The effect of tea derived epigallocatechin on mouse intestinal motility in vitro.

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Summary
Since the data concerning the effects of catechins on gastrointestinal transit are contradictory the effect on the motility of the isolated mouse jejunum, of an active component in green tea, epigallocatechin gallate (EGCg) was investigated. EGCg induced a reversible decrease in the spontaneous motility of isolated segments of mouse jejunum. The effect was dose dependent and significant inhibition was observed at a concentration of 3x10^{-4} M EGCg. Both the frequency and amplitude of spontaneous contractions decreased significantly in the presence of EGCg. The effect of EGCg was observed over a duration of 10-15 minute period. The effect of 10^{-3} M EGCg was partly inhibited by methylene blue, while tetrodotoxin, L-NAME, and N-ethylmaleimide were not effective. These data suggest that EGCg induces alteration of spontaneous motility through a direct cellular mechanism which is in part related to the activation of guanylate cyclase.

Key words
Epigallocatechin gallate, small intestine, motility

Introduction
Catechins originate from various plants. They are easily absorbed in the gastrointestinal tract from ingested cocoa (3), green (13), or black tea (16). Tea is the main source of catechins in humans. Catechins that are present in green tea include epigallocatechin gallate (EGCg), epigallocatechin, etc.

EGCg performs a variety of biological activities, including antitumor as well as antimicrobial activity against some pathogens. Prevention of UV-B-induced infiltrating leukocytes, antigen-presenting cells, and oxidative stress by EGCg treatment of mouse skin has been reported to be associated with the prevention of UV-B-induced immunosuppression and photocarcinogenesis (8). Moreover, pretreatment with EGCg has been found to restore an UV-induced decrease in total glutathione level and to provide protection to the glutathione peroxidase (antioxidant enzyme) (8). EGCg showed the strongest activity among the six tea catechins tested against Helicobacter pylori (11). EGCg selectively upregulated the production of IL-12 and of tumor necrosis factor alpha and downregulated the IL-10 production of macrophages induced by Legionella pneumophila infection (12). Meclidanol (O-methyl-3(+)-catechin), a synthetic flavonoid, protects against ulcerogens and does not affect either gastric acid secretion or pepsin output in rats (7, 10). It has been shown that catechins inhibit gastric H^+, K^+-ATPase activity, with EGCg as the most potent inhibitor. These findings suggest that the anti-secretory and anti-ulcerogenic effects of catechins are due to their inhibitory activity on gastric H^+, K^+-ATPase (14).

Catechins also influence gastrointestinal motility and transit. An early study showed that gossypin, epicatechin and hydroxyethyl rutosides delayed small intestinal transit in a dose-dependent manner in mice. Naloxone, yohimbine, and phentolamine antagonized the effect of these compounds. Prazosin, propranolol, atropine, physostigmine, hexamethonium, pheniramine or metiamide had no effect. Thus it has been suggested that opiate and alpha-2 adrenergic receptors mediate flavonoid-induced delay in small intestinal transit (15). Another study concerning the effects of polyphenolic compounds on gastrointestinal transit has shown that kampferol morin, myricetin, and rutin have a strong, dose-dependent inhibitory effect on charcoal transit in mice, whereas catechin did not show significant effects (5). In vitro study has shown that EGCg has a direct depolarizing effect on myenteric neurons in guinea pig small intestine (6).

Since catechins are compounds that may be used as medicines, it is necessary to investigate their effects on various organs. However, there has been no report showing the effect and mechanism of catechins
on mechanical activity of intestine in vitro. Thus, the aim of the present study was to characterize the effect and mechanism of EGCg action on the mechanical activity of mouse small intestine.

Materials and methods

Animals

Female, ddy mice (25 - 65 g) were used in all experiments. The animals had free access to laboratory chow and tap water before the experiment and were kept under artificial lighting 12 hours a day at 20°C to 22°C. Experimental protocols were carried out according to the principles and guidelines of the National Institute for Physiological Sciences in Japan.

In vitro gastric motility recording

The animals were anesthetized with ether and then stomach tissue was harvested. Segments of proximal jejunum (approximately 7 mm long) were prepared and mounted in a 10 ml organ bath filled with Krebs buffer. The solution contained (in mM): NaCl 118, KCl 4.8, KH2PO4 1.2, MgSO4 1.2, CaCl2 1.9, NaHCO3 25, and glucose 10.1. The buffer was constantly gassed with an O2 95%, CO2 5% mixture. The temperature of the buffer was maintained at 37°C. Changes in the tension of the strips were recorded isometrically with NEC San-ei type 45196A force transducers (NEC-San-ei, Japan) coupled with a MacLab recording system (ADInstruments, Australia).

Mechanical activity of jejunal segments was recorded in accordance with the following schedule. The strips were allowed to equilibrate for 30 minutes. Next, acetylcholine (ACh; 10⁻⁶M) was added to the bath. Then EGCg was added to the chamber in a non-cumulative manner in concentrations 10⁻⁷, 10⁻⁶, 10⁻⁵, and 10⁻⁴ M. The response to each administration of catechins was recorded for 10 minutes, and then the chamber was washed for another 10 minutes in preparation for the next administration of EGCg. At the end of the experiment ACh was added in the same manner as at the beginning of the experiment.

Mechanism of catechins’ action

After equilibration, EGCg 10⁻³ M was added to the chamber. Next EGCg was added in the presence of tetrodotoxin (TTX, sodium channel blocker), methylene blue (MB, guanylate cyclase inhibitor), L-nitro arginine methyl ester (L-NAME, nitric oxide synthase inhibitor), or N-ethylmaleimide (NEM, adenylate cyclase inhibitor) in order to investigate the mechanism of EGCg action.
Results

The effect of catechins on spontaneous contractions

EGCg induced a decrease in the spontaneous activity of the jejunum. The effect was observed as a decrease in the frequency, and the amplitude of contractions (Fig. 1 and 2). The decrease in frequency induced by EGCg was dose dependent. However, a statistically significant decrease in amplitude was observed only at the highest concentration (Fig. 3).

The mechanism of catechins action

In the present study TTX, 10^{-6} M and L-NAME, 10^{-4} M did not significantly affect an EGCg-induced decrease in spontaneous jejunal activity. On the other hand, guanylate cyclase inhibitor MB, 10^{-4} M had an effect on the EGCg-induced decrease in the amplitude of jejunal motility; however, the effect was very small. Moreover, MB did not significantly influence the frequency of contractions. NEM 10^{-5} M had no significant effect on the EGCg-induced alteration in intestinal motility (Fig. 4).

Discussion

The present study shows that EGCg derived from tea leaves can alter the spontaneous activity of the small intestine. However, the effect appears only at quite high concentrations. On the other hand, the level of availability of catechins has been confirmed at lower concentrations (4, 16). Plasma concentrations and doses presented in recent studies are much lower than those presented as effective in the present study. Thus, the proposed antioxidative action that has been reported at concentrations in the range of 10^{-6} M (1, 13) may be reached without the disturbance of gastrointestinal motility. However, a potential negative influence is possible in the case of longer exposures to higher concentrations. The present study shows that EGCg induces a long-lasting disruption of spontaneous activity in the small intestine. The mechanism of this action most likely involves direct cellular action since TTX and L-NAME were ineffective to prevent the inhibiting effect of EGCg. The small effect of MB suggests that, in part,
inhibition of spontaneous motility is induced by the activation of guanylate cyclase, while adenylyl cyclase is not involved in this action. However, the precise mechanism of this effect remains to be investigated. The present study shows that EGCg effects (myenteric neuron depolarization) observed at the cellular level at concentrations in a range of 10⁻⁰⁷ M do not induce significant effects at a tissue level. It is possible that, in the experimental design of the present study, local concentrations of EGCg were smaller than the general concentration in the chamber, but still the lack of TTX effect suggests the direct action of EGCg on intestinal smooth muscle. Catechins directly activate superoxide dismutase in rat brain. On the other hand, it has been reported that catechins inhibit xanthine oxidase in the liver. Thus, it is possible that in high concentrations, EGCg induces profound, non-selective inhibition of intracellular functions that results in the inhibition of spontaneous motility of the intestine.

References


