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In vitro transdermal delivery of the major catechins and caffeine from extract of *Camellia sinensis*

Rachel J. Batchelder, Richard J. Calder, Chris P. Thomas, Charles M. Heard*

Welsh School of Pharmacy, Cardiff University, Cardiff CF10 3XF, UK

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Abstract

The aim of this study was to investigate the feasibility of the transdermal delivery of catechins and caffeine from green tea extract. Drug-in-adhesive patches containing 1.35, 1.03, 0.68, and 0.32 mg cm^{-2} green tea extract were formulated and the dissolution of (−)-epigallocatechin gallate (EGCg), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC) from these was determined. Transdermal delivery was determined across full thickness pig ear skin from saturated solutions of green tea extract in pH 5.5 citrate–phosphate buffer, polyethylene glycol 400 and a 50:50 mixture of the citrate phosphate buffer and polyethylene glycol in addition to patches containing 1.35 mg cm^{-2} green tea extract. Dissolution experiments indicated first order release which was dose dependent in respect of the loading level, although the amounts permeated were not always proportional to the amounts in the formulation. The highest percentage permeation of EGCg was found to be from the patch formulation. EGCg, EGC and EC were all successfully delivered transdermally from saturated solutions and adhesive patches containing green tea extract in this study. There was some evidence for the dermal metabolism of EGCg, but after 24 h 0.1% permeated from the patches containing 1.35 mg cm^{-2} green tea extract. This was equivalent to the percentage absorbed after intragastric administration of green tea extract in rats. In addition, the concentration of EGCg in the Franz cell receptor chamber after 24 h permeation from the 0.9 cm diameter patch containing 1.35 mg cm^{-2} was within the range of C_{max} plasma levels achieved after oral dosing of 2.2–4.2 g m^{-2} green tea extract. Caffeine was also delivered at concentrations above those previously reported.

Keywords: Tea; Catechins; Polyphenols; Transdermal delivery; Drug-in-adhesive patch

1. Introduction

The leaves of *Camellia sinensis* have been consumed as a beverage for thousands of years, and the health benefits of tea, in particular green or unfermented tea, have been recognised for many centuries, historically in the Far East and more recently in Western societies (Hara, 2001). Green tea contains relatively high proportions of a class of polyphenolic compounds, catechins, and it has recently been demonstrated that these compounds inhibit cancer initiation, promotion and progression (Dufresne and Farnworth, 2001). Protective effects in cardiovascular disease have also been identified, including reduced oxidation of low-density lipoprotein and anti-inflammatory action (Blache et al., 2002).

Four major catechins are found in tea: (−)-epicatechin (EC), (−)-epicatechin gallate (ECg), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCg) (Fig. 1). Of these, EGCg possesses the great-

* Corresponding author. Tel.: +44-29-2087-5819; fax: +44-29-2087-5819.
E-mail address: heardl@cf.ac.uk (C.M. Heard).

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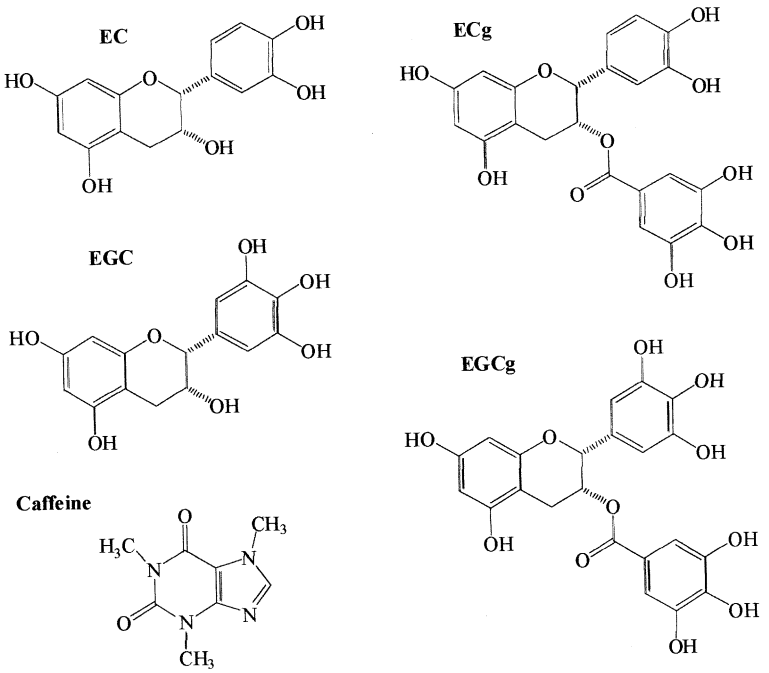

Fig. 1. Chemical structures of catechin (EC, mw 290.3), epicatechin gallate (ECg, mw 442.37), epigallocatechin (EGC, mw 306.27), epigallocatechin gallate (EGCg, mw 458.37) and caffeine (mw 194.19).

est anti-oxidant activity, possibly as a consequence of the greatest number of hydrogen radical donors in its structure (Koketsu, 1997). Although many of the effects of catechins have been attributed to their high anti-oxidant activity, a more specific anti-cancer effect has also been hypothesised (Cutter et al., 2001), more recently linked to blockade of the aryl hydrocarbon hydroxylase (AHH) receptor (Accessed, 2003a http://news.bbc.co.uk/l/hi/health/3125469.stm). Moreover, catechins are believed to exert synergistic effects with other catechins and caffeine suggesting that green tea extract has a stronger effect than any individual tea component (Wang et al., 2000). Topical application of EGCg and caffeine has also been shown to decrease the number of non-malignant and malignant skin tumours in mice (Lu et al., 2002). A lotion containing green tea has recently been proposed to block the development of non-melanoma skin cancers (Accessed, 2003b http://news.bbc.co.uk/l/hi/health/3090190.stm).

A study in rats found that only 0.1% of EGCg was bioavailable after intragastric administration (Chen et al., 1997), thus most of the efficacious catechins are either subject to first-pass metabolism by the liver or are poorly absorbed in the GI tract (Ioannides and Yoxall, 2003). A transdermal system would provide an alternative delivery mechanism for catechins thus giving consumers an option to formalise dosing. The aim of this study was therefore to investigate the feasibility of the transdermal delivery of the major tea catechins and caffeine in green tea extract. Transdermal delivery from patches is highly popular among consumers and administration of tea catechins via the skin would have numerous distinct advantages, including elimination of first pass liver metabolism and food interactions, and easy, controlled dosing with constant plasma concentrations (Williams, 2003).

Green tea consumption in UK has grown markedly in recent years as more and more people become aware of its demonstrable health benefits, in addition to its appeal as a beverage. In this work the transdermal delivery of the major catechms and caffeine from green tea extract was studied in vitro. Rather than seeking to replace green tea consumption, a transdermal product would aim to offer consumers a more formalised dos-

ing stratagem and potentially widen the scope of the benefits of this herb.

2. Methods

2.1. Materials

Green tea extract (51.2% total polyphenols [EGCg, 23.2%; ECg, 9.5%; EC, 1.5%, EGC, 1.1%, other 15.9%], 5.4% caffeine) was supplied by Handa Fine Chemicals (Nottingham, UK). Transdermal adhesive (Duro-tak 387-2516) was a gift from National Starch & Chemical (Zutphen, The Netherlands). Epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, caffeine, polyethylene glycol 400 (PEG 400), all buffer salts and trifluoroacetic acid (TFA) were supplied by Sigma (Poole, UK). HPLC grade solvents were obtained from Fisher scientific (Loughborough, UK). Pig ears were obtained from a local abattoir, prior to steam cleaning.

2.2. Saturated solutions

Saturated solutions of green tea extract were prepared at 32 °C in three solutions: pH 5.5 citrate–phosphate buffer (CPB), PEG 400 and 50:50 mixture of CPB and PEG 400. It was reported that EGCg was relatively stable in aqueous solutions of low pH (Proniuk et al., 2002). Consequently, pH 5.5 CPB (18.1 g $Na_2HPO_4{\cdot}7H_2O$, 9.42 g citric acid in 1 L de-ionised water, adjusted to pH 5.5 with TFA) was used, which is also similar to the surface pH of the skin. Also, PEG 400 is a commonly used excipient in topical formulations. Excess extract was added to 5 ml solution and rotated at 32 °C overnight, before centrifugation and sampling of the supernatant.

2.3. Drug-in-adhesive patches

Green tea extract was suspended in methanol and added to Duro-tak® 387–2516 transdermal adhesive for the manufacture of patches containing 1.35, 1.03 and 0.68 mg cm^{-2} green tea extract. Coatings of 50 g m^{-2} were prepared on polymer lining using a laboratory-scale film coat applicator (Medifix, Luton, UK).

2.4. Dissolution from drug-in-adhesive patches

Dissolution of catechins from 0.9 cm diameter patches containing 1.35, 1.03 and 0.68 mg cm^{-2} green tea extract was determined following addition of 0.9 cm^2 patch samples to 30 ml degassed CPB in screw-capped vials set up on a laboratory rocker (Chedgzoy et al., 2002). Samples (0.5 ml) were taken at appropriate timepoints and replaced with fresh solution.

2.5. Transdermal delivery

Transdermal delivery was determined in vitro using Franz-type diffusion cells incorporating full thickness pig ear skin, a representative model for permeation across human skin (Simon and Maibach, 2000; Schmook et al., 2001). The skin was carefully excised from the underlying cartilage by blunt dissection, cut into 2 cm^2 sections, and inspected to ensure no holes or other imperfections were present. These were then mounted between the pre-greased flanges of the cell and pinch-clamped. Receptor phase (de-gassed CPB) was added along with a magnetic stirrer bar and the complete cells placed in a water bath maintained at 37 °C. After a 10 min equilibration period, cells were dosed with patch sample (pressed onto the skin surface with a glass rod) or 500 μl of saturated solution of green tea extract. Donor chambers were occluded. At appropriate timepoints 200 μl of receptor phase was removed and replaced with fresh solution.

2.6. Analysis

The presence of the major catechins and caffeine in the extract was confirmed by location of the relevant molecular ions by mass spectrometry using a ThermoFinnigan LCQ-Deca and APCI ionisation. Quantitative analysis of samples from the skin permeation and dissolution experiments was by reverse-phase HPLC using an Agilent 1100 automated chromatograph, fitted with a Sphereclone 5 μm ODS 4.6 mm × 250 mm column (Phenomenex, Macclesfield, UK). A mobile phase comprising of 13:87 acetonitrile: 0.05% trifluoroacetic acid was used at a flow rate of 1 ml min^{-1} and UV detection at 210 nm. Calibration curves, prepared in receptor phase, were linear over the range 0.5–20 ng ml^{-1}, with r^2 values of ≥0.9999.

Analytes were identified through a combination of comparison of retention times and sample spiking. Retention times for EGC, EC, caffeine and EGCg were 6.5, 8.4, 10.5 and 14.9 min respectively. All analytes were baseline resolved under these conditions.

2.7. Data processing

Data obtained by HPLC analysis were corrected for sampling effects. For permeation data, cumulative permeation (cm^{-2}) was plotted as a function of time and steady state flux (J_{ss}) determined from the linear portion of the curve. Permeability coefficient was obtained by dividing J_{ss} by the donor concentration. For release data, cumulative permeation (cm^{-2}) was plotted as a function of time square root of time, where linearity is indicative of first order release (Higuchi, 1962; Gallagher et al., 2003). The amounts permeated or released at 24 and 48 h are stated as Q_{24} and Q_{48} values.

3. Results and discussion

3.1. Dissolution from model patches

Profiles for the simultaneous release of EGCg, EGC and EC were linear from each of the patches containing three different loadings of green tea extract, demonstrating first-order kinetics and that the concentration of catechin in the patch was rate-limiting. Q_{24} and Q_{48} data are also shown in Table 1. As expected, there was a dose-dependent relationship between the patch loading and the catechin release rate suggesting that release rate is controllable (Fig. 2). However, as the extract incorporated into the formulation is a complex mixture, altering the release of a particular compound will clearly affect release of other compounds.

