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Effects of Tea Catechins on the Gastrointestinal Mucosa in Rats

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Although tea catechins are known to exert a potent antiulcer effect on the alimentary tract, there is scant information concerning their effects on normal mucus cell functions. Using original anti-mucin monoclonal antibodies, we studied the influences of long-term administration of catechins on the quantity and quality of mucin in rat gastrointestinal mucosa. Administration of 0.5% tea catechins significantly increased the mucin content of the ileum, but not the stomach. An enzyme-linked immunosorbent assay (ELISA) showed no remarkable qualitative changes in gastric mucin, but a selective increase and decrease in sulfo- and sialomucins, respectively, in the ileum of rats administered catechins. The ELISA results were consistent with both the immunohistochemical findings and the high-iron diamine-alcian blue staining pattern. These findings indicate that tea catechins modulate ileal mucin metabolism in the ileal mucosa, suggesting that further studies focusing on the ileal epithelium will assist in further elucidation of the mechanism of catechin effects.

KEYWORDS: Immunohistochemistry; rat ileal mucus; sialomucin; sulfomucin; tea catechins

INTRODUCTION

In recent years, possible health benefits of drinking green tea have received a great deal of attention, much of it directed at a group of polyphenolic compounds called catechins (*1*−*5*). These are condensed into tannins in black tea and are also found in sources other than green tea, such as grape skins and seeds. The most abundant of the polyphenolic compounds in green tea is epigallocatechin gallate (EGCG), with other catechins such as epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin (EGC) also present (**Figure 1**). As green tea is a candidate for primary prevention of gastrointestinal (GI) ulceration, much attention has been paid to the antiulcer effects of these catechins. Administration of tea catechins has been reported to attenuate GI mucosal injury induced by various sources such as *Helicobacter pylori*, ethanol, and stress in experimental models (*1, 5*−*7*). However, there is scant information regarding the influence of the catechins on normal GI mucosa, especially on mucus cells present throughout the alimentary tract.

Mucin, a major component of mucus produced by these cells, is considered to be a principal factor in the physiological defense of the GI tract, in particular, the gastric mucosa (*8*). Considerable interest has been stimulated by reports of selective changes in human GI mucins in both physiological and pathological circumstances (*9, 10*). We have already shown that alterations in human gastric mucin accumulation caused by certain agents were consistent with the results from a rat model (*11, 12*). Moreover, we identified several monoclonal antibodies (mAbs) that react with mucin synthesized and secreted from specific mucus cells of the rat GI tract (*13*−*15*).

The purpose of the present study was to use our biochemical and histological methods to determine the effects of long-term administration of tea catechins on the mucus cells in the rat GI mucosa.

MATERIALS AND METHODS

Tea Catechins. We used a crude catechin extract (Teafuran 90S; Itoen Ltd., Tokyo, Japan) containing 55.5% (w/w) EGCG, 16.7% ECG, 2.3% EGC, and 1.4% EC. According to the manufacturer, the total polyphenol content was 94% and the caffeine content, 0.6%.

Animals. We used 7-week-old male Wistar rats purchased from CLEA-Japan (Tokyo, Japan) in this study. The animals were housed in our animal care facility for 1 week while their body weight stabilized. Animals were housed in individual cages with raised mesh bottoms in a temperature- and humidity-controlled environment with a 12 h dark−light cycle (6:00 p.m.−6:00 a.m. dark cycle). They were divided into three groups of six animals each, with all fed a normal diet (Clea Rodent Diet CE-2: 24.5% crude protein, 4.8% crude fat, 51.4%

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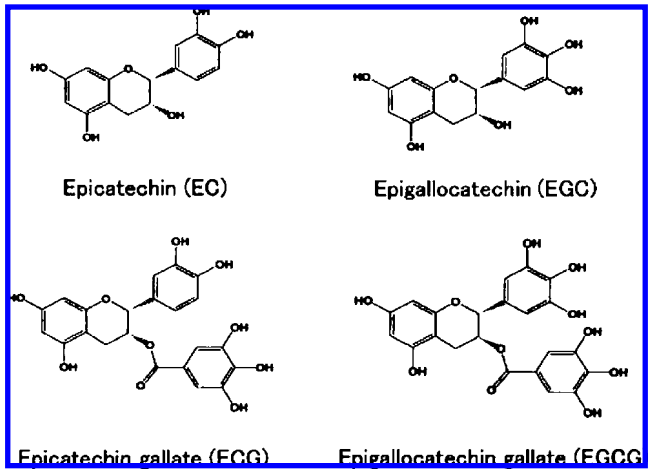

Figure 1. Structures of principal tea catechins.

nitrogen-free extract, 3.7% crude fiber). The control group was given tap water, and the treatment groups were given a 0.1 or 0.5% solution of catechins. These solutions of catechins were prepared fresh every day. The content of catechins in the drinking water changed by a negligible amount in this study. Following a 24 h fast, animals were euthanized with carbon dioxide at 22 days (3 weeks) after commencement of the study, and their stomachs, proximal and distal small intestines (corresponding to the jejunum and ileum, respectively), and large intestines were removed.

This study was conducted in accordance with the guidelines of the Animal Laboratory Center of Kitasato University School of Medicine.

Body Weight and Food Intake. Body weight was measured on day 0 (start of catechin administration) and day 21. Daily intakes of food and water were measured on the seventh day after commencement of the study.

Biochemical Examination. GI mucosal samples were separated into stomach, jejunum, ileum, and colon for measurement of mucin content. Each specimen was lyophilized and powdered for extraction of mucin according to the previously described method (*16*). Each sample was suspended in Triton−Tris buffer, homogenized, and then incubated at 37 °C for 1 h. After centrifugation at 8000*g* for 30 min at 4 °C, the supernatant was collected, and an aliquot was applied to a Bio-Gel A-1.5m column and eluted with the Triton−Tris buffer. The void volume fraction (Fr-1) monitored by hexose measurement was collected as mucin. The hexose content in this fraction was measured using the phenol−sulfuric acid method with galactose as the standard. Mucin content (Fr-1 hexose value) was expressed as micrograms of hexose per tissue.

An enzyme-linked immunosorbent assay (ELISA) was used to evaluate the involvement of certain mucus cells in quantitative changes in mucin. Ten times serial-doubling dilution of the partially purified mucin from each specimen was prepared from 100 ng of mucin hexose per well. Microtiter plates with ELISA wells were kept overnight at 4 °C, followed by blocking with 2% (w/v) skimmed milk (*14*). After washing, a specific amount of anti-mucin mAbs (RGM21, RGM26, HIK1083, HCM31, and PGM34) was added to each well, followed by incubation at room temperature for 1 h. Wells were successively incubated with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Dako, Kyoto, Japan) and 2,2′-azinobis[3-ethylbenzthiazoline-6-sulfonate] (ABTS)/H_2O_2 solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD), and color was allowed to develop. The optical density (OD) at 405 nm was measured using a model 680 microplate reader (Bio-Rad Laboratories Inc., Tokyo, Japan).

Histological Examination. Resected specimens were immediately fixed for 3 h in freshly prepared Carnoy's solution following the method described previously (*17*). After fixation, specimens were dehydrated using ethanol, cleared in xylene, and embedded in paraffin. From these specimens, 3 μm paraffin sections were prepared for immunostaining with anti-mucin mAbs and conventional staining with high-iron diamine-alcian blue (HID-AB) (pH 2.5). Immunohistochemical staining was performed with the avidin−biotin peroxidase method using an LSAB2 Kit (Dako, Carpinteria, CA). Briefly, endogenous peroxidase activity was blocked with 0.3% H_2O_2, and then specimens were sequentially incubated with 10% (v/v) normal swine serum, the anti-mucin mAb (HCM31, PGM34), biotinylated anti-mouse immunoglobulins, streptavidin horseradish peroxidase (HRP), and 0.02% 3,3-diaminobenzidine in 50 mM Tris-HCl, pH 7.6, containing 0.005% H_2O_2. Counterstaining was performed using hematoxylin. The immunohistochemical reactivity of each mAb was observed using light microscopy.

Table 1. Weight Changes in Rats before and after Oral Administration of Tea Catechins

	Body Weight		
	Before	After	
Control	217.0 (±2.5)	315.8 (±27.7)	*
0.1% Tea Catechins	212.8 (±7.3)	303.3 (±10.0)	
0.5% Tea Catechins	220.0 (±7.8)	273.7 (±15.7)	

[a] Mean (± SD); *, $p < 0.05$; $n = 6$.

Statistical Analysis. Biochemistry test results are expressed as mean ± SD relative to the mean value of the corresponding control. One-way analysis of variance (ANOVA) and the Scheffé test were used for statistical analysis. A difference of $p < 0.05$ was considered to be statistically significant.

RESULTS

Body Weight, Food Intake, and Fluid Consumption. Changes in body weight in rats in each experimental group after free access to food and water for 3 weeks are shown in **Table 1**. Weight gain in the catechin groups was less than that in the control group. The mean body weight of the 0.5% catechin group after 3 weeks was significantly lower than that of the controls. Food and fluid intakes did not differ between the control and catechin groups (data not shown).

Changes in the Total Mucin Content. Figure 2 shows the mucin content in the four different regions of the rat GI tract after treatment with tea catechins compared with the control. The mucin contents of the controls were 1166 ± 159, 665 ± 101, 618 ± 62, and 896 ± 18 μg of hexose/100 mg of dry tissue weight in the stomach, jejunum, ileum, and colon, respectively. As shown in **Figure 2C**, administration of 0.5% tea catechins significantly increased the mucin content of the ileal mucosa in the rat GI tract. In contrast, the administration of neither 0.1 nor 0.5% catechins significantly influenced the total mucin content of the gastric, jejunal, and colonic mucosa (**Figure 2A**,**B**,**D**).

Selective Changes in Specific Mucins. In the gastric specimens, ELISA OD values of HIK1083 mAb did not differ significantly between groups (OD range = 0.512−0.531). Similarly, no remarkable changes in gastric mucin were detected in the OD values of either RGM21 or RGM26 mAbs following the administration of tea catechins (OD range for RGM21 = 0.281−0.292; OD range for RGM26 = 0.095−0.114).

In the ileal samples, the control OD values of PGM34 and HCM31 mAbs were 0.203 ± 0.011 and 0.363 ± 0.030, respectively (**Figure 3**). As shown in **Figure 3A**, the relative antigenic activities in the PGM34 mAb were significantly increased by the long-term administration of tea catechins. In contrast, the antigenic activities of the HCM31 mAb were suppressed in specimens from both the 0.1 and 0.5% catechin groups.

Histological Finding. Figure 4 shows microphotographs of ileal sections from the small bowel mucosa of rats from each

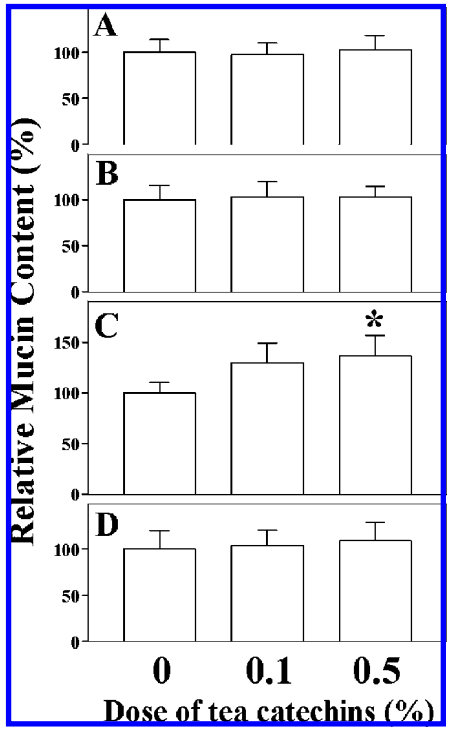

Figure 2. Influence of tea catechins on gastric (**A**), jejunal (**B**), ileal (**C**), and colonic (**D**) mucosal mucin accumulation in the rat. Each specimen was lyophilized and powdered. The extraction and isolation of mucin were performed as described in the text. Fr-1 hexose values were measured as the mucin content and expressed as micrograms of hexose per tissue. Data are expressed as a percentage of control and represent the mean ± SD from six different specimens derived from six different rats. Asterisks indicate the statistical significance (∗, $p < 0.05$) versus the control value. The mucin contents of the controls were 1166 ± 159, 665 ± 101, 618 ± 62, and 896 ± 18 μg of hexose/100 mg of dry tissue weight in the stomach, jejunum, ileum, and colon, respectively.

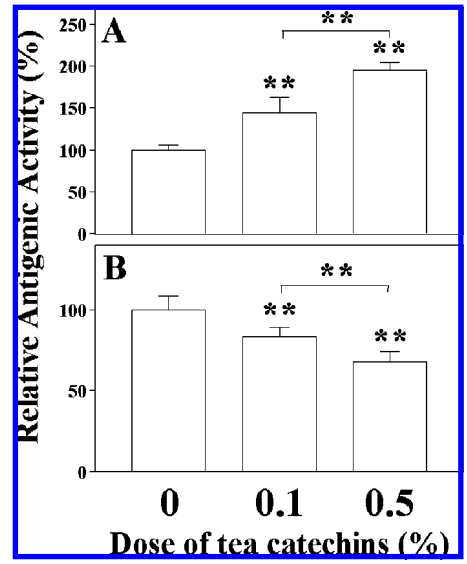

Figure 3. Immunoreactivities for PGM34 (**A**) and HCM31 (**B**) in ileal mucin from rats administered or not administered tea catechins. Using an enzyme-linked immunosorbent assay system, 6.25 and 25 ng of partially purified mucin from each specimen was reacted with PGM34 and HCM31 monoclonal antibodies, respectively. The optical density (OD) at 405 nm was measured using a microplate reader. Each value is expressed as the percentage of control and represents the mean ± SD for six different samples derived from six different rats. Asterisks indicate statistical significance (∗∗, $p < 0.01$), those just above the SD bar showing the significance versus the control value. PGM34- and HCM31-antigenic activities in the control expressed as OD were 0.203 ± 0.011 and 0.363 ± 0.030, respectively.

group stained with two distinct anti-mucin mAbs. In the control rat, immunohistochemical reactivity for both PGM34 and HCM31 could be detected in goblet cells, as well as the surface mucus gel layer and partial goblet cells, respectively (**Figures 4A**,**D**). As shown in panels **B** and **C** of **Figure 4**, goblet cells in the ileal mucosa were strongly stained with PGM34 after administration of tea catechins. In contrast, the number of goblet cells stained with HCM31 was specifically decreased by tea catechins (**Figure 4E**,**F**).

Sulfomucins and sialomucins could be differentiated in the ileal mucosa of control rats, according to the color reaction obtained with HID-AB stain (**Figure 5A**). Ileal samples from rats administered tea catechins showed a predominance of sulfomucin, stained black in this image (**Figures 5B**,**C**). In contrast, administration of catechins caused a marked decrease in the staining intensity of sialomucin, staining blue with the HID-AB method.

DISCUSSION

In this study, we demonstrated marked quantitative and qualitative alterations in ileal mucin in rats given drinking water containing crude catechin extract at doses (0.1 and 0.5%) within the normal limits for routine green tea drinking (*18, 19*), with virtually no inhibitory effect on the intake of fluid and food. A recent study (*1*) using the same extract demonstrated that oral administration at 50, 100, and 200 mg/kg dose-dependently prevented decreases in rat gastric mucin content induced by absolute ethanol. Furthermore, EGCG, a major polyphenolic component of green tea, has been reported to inhibit interleukin-1β-induced mucin secretion by normal human nasal epithelial cells (*20*). Although the difference in route of administration should be kept in mind, taken together these findings confirm that catechins, at clinically appropriate doses, affect certain mucus cell functions.

In the stomach, mucin is a key element in protecting the gastric epithelium against various irritants, and its accumulation in the gastric mucosa is closely related to the mucosal protective capability (*11, 16*). Several studies have indicated a protective effect for tea catechins against gastric mucosal injury (*1, 5*−*7*). Unfortunately, a significant increase in the total mucin content could not be detected in the gastric mucosa of rats treated with tea catechins in this study. In the mammalian GI mucosa, specific types of mucin are expressed in distinct mucus cells and are thought to have distinct individual functions (*17, 21*). In the gastric mucosa of normal rats, the corpus surface mucus cells, the antral surface mucus cells, and the gland mucus cells produce different types of mucin molecules recognized by the anti-mucin mAbs RGM21, RGM26, and HIK1083, respectively (*14*). Recently, Kawakubo et al. (*22*) reported a natural antibiotic effect of HIK1083-positive mucin against *H. pylori*. Because orally administered tea catechins also possessed an antibacterial effect against *H. pylori* in infected Mongolian gerbils (*23, 24*), it is possible that an increase in the ratio of this type of mucin will contribute to an anti-*H. pylori* effect. However, in the present study, there was no significant difference in the antigenic activities of anti-mucin mAbs including HIK1083 in the gastric mucin between controls and rats administered tea catechins, indicating that these compounds do not alter gastric mucus cell function in the physiological state.

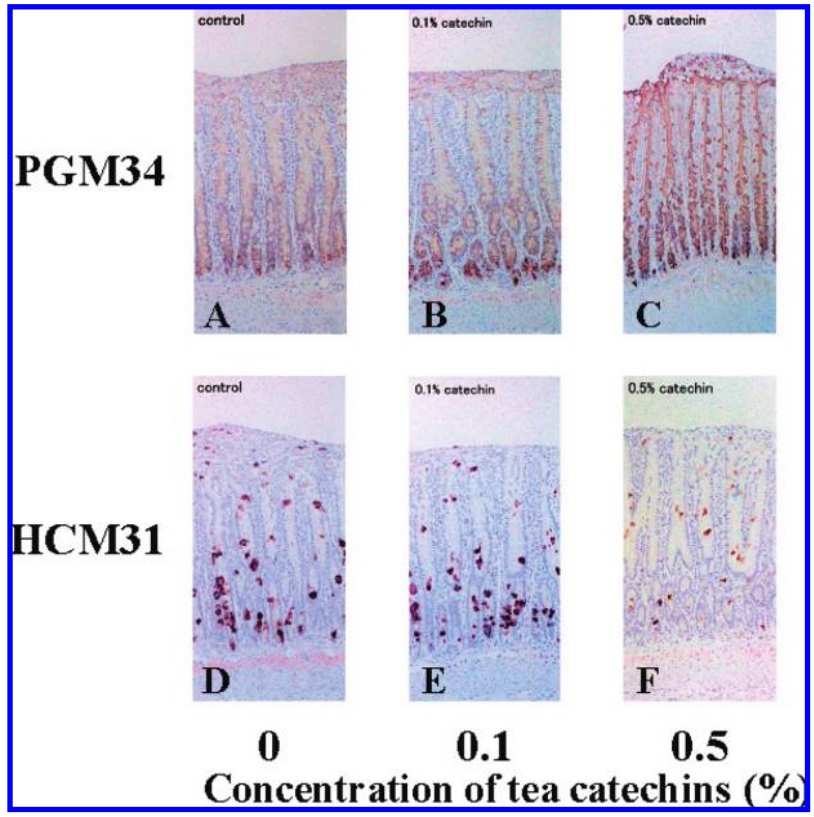

Figure 4. Immunostaining of the rat ileal mucosa with PGM34 (**A**−**C**) and HCM31 (**D**−**F**) anti-mucin monoclonal antibodies. Specimens were from control rats (**A**, **D**), rats administered 0.1% catechins (**B**, **E**), and rats administered 0.5% catechins (**C**, **F**). Note that sulfo- and sialomucins in the ileum show positive staining with PGM34 and HCM31, respectively. Original magnification ×60.

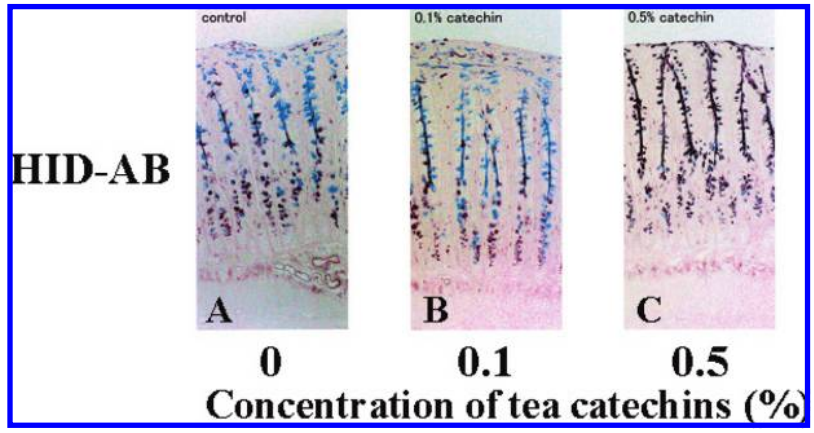

Figure 5. Microphotographs of ileal specimens from rats from each group stained with high-iron diamine-alcian blue (HID-AB). Specimens were from control rats (**A**), rats administered 0.1% catechins (**B**), and rats administered 0.5% catechins (**C**). Note that sulfo- and sialomucins are stained black and blue, respectively, in this image. Original magnification ×60.

The most striking finding of this study was that ileal mucus cell function was selectively affected by the administration of tea catechins. The PGM34 and HCM31 mAbs that we used in this study recognize certain carbohydrate structures attached to specific mucin molecules. Our previous study demonstrated that the epitopes of these mAbs involve the sulfuric acid residue and sialic acid residue, respectively (*13, 15*). Furthermore, a specific type of sialomucin that reacts with HCM31 plays an important role in the infection by and rejection of certain parasites (*13*). In this study, ELISA using these mAbs showed a decrease in HCM31 immunoreactivity and an increase in PGM34 reactivity, consistent with both the immunohistochemical findings and HID-AB staining patterns. Although further studies are needed to clarify the specific functions of each mucin, the opposing changes in the amount of PGM34- and HCM31-positive mucins may contribute to the physiological effects of tea catechins on the intestinal epithelial function. Meyer-Hoffert et al. (*25*) recently reported that Paneth cell-derived antibacterial peptides such as a-defensins are retained by the surface-overlaying mucus and thereby provide a combined physical and antibacterial barrier to prevent bacterial attachment and invasion. Changes in acidic mucin distribution (PGM34-and HCM31-reactive mucins) induced by tea catechins may be associated with electrostatic binding to positive charged defensins, resulting in different circumstances in the small intestine.

Our data show that the body weight of rats administered tea catechins tended to be lower than that of the control subjects, even though the average daily food intake did not differ between the groups during the study period. We have already reported that administration of catechins in the same manner caused a

decreased weight gain (*26*). Polyphenolic compounds are reported to inhibit the absorption of nutrients and minerals in the isolated rat ileum (*27*). A recent study documents that the uptake of certain minerals is significantly affected by the application of mucin to an in vitro cell culture system (*28*). On the basis of these findings, it would appear that alterations in the quantity and quality of mucin are confined to the ileal mