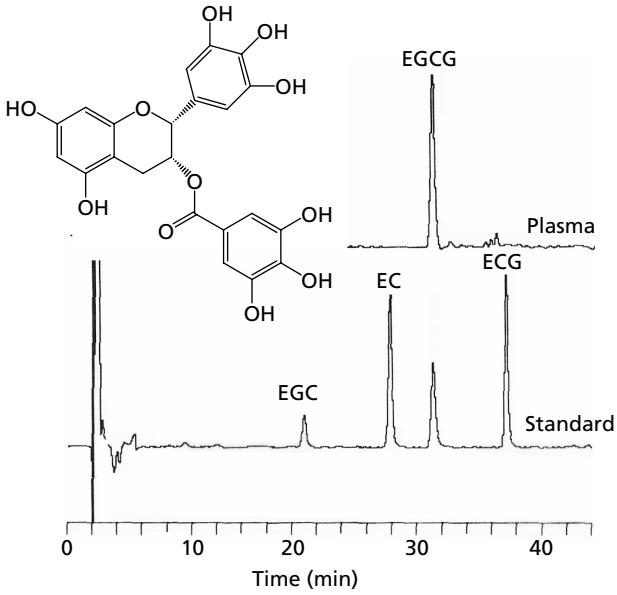

Figure 1 Structure and HPLC analysis of EGCG in transdermal gel. Representative chromatogram shows a standard catechin solution ($100\,ng\,mL^{-1}$ EGC, EC, EGCG and ECG) and total EGCG extracted from mouse plasma.

small intestine and liver, may play a role in limiting the bioavailability of orally administered EGCG. We hypothesized that transdermal delivery (t.d.) of EGCG might represent a means of bypassing the first-pass metabolism of EGCG and improving its bioavailability.

Transdermal formulations deliver compounds across the skin and into the systemic circulation and thereby represent a means of bypassing poor absorption from the gut and first-pass metabolism (Willams 2003). Transdermal formulations of steroid hormones (Climara; Berlex, Inc.), nicotine (Nicoderm CQ; GlaxoSmithKline, Corp.) and analgesics, such as fentanyl (Duragesic; JannsenPharmaceutica), are currently in clinical use where oral administration is impractical. Dvorakova et al (1999) have reported that topical administration of EGCG formulated in USP hydrophilic ointment in-vitro resulted in good penetration into both mouse and human skin, but only negligible systemic availability. Recently, Batchelder et al (2004) have reported the in-vitro permeation of tea catechins from a transdermal patch and found that the permeation of EGCG over 24 h was 0.1% of the amount of EGCG in the patch. Although a green tea patch (Green Tea300, 1800patches; Salt Lake City, UT) is on the market, there are no published in-vivo experiments with transdermal formations of green tea polyphenols.

In this paper, we provide proof of principle for transdermal delivery of EGCG to mice. We have determined the stability of the formulation and the epidermal, dermal, plasma and tissue levels of EGCG in mice treated with a single transdermal dose of EGCG. Herein we report the results of this study.

Materials and Methods

Chemicals

EGCG (100% pure) was provided by Mitsui Norin Co. Ltd (Fujieda City, Japan). β-D-Glucuronidase (G-7896, EC 3.2.1.31, from *Escherichia coli* with 9×10^6 U (g solid)$^{-1}$) and sulfatase (S-9754, EC 3.1.6.1, from Abalone entrails with 2.3×10^5 U (g solid)$^{-1}$) were purchased from Sigma Chemical Co. (St Louis, MO). All other reagents were of the highest grade commercially available. For analytical purposes, a standard stock solution of EGCG, epigallocatechin (EGC), epicatechin (EC) and epicatechin-3-gallate (ECG) ($10\,mg\,L^{-1}$ each) was prepared in 0.2% ascorbic acid−0.005% EDTA (pH 3.8) and stored at −80°C.

Mice

Female SKH-1 mice, 25–30 g, were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and allowed to acclimate for at least one week before the start of the experiment. The mice were housed 10 per cage, and maintained in air-conditioned quarters with a room temperature of 20 ± 2°C, relative humidity of 50 ± 10% and an alternating 12-h light–dark cycle. Mice were given free access to Purina Rodent Chow 5001 (Research Diets, New Brunswick, NJ, USA) and water. Animal experiments were approved by the Institutional Animal Care and Use Committee (Piscataway, NJ, USA, Protocol #91-024).

Transdermal formulation of epigallocatechin-3-gallate

EGCG ($10\,mg\,mL^{-1}$) was formulated in a proprietary transdermal gel base (Lipoderm Base; PCCA, Houston, TX, USA) containing 0.1% DMSO and 0.2% D, L-α-lipoic acid. In brief, EGCG was wetted with DMSO and combined with D, L-α-lipoic acid in a mortar. This suspension was combined with the Lipoderm Base and mixed by passing the gel between two syringes. The prepared gel was stored at 4°C and used within 14 days of preparation.

Treatment of mice and sample collection

For transdermal dosing, mice were given a single application of $50\,mg\,kg^{-1}$ EGCG (equivalent to $28.6\,\mu g\,cm^{-2}$) to the dorsal surface. At different time points, mice were anaesthetized and blood was collected by cardiac puncture (0.25–24 h). Plasma was separated by centrifugation and combined with a 0.1 volume of ascorbate preservative (20% ascorbic acid–0.1% EDTA) and frozen at −80°C for future analysis. Dorsal skin was removed from euthanized mice, frozen at −80°C, and fractionated into epidermis and dermis by scraping with a razor blade. These fractions were stored at −80°C for later analysis. Liver, small intestine

and colon tissues were collected, washed with 0.9% NaCl and frozen at −80°C. Urine samples were collected upon spontaneous voiding during euthanasia. Samples were combined with 0.1 volumes of ascorbate preservative.

Extraction and quantification of epigallocatechin-3-gallate in biological samples

Plasma samples were analysed by previously described methods (Lee et al 2000). In brief, plasma (100 μL) was extracted with methylene chloride and ethyl acetate, and the ethyl acetate fraction was dried in-vacuo. The sample was then resuspended in 10% acetonitrile and analysed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The results represented unconjugated levels of EGCG in the plasma. To determine the levels of total EGCG (i.e. conjugated plus unconjugated EGCG), plasma samples were hydrolysed with β-glucuronidase (250 U) and sulfatase (1 U) before the solvent extraction procedure. Urine samples were prepared in an analogous manner with the only difference being omission of the methylene chloride extraction.

Epidermis, dermis, small intestine, colon and liver samples were prepared using the method of Chu et al (2004) with modifications. In brief, tissue samples were homogenized in 5 volumes of methanol–ethyl acetate–dithionate (2:1:3, v/v/v) with 14 passes of a mechanical dounce homogenizer. Samples were centrifuged for 4 min at 14 000 rev min^{-1} and 400 μL of supernatant was dried in-vacuo. The resulting residue was resuspended with water (200 μL), then hydrolysed and extracted in a manner analogous to plasma.

EGCG levels were analysed using an HPLC system consisting of two ESA model 580 dual-piston pumps (Chelmsford, MA, USA), a Waters Model 717plus refrigerated autosampler (Milford, MA, USA) and an ESA 5500 coulochem electrode array system (CEAS). The potentials of the CEAS were set at −100, 100, 300 and 500 mV. Separation was achieved using previously described methods (Lee et al 2000). EGCG was detected as a single peak with $t_R = 31$ min (Figure 1). The exposure (AUC), t½ and C_{max} of EGCG were determined using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Stability of transdermal epigallocatechin-3-gallate

The stability of the EGCG transdermal gel was determined at 4°C, 25°C and 37°C and relative humidity 50%. Samples of the gel were taken at 0–48 h and combined with a 1/10th volume of ascorbate preservative. Samples were diluted 1:100 with de-ionized water, and extracted and analysed in a manner analogous to plasma samples.

Statistical analysis

C_{max}, AUC and t½ were calculated using Microsoft Excel (Redmond, CA, USA). Values are expressed as the means and error bars represent the standard error of the mean. Differences between values were determined by analysis of variance with Tukey's Multiple Comparison test to compare values between tissues. Significance was achieved at $P < 0.05$.

Results and Discussion

Cutaneous levels of EGCG

The levels of EGCG in the epidermis and dermis of mice were determined following a single application of EGCG transdermal gel to the dorsal surface of the mice. EGCG in both skin fractions declined in a time-dependent manner with maximal concentrations of 1365.7 ng mL^{-1} and 411.2 ng mL^{-1} at 15 min in the epidermis and dermis, respectively (Figure 2). The $AUC_{0\rightarrow 24h}$ of EGCG was 5978.3 ng g^{-1} h and 1729.5 ng g^{-1} h in the epidermis and dermis, respectively. The t½ of EGCG was 9.3 and 10.9 h in the epidermis and the dermis, respectively (Table 1).

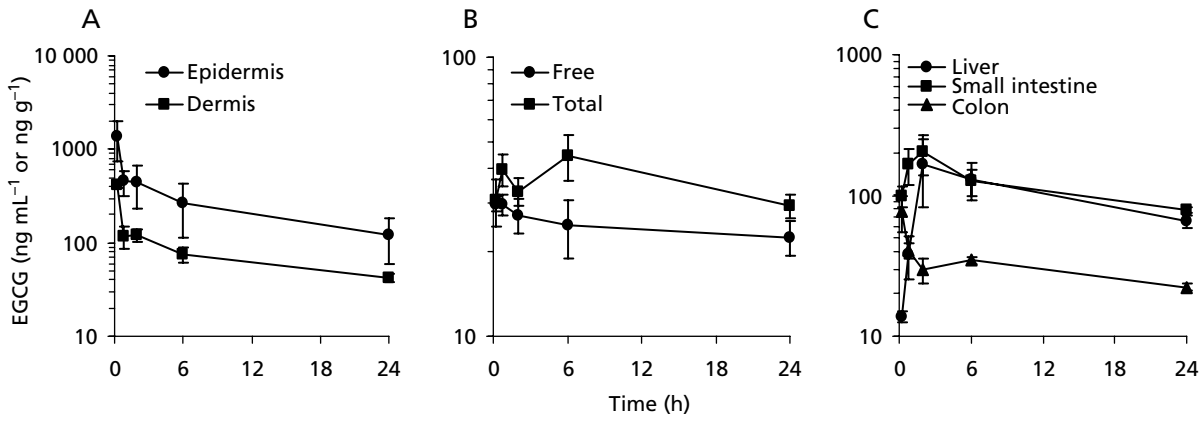

Figure 2 Plasma and skin level of EGCG in female SKH-1 mice after 50 mg kg^{-1} transdermal EGCG. Total EGCG in the epidermis and dermis (A); unconjugated and total EGCG in the plasma (B); and total EGCG in the liver, small intestine, and colon (C). Each point represents the average of 4–6 mice. Error bars represent the s.e.m.

Table 1 Pharmacokinetic parameters of EGCG in the skin and plasma following transdermal application of EGCG to male SKH-1 mice

	C_{max} (ng mL^{-1} or ng g^{-1})	t½ (h)	$AUC_{0\rightarrow 24h}$ (ng mL^{-1} h or ng g^{-1} h)
Plasma-free	29.8 ± 1.9^{a}	64.8 ± 8.4^{a}	582.1 ± 75.7^{a}
Plasma-total	44.5 ± 8.4^{a}	94.4 ± 13.2^{a}	881.5 ± 123.4^{a}
Epidermis	1365.7 ± 613.8^{b}	9.3 ± 4.3^{b}	5978.3 ± 2779.9^{b}
Dermis	411.2 ± 21.5^{a}	10.9 ± 1.6^{b}	$1729.5 \pm 259.4^{a,b}$
Liver	164.8 ± 83.0^{a}	74.6 ± 20.1^{a}	$2494.8 \pm 673.6^{a,b}$
Small intestine	203.1 ± 64.0^{a}	26.8 ± 5.6^{b}	$2802.2 \pm 588.5^{a,b}$
Colon	77.0 ± 22.4^{a}	21.3 ± 3.2^{b}	$715.0 \pm 107.3^{a,b}$

C_{max}, maximum concentration; t½, half-life; $AUC_{0\rightarrow 24h}$, area under the curve over 24 h; free, unmetabolized EGCG; total, unmetabolized + conjugated EGCG. Values represent the mean ± s.e.m., n = 6. Values within the same column that have different superscript letters are significantly different by analysis of variance with Tukey Multiple Comparison Test ($P < 0.05$).

Plasma, tissue and urine levels of EGCG

EGCG levels were determined in the plasma, liver, colon and small intestine following transdermal administration in mice. EGCG could be detected for at least 24 h in all tissues (Figure 2). The C_{max} of total and unconjugated EGCG in plasma was 44.5 and 29.8 ng mL^{-1}, respectively. The t½ and $AUC_{0\rightarrow 24h}$ were greater for total EGCG than for unconjugated EGCG in the plasma (Table 1), suggesting that EGCG is rapidly conjugated following absorption through the skin. The plasma C_{max} following transdermal administration was approximately 34% and 3.6% of that which we previously reported following oral (75 mg kg^{-1}) or intravenous (10 mg kg^{-1}) administration to mice (Table 2) (Lambert et al 2003). By contrast, the $AUC_{0\rightarrow\infty}$ and t½ observed following transdermal administration of EGCG were significantly larger than the values we previously reported for oral or intravenous administration of EGCG to mice (Table 2) (Lambert et al 2003).

Table 2 Comparison of pharmacokinetic parameters of plasma EGCG following tansdermal, oral or intravenous administration of EGCG to mice

	Transdermal (50 mg kg^{-1})	Intragastric (75 mg kg^{-1})*	Intravenous (10 mg kg^{-1})*
C_{max} (ng mL^{-1})	44.5 ± 8.4^{a}	130.0 ± 37.1^{a}	1250.0 ± 324.0^{b}
$AUC_{0\rightarrow\infty}$ (ng mL^{-1} h)	4856.4 ± 679.4^{a}	533.3 ± 151.8^{b}	268.3 ± 72.4^{b}
t½ (h)	94.4 ± 13.2^{a}	1.4 ± 0.39^{b}	3.5 ± 0.94^{b}

*Data from Lambert et al (2003). C_{max}, maximum concentration; $AUC_{0\rightarrow\infty}$, area under the curve over 24 h; t½, half-life. Values represent the mean ± s.e.m., n = 6. Values within the same row that have different superscript letters are significantly different by analysis of variance with Tukey Multiple Comparison Test ($P < 0.05$).

EGCG in the liver, small intestine and colon reached peak concentrations of 77.0–203.1 ng g^{-1} (Table 1). The t½ of EGCG was 21.3, 26.8 and 74.6 h in the colon, small intestine and liver, respectively. The $AUC_{0\rightarrow 24h}$ for the liver, colon and small intestine are shown in Table 1. The C_{max} for EGCG in the liver was 3.2-fold greater than the previously reported values following oral administration of EGCG in mice (Lambert et al 2003). By contrast, only the t½ was significantly greater in the small intestine and colon as compared with values observed following oral administration (Lambert et al 2003). The C_{max} in the small intestine and colon was 0.9 and 1.9%, respectively, of that observed following oral administration of EGCG to mice (Lambert et al 2003). These results suggest that whereas oral administration is better at achieving high peak levels of EGCG in the small intestine and colon, transdermal administration increases the levels of EGCG available to internal organs (i.e. the liver) and increases the t½ in all organ sites tested. The C_{max}, $AUC_{0\rightarrow 24h}$ and t½ of EGCG in the urine was 177 ng mL^{-1}, 3427.9 ng mL^{-1} h, and 70.0 h, respectively.

We believe that the larger AUC and longer t½ following transdermal administration are due to the continuous movement of EGCG from reservoirs in the skin into the plasma. This is supported by the fact that relatively high concentrations of EGCG are detected on the skin over the entire course of the experiment and thus are available for movement into the plasma and internal organs. Interestingly, we found that the levels in the plasma and tissues converge at the later time points. We believe that this is due to the fact that the t½ in the skin is shorter than that in the other tissues due to movement from the skin into the bloodstream. Further, these parallel levels may result from the fact that EGCG is expected to accumulate in the liver and small intestine during the excretory process. This would result in higher than expected levels in these tissues.

We would predict that if the linear dissolution relationship observed by Batchelder et al (2004) is observed in-vivo, then it is conceivable that increasing the concentration of EGCG in the transdermal gel could result in a linear increase in the plasma and tissue concentrations. Further experiments are required to determine the dose-dependence of plasma and tissue levels of EGCG following transdermal administration.

One potential drawback to the present formulation is the use of DMSO as an excipient. Although DMSO is effective as a penetration enhancer, high concentrations (greater than 60%) of the compound applied for long periods of time have been shown to induce erythema and other skin reactions (Williams & Barry 2004). The concentration used in the present formula is only 0.1% and is not expected to cause adverse effects. We are, however, in the process of developing an alternative transdermal formulation of EGCG that omits DMSO as the excipient.

Stability of transdermal epigallocatechin-3-gallate

The stability of EGCG transdermal gel was determined at 4°C, 25°C and 37°C. Previously we have shown that EGCG

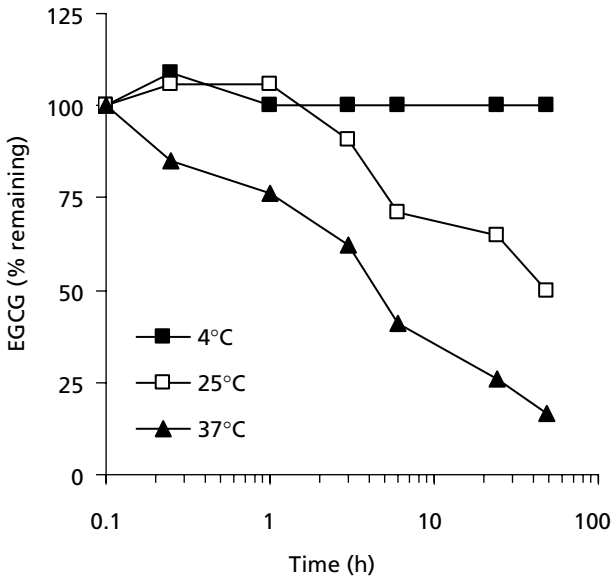

Figure 3 Stability of EGCG in transdermal gel. The stability of EGCG in the transdermal gel was determined at 4°C, 25°C and 37 °C. Each point represents the average of two separate experiments, n = 4. Error bars represent the standard deviation, but are smaller than the symbols.

is relatively unstable under cell culture conditions and neutral and basic conditions (Hou et al 2005). In this study we found that EGCG in the transdermal formulation is stable at 4°C, but undergoes time-dependent degradation with a t½ of 20.2 and 47.1 h at 37°C and 25°C, respectively (Figure 3). Further studies are required to optimize the stability of transdermal formulations of EGCG at higher temperatures, including studies over much longer periods of time.

Conclusions

EGCG is the most abundant and well-studied catechin in green tea. We and others have shown that its oral bioavailability is relatively poor, and that this is due to poor absorption and extensive metabolism (Lambert & Yang 2003b). In this report, we demonstrate, for the first time, the systemic delivery of EGCG using a transdermal formulation. We found that this route of administration resulted in significantly longer t½ and larger AUC (67- and 8-fold, respectively), but lower C_{max}, in plasma and tissues than oral administration of EGCG in mice (Lambert et al 2003). The formulation used in this study was stable under storage conditions (i.e. 4°C) but underwent time-dependent degradation at room temperature and 37°C (t½ = 47.1 and 20.2 h, respectively). Further refinement of the formulation could result in improved stability, which may result in even larger systemic AUC and longer t½.

Although tea is a convenient method for the delivery of catechins, including EGCG, it has several inherent flaws for use as a cancer-preventive agent. These include differences in green tea preparations available from different manufacturers, in brew-time and tea strength and in frequency of consumption (Yang et al 2002). Additionally, the side effects most often associated with consumption of high doses of green tea involve the gastrointestinal system (e.g. stomach-ache, nausea, etc.) (Chow et al 2003; Jatoi et al 2003; Laurie et al 2005). Such flaws could be eliminated by the further development and use of a transdermal gel. In summary, this study is the first to demonstrate plasma and tissue levels in-vivo following transdermal administration of EGCG, and suggests that transdermal formulations may be a viable sustained release system for EGCG and a potential alternative to orally administered formulations. Further studies, including studies with human subjects, are needed to fully elucidate the usefulness of this alternative route of administration.

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