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Note

Development of (−)-epigallocatechin-3-gallate (EGCG)-loaded enteric microparticles with intestinal mucoadhesive property

Satomi Onoue*, Masanori Ochi, Shizuo Yamada

Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

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ABSTRACT

The main purpose of the present investigation was to develop a novel enteric mucoadhesive formulation of (−)-epigallocatechin-3-gallate (EGCG), a green tea catechin, with the aim of avoiding its severe degradation in the gastrointestinal tract and therefore improving pharmacological effects. The EGCG-loaded microspheres (EGCG/MS), containing Eudragit® S100, were prepared with an emulsion solvent diffusion method in aqueous PVA solution. The EGCG/MS with a diameter of 16 μm exhibited pH-dependent controlled release of EGCG with limited initial burst release, and the Eudragit® S100-based MS also had moderate bioadhesive property in isolated small intestine of rats. There appeared to be marked degradation of EGCG in acidic solution (pH 1.2) and neutral buffer (pH 6.8) containing intestinal microsomal fraction, although significant improvement in chemical and metabolic stability of EGCG was observed in the EGCG/MS, possibly due to the controlled release and/or bioadhesion. From these findings, newly prepared EGCG/MS might be of clinical importance in both stabilizing and delivering EGCG for treatment of intestinal diseases.

Tea polyphenols, known as catechins derived from tea (*Camellia sinensis*), account for ca. 40% of the dry weight of brewed green tea (Zaveri, 2006). (−)-Epigallocatechin-3-gallate (EGCG) has been identified as a major tea polyphenol, showing health benefits for a variety of disorders, ranging from cancer to obesity. EGCG appears to be the most powerful of all the catechins with an antioxidant activity about 25–100 times more potent than those of vitamins C and E (Zaveri, 2006), so many beneficial effects may be attributed to its potent anti-oxidative activity. Recently, a considerable number of *in vivo* studies have demonstrated the therapeutic potential of EGCG for the treatment of intestinal diseases, such as chronic inflammatory bowel disease (IBD) (Ran et al., 2008) and intestinal cancer (Lambert and Yang, 2003).

In spite of its notable therapeutic potential, EGCG tends to undergo intensive acid hydrolysis in gastric fluid and metabolic degradation in the gastrointestinal (GI) tract (Spencer, 2003). As for the biotransformation mechanism of EGCG, methylation, glucuronidation, sulfation, and ring-fission metabolism in the GI tract have been reported, and degalloylation of flavanol gallate esters has also been observed in human saliva. Rapid elimination of EGCG, due to chemical and enzymatic degradation, might thus lead to limited clinical outcomes from EGCG-based therapy for intestinal diseases. Therefore, improvement in metabolic stability of EGCG in the GI tract may be key for its clinical application.

Enteric formulation strategy has been used to avoid the degradation of drugs in gastric fluid, and nano- and/or microsphere (MS) technologies are often employed with the aim of enhancing topical pharmacological effects in intestinal organs, as well as bioavailability. Although these formulation strategies might be of great importance for improved therapeutic potential of EGCG, the feasibility of these approaches has never been fully elucidated. The present study aimed to develop an efficacious microsphere system of EGCG (EGCG/MS) using Eudragit® S100, an enteric and bioadhesive polymer.

In the present study, EGCG/MS was prepared by an emulsion solvent diffusion method in polyvinyl alcohol (PVA) solution (Kietzmann et al., 2009). Briefly, Eudragit® S100 (150 mg) and EGCG (50 mg) were dissolved in 6 mL of *n*-butanol in an ultrasonic bath for 15 min at 60 °C, and the mixture was added to 30 mL of 1% PVA solution at 45 °C to form an O/W emulsion. The resultant emulsions were washed using 0.1% PVA solution (pH 4.0), centrifuged at 3000 rpm for 5 min, and lyophilized to obtain the EGCG/MS. SEM image of the EGCG/MS revealed that they were spherical and uniformly dispersed with a diameter of 5–30 μm (Fig. 1A). A smooth outer surface and a regular spherical shape could be important for extended drug release, and any irregularities would cause an increase in surface area, creating a much faster diffusive release of EGCG from the EGCG/MS. The EGCG/MS formulation was readily re-dispersed into HCl solution (pH 1.2) by shaking manually,

* Corresponding author. Tel.: +81 54 264 5633; fax: +81 54 264 5635.
E-mail address: onoue@u-shizuoka-ken.ac.jp (S. Onoue).

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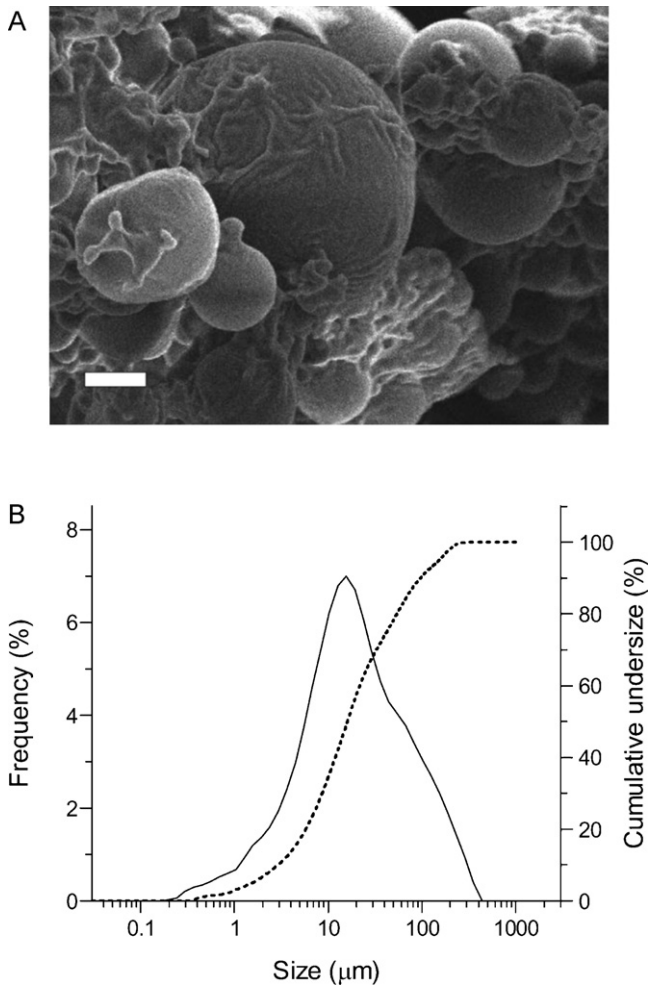

Fig. 1. Morphology of EGCG/MS. (A) Scanning electron microscopic image of EGCG/MS. Bar represents 5 μm. (B) Particle size distribution of EGCG/MS redispersed in HCl solution (pH 1.2). Solid line, frequency; dotted line, cumulative undersize fraction curve.

reproducing the MS with a diameter of ca. 16 μm as determined by laser diffraction analysis (Fig. 1B). The recovery and EGCG content of the EGCG/MS were calculated to be 10.8% and 3.2%, respectively, by UPLC/ESI-MS analysis using a Waters Acquity UPLC system equipped with single quadrupole mass detectors (Waters, Milford, MA). The EGCG can be easily dispersed into the water phase upon mechanical agitation, and the EGCG may undergo chemical degradation by hydrolysis and/or oxidation during preparation of EGCG/MS formulation. They might partly explain the limited recovery and loading amount of EGCG. Although suitable dose setting of EGCG for the treatment of intestinal diseases still remained a matter of controversy, further improvement in loading amount of EGCG would be beneficial for enhancing developability of the EGCG/MS for clinical use.

Fig. 2 illustrates the *in vitro* release profiles of EGCG/MS in aqueous media by representing the percentage of EGCG release with respect to the amount of EGCG encapsulated. The dissolution testing was carried out in HCl solution (pH 1.2) and 20 mM sodium phosphate buffer (pH 6.8) to simulate gastric and intestinal conditions, respectively (Kalantzi et al., 2006). Release behavior of EGCG/MS was found to be pH-dependent as expected. In an acidic condition, only slight initial leakage of EGCG was observed followed by efficient retention of the EGCG inside the MS matrix over a tested period of 2 h. As observed for other controlled release MS formulations, the initial burst can most likely be attributed to EGCG

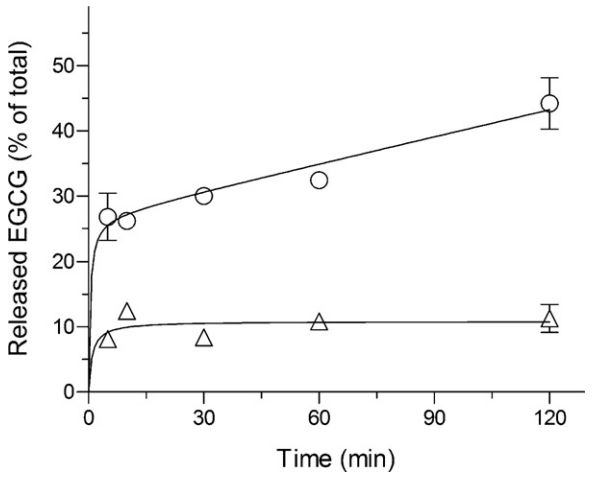

Fig. 2. Cumulative *in vitro* release of EGCG from EGCG/MS. (○), release profile at pH 6.8; (△), that at pH 1.2. Data represent mean ± SE of three experiments.

adsorbed to the surface of the particles. In contrast, release of EGCG at pH 6.8 occurred in two phases: a first initial burst release and a slow release of EGCG resulting from the diffusion of the EGCG through the polymer matrix. There was ca. 27% burst release of total encapsulated EGCG within the first 5 min. In the second phase, a constant slow release of EGCG until release of ca. 44% of the total loaded amount was observed within 2 h, showing a typical pH-sensitive and sustained release of EGCG. The pH-sensitive and sustained release behavior of the EGCG/MS might contribute to the avoidance of rapid oxidative and enzymatic degradation in gastric fluid with enhanced duration of action in the intestine.

Anionic polymers have been widely employed as bioadhesive carriers within pharmaceutical formulations because of their high mucoadhesion and low toxicity (Andrews et al., 2009). Thus, the EGCG/MS may also be adhesive to the mucosal layer of the intestinal wall as long as it contains anionic Eudragit® S100. To clarify the possible interaction between Eudragit® S100-based MS and mucosal epithelia, *in vitro* bioadhesion testing was carried out using isolated small intestine of rats as reported previously (Ranga Rao and Buri, 1989). EGCG/MS containing 0.02% FITC (FITC-EGCG/MS) was prepared for fluorescent monitoring with high sensitivity, and FITC-EGCG/MS (5 mg) or FITC (1 μg) was placed on dissected small intestine of rats (ca. 5 cm^2) under a humid environment. The tissues were then washed using 20 mM sodium phosphate buffer (NaPB) (pH 6.0) with a flow rate of 15 mL/min and the mucoadhesive behavior of MS was expressed as the percentage of MS remaining on the small intestine after washing. According to the fluorometric analyses on the lavage fluids after 5- and 15 min washing (Table 1), the remaining MS in the small intestine of rats was found to be ca. 2-fold higher than that with FITC alone. These data support the mucoadhesive property of FITC-EGCG/MS and are in agreement with previous observations demonstrating that polyanionic polymers could adhere strongly to the mucus (Ranga Rao and Buri, 1989). These polymers are characterized by the presence of

Table 1
Bioadhesive property of Eudragit S100-based microspheres in isolated small intestine of rats.

	FITC adhering to rat intestinal wall (% remaining)	
	After washing for 5 min	After washing for 15 min
FITC	25.8 ± 0.7	12.5 ± 2.4
FITC-EGCG/MS	42.1 ± 3.0	28.7 ± 4.8

Data represent the mean ± standard error (SE) of three determinations.

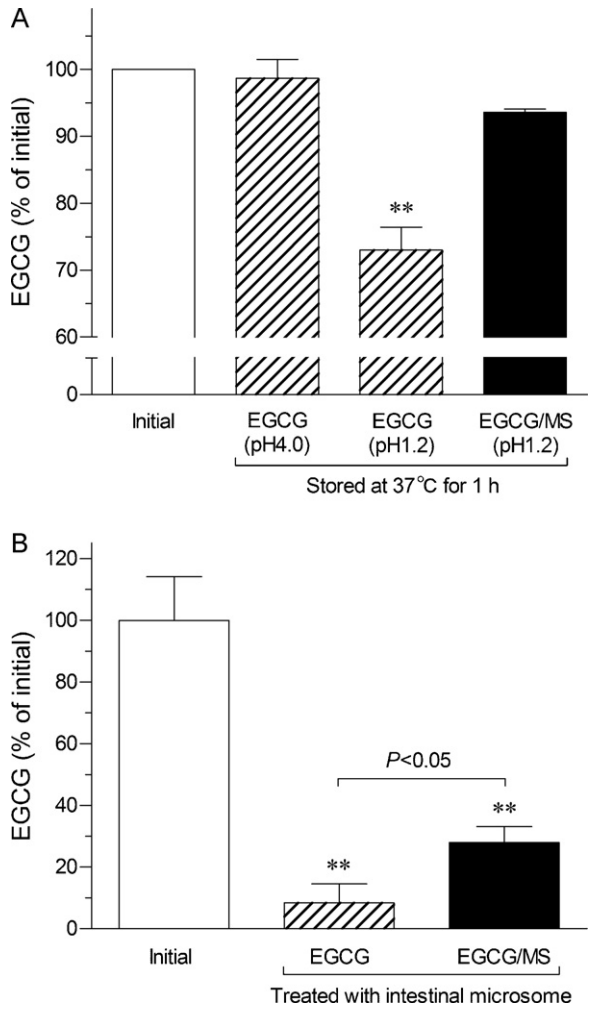

Fig. 3. Chemical and metabolic stability of EGCG and EGCG/MS. (A) Degradation of EGCG after 2 h incubation at 37 °C in solutions of pH 1.2 and 4.0. (B) Metabolic degradation of EGCG after 30 min incubation at 37 °C in 20 mM NaPB (pH 6.8) containing microsomal fraction of the rat small intestine (600 μg protein/mL). Data represent mean ± SE of three experiments. $^{**}P < 0.01$ with respect to the control (initial) group.

carboxyl or sulphate functional groups, and hydrogen bonding by these hydrophilic functional groups might play a significant role in mucoadhesion. The increased residence time for the EGCG/MS might lead to the improved duration of action and/or the moderate avoidance of first-pass metabolism in the intestinal tract.

In solution-state stability studies on EGCG, gradual degradation of EGCG was observed during storage under an acidic condition (pH 1.2) as determined by UPLC/ESI-MS analysis, although no significant degradation of EGCG dissolved in 10 mM sodium acetate/acetic acid buffer (pH 4.0) stored at 37 °C was observed (Fig. 3A). When the decomposition of EGCG in HCl solution (pH 1.2) was plotted in a typical logarithm of percent remaining versus time (0, 1 and 2 h), a linear relationship was observed with a correlation coefficient of 0.99 (data not shown). This indicated that the degradation of EGCG under an acidic condition followed apparent first-order degradation kinetics, and the apparent first-order degradation rate constant was calculated to be $1.59 \pm 0.20 \times 10^{-1}\ h^{-1}$. The results could be indicative of less chemical stability of EGCG in the gastric fluid as reported previously (Spencer, 2003), possibly leading to marked reduction of therapeutic efficacy. In contrast, storage of the EGCG/MS for 2 h under an acidic condition (pH 1.2) resulted in only 5% degradation of EGCG, the degradation rate constant of which was found to be $3.32 \pm 0.26 \times 10^{-2}\ h^{-1}$. Thus, there was significant improvement in the chemical stability of EGCG with a decrease in degradation kinetics by ca. 5-fold; this was in agreement with the *in vitro* release profile of EGCG/MS.

In addition to the chemical stability, metabolic stability of the EGCG/MS was also assessed using rat intestinal microsome (Fig. 3B). The microsomal fraction of rat small intestine was obtained in accordance with a previous report (Zhang et al., 2007), and 300 μg of small intestinal microsomal protein was added to 500 μL of EGCG solution or EGCG/MS suspension (pH 6.8, 40 μg EGCG/mL). After 30 min incubation at 37 °C, the reactions were stopped by addition of 5 mL of ethyl acetate, and the remaining amount of EGCG was determined by UPLC/ESI-MS analysis. Although treatment of EGCG with rat intestinal microsome for 30 min resulted in marked metabolism of EGCG as evidenced by only 8.5% of EGCG remaining, intact EGCG in the EGCG/MS exposed to microsome was calculated to be ca. 28%. The improved metabolic stability of EGCG/MS may be due to the delayed release of EGCG from Eudragit® S100-based MS system. As such, pH-sensitive controlled release system of EGCG using Eudragit® S100 is expected to be effective to avoid gastric degradation and improve metabolic stability in the intestinal tract after oral administration.

In conclusion, the MS formulation of EGCG with a mean diameter of 16 μm has been successfully prepared by an emulsion solvent diffusion method in PVA solution. Better stability for EGCG in the GI tract and bioadhesion with mucosal layer can be achieved with Eudragit® S100-based MS system, and the developed formulation of EGCG may be suitable for clinical treatment of intestinal diseases such as IBD and intestinal cancer.

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