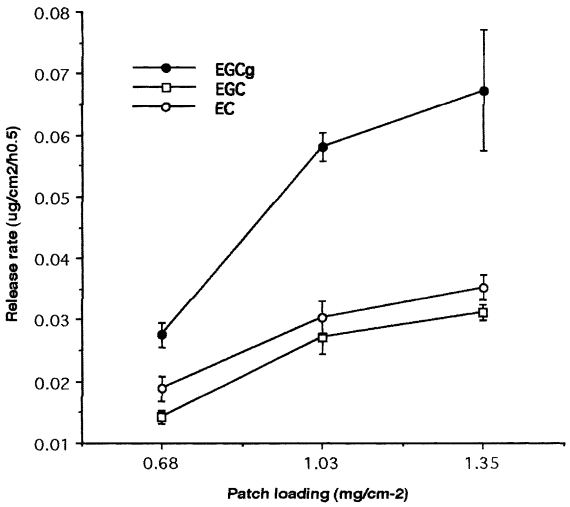

Fig. 2. Release rates of EGCg, EGC and EC from drug-in-glue patches as a function of green tea extract loading ($n = 4$, ±S.E.M.).

3.2. Permeation across porcine ear skin

Cumulative permeation profiles of EGCg, EGC and EC illustrated the attainment of steady state flux for the catechins across full thickness pig ear skin over the period of the experiment. The mean steady state fluxes (J_{ss} and permeability coefficients (k_p) for the catechins investigated are listed in Table 2.

Different amounts of EGCg, EGC and EC permeated across pig ear skin from the different formulations. EGCg, EGC and EC permeation was greatest from the saturated solution of green tea extract in CPB and least from the saturated solution in PEG. This

Table 1
Amounts and percentages of EGCg, EGC and EC released (Q) or permeated across skin from adhesive patches containing 1.35 mg cm^{-2} green tea extract after 24 and 48 h (±S.E.M.)

Polyphenol	Q_{24}	Permeated @ 24 h	Q_{48}	Permeated @ 48 h	Rel/perm @ 24 h	Rel/perm @ 48 h
EGCg (μg cm^{-2})	2.67 ± 0.27	0.27 ± 0.15	3.15 ± 0.49	0.66 ± 0.30	9.73	4.73
EGC (μg cm^{-2})	1.69 ± 0.04	0.06 ± 0.02	1.91 ± 0.09	0.10 ± 0.03	29.1	19.8
EC (μg cm^{-2})	1.50 ± 0.18	1.32 ± 0.22	2.11 ± 0.18	2.34 ± 0.34	1.14	0.90
EGCg (%)	0.67 ± 0.07	0.07 ± 0.03	0.79 ± 0.12	0.17 ± 0.08	9.73	4.73
EGC (%)	4.63 ± 0.11	0.16 ± 0.07	5.23 ± 0.27	0.26 ± 0.08	29.1	19.8
EC (%)	11.0 ± 1.36	9.67 ± 1.61	15.5 ± 1.33	17.2 ± 2.49	1.14	0.90

Table 2
Mean solubility, steady state flux and permeability coefficient data for EGCg, EGC, EC and caffeine from different formulations across full thickness pig ear skin ($n = 6$, ± S.E.M.)

Vehicle	Analyte	Solubility ($mg\,cm^{-3}$)	J_{ss} ($\mu g\,cm^{-2}\,h^{-1}$)	k_p ($cm\,h^{-1}$)	Permeated @ 24 h ($\mu g\,cm^{-2}$)	Permeated @ 48 h ($\mu g\,cm^{-2}$)
Saturated solution of GT in CPB	EGCg	13.8	$5.90 \times 10^{-2} \pm 2.09 \times 10^{-2}$	$4.26 \times 10^{-6} \pm 1.51 \times 10^{-6}$	1.37 ± 0.40	1.88 ± 0.45
	EGC	7.79	$5.68 \times 10^{-3} \pm 1.38 \times 10^{-2}$	$7.20 \times 10^{-7} \pm 1.75 \times 10^{-7}$	$0.189 \pm 4.10 \times 10^{-2}$	$0.342 \pm 7.48 \times 10^{-2}$
	EC	3.00	1.49 ± 0.55	$4.97 \times 10^{-4} \pm 1.82 \times 10^{-4}$	32.4 ± 11.3	71.2 ± 35.2
	Caffeine	15.96	$5.5 \times 10^{-3} \pm 5.0 \times 10^{-4}$	$3.47 \times 10^{-7} \pm 3.44 \times 10^{-8}$	0.32 ± 0.05	0.49 ± 0.01
Saturated solution of GT in PEG400	EGCg	8.31	<LoD	<LoD	<LoD	<LoD
	EGC	4.07	<LoD	<LoD	<LoD	<LoD
	EC	1.09	0.27 ± 0.13	$2.47 \times 10^{-4} \pm 1.20 \times 10^{-4}$	1.34 ± 0.35	4.96 ± 0.85
	Caffeine	50.74	1.64 ± 0.23	$3.24 \times 10^{-5} \pm 4.53 \times 10^{-6}$	46.8 ± 3.43	88.9 ± 0.08
Saturated solution of GT in 50:50 CPB:PEG400	EGCg	12.8	$4.57 \times 10^{-2} \pm 1.14 \times 10^{-2}$	$3.58 \times 10^{-6} \pm 8.93 \times 10^{-7}$	1.27 ± 0.38	1.62 ± 0.18
	EGC	6.93	$7.75 \times 10^{-3} \pm 4.29 \times 10^{-3}$	$1.12 \times 10^{-6} \pm 6.20 \times 10^{-7}$	$0.128 \pm 1.71 \times 10^{-3}$	0.329 ± 0.04
	EC	1.49	0.99 ± 0.64	$6.65 \times 10^{-4} \pm 4.31 \times 10^{-4}$	22.2 ± 17.3	40.2 ± 43.8
	Caffeine	31.09	7.78 ± 1.39	$2.50 \times 10^{-4} \pm 4.47 \times 10^{-5}$	173 ± 24.6	368 ± 52.9
Patch containing $1.35\,mg\,cm^{-2}$ GT	EGCg	–	$2.24 \times 10^{-2} \pm 1.10 \times 10^{-2}$	$1.76 \times 10^{-6} \pm 8.58 \times 10^{-7}$	0.27 ± 0.15	0.66 ± 0.30
	EGC	–	$5.02 \times 10^{-2} \pm 9.14 \times 10^{-3}$	$4.25 \times 10^{-6} \pm 8.04 \times 10^{-7}$	0.06 ± 0.02	0.10 ± 0.03
	EC	–	$5.43 \times 10^{-2} \pm 9.14 \times 10^{-3}$	$1.24 \times 10^{-4} \pm 2.08 \times 10^{-5}$	1.32 ± 0.22	2.34 ± 0.34
	Caffeine	–	1.05 ± 0.09	$1.43 \times 10^{-3} \pm 1.19 \times 10^{-4}$	28.4 ± 2.46	50.2 ± 1.54

LoD, limit of detection.

decrease corresponded with the decrease in the concentrations of green tea extract in the different saturated solutions and was consistent with Fickian diffusion. Only EC permeated across the skin from the saturated solution of green tea extract in PEG, although it was probable that the concentrations of permeated EGCg and EGC were below the limit of detection. It was indicated that more EGC permeated using the solvent mixture compared to CPB and may reflect a degree of penetration enhancement by the PEG 400. The highest amount of EGCg to permeate at 24 h was 1.4 $\mu g\,cm^{-2}$ from saturated solution in CPB, from the model patch the amount was approximately a quarter of this value.

Caffeine delivery from the different formulations through pig ear skin also displayed typical permeation profiles. Mean steady state flux values and permeation coefficients are listed in Table 2. Maximum flux of 7.78 $\mu g\,cm^{-2}\,h^{-1}$ was observed from a saturated green tea solution in 50:50 CPB:PEG where 172.9 $\mu g\,cm^{-2}$ permeated after 24 h. The least permeation of caffeine was from CPB alone where 0.32 $\mu g\,cm^{-2}$ permeated after 24 h.

The dissolution and transdermal delivery of EGCg, EGC and EC from patches containing 1.35 $mg\,cm^{-2}$ green tea extract were compared (Fig. 3). The release of a drug from the adhesive layer of a patch is an important parameter in its transdermal delivery. However, it is not expected that all of the drug released will reach the systemic circulation due to retention in the stratum corneum, i.e. permeation through the stratum corneum being rate limiting and/or reservoir deposition in the stratum corneum. As expected, the amounts of EGCg and EGC permeated were considerably less than those released. After 24 h the amount of EC permeated was also less than that released but the ratio between these values was less than between the release and permeation of EGCg and EGC. After 48 h the amount of EC permeated appeared to be more than the amount released. This difference can be explained by the metabolism of EGCg in the skin, whereby EGCg was hydrolysed by dermal esterase (Hotchkiss, 1998) leading to increased levels of EC in the receptor phase.

The mean steady state flux of caffeine from a patch containing 1.35 $mg\,cm^{-2}$ green tea extract (1.05 $\mu g\,cm^{-1}\,h^{-1}$) far exceeded that found for any of the catechins, as expected due to the known penetrating capability of caffeine. Permeation was found to be 28.39 $\mu g\,cm^{-2}$ after 24 h. This was greater than the permeation of 1.1 $\mu g\,cm^{-2}$ from a topical formu-

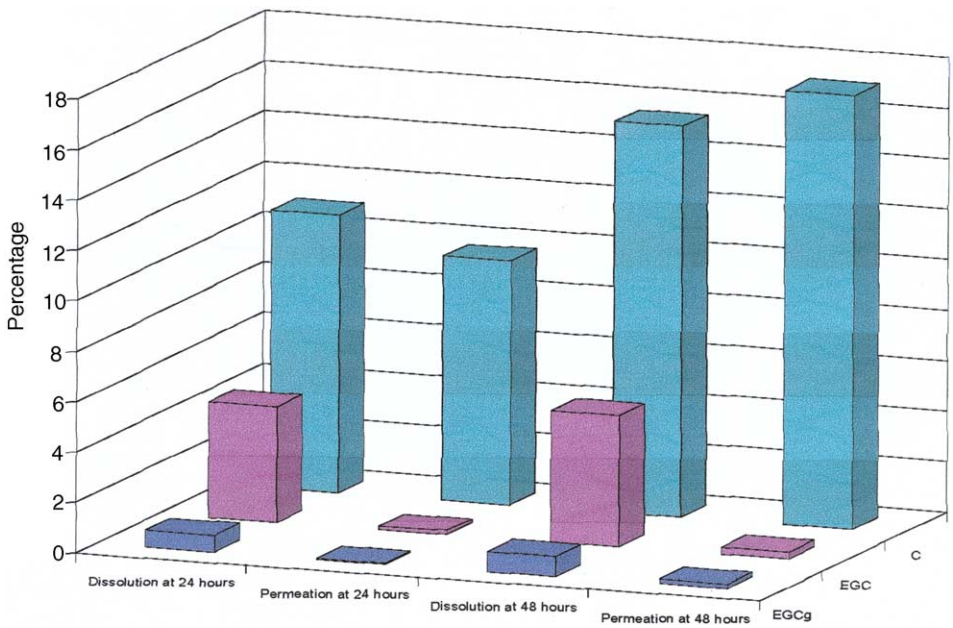

Fig. 3. Comparison of the mean percentage dissolution and permeation of EGCg, EGC and C from adhesive patches containing 1.35 $mg\,cm^{-2}$ green tea extract after 24 and 48 h.

lation containing 3% caffeine (Potard et al., 1999) and below concentrations where caffeine related side effects of green tea consumption are observed (Pisters et al., 2001).

4. Conclusion

From the patches containing 1.35 mg cm^{-2} green tea extract, it was found that 0.1% of EGCg permeated the skin after 24 h and is equivalent to the percentage absorbed after intragastric administration of green tea extract in rats (Chen et al., 1997). Despite the apparent decomposition of EGCg in the skin, the amount of EGCg permeated from the 0.9 cm diameter patch containing 1.35 mg cm^{-2} after 24 h, was within the range of C_{max} plasma levels previously reported after oral dosing of 2.2–4.2 g m^{-2} green tea extract (Pisters et al., 2001). Furthermore, caffeine remains at concentrations at which no side affects are observed. This demonstrates transdermal delivery as an alternative means of delivering catechins and caffeine at effective and safe concentrations.

Acknowledgements

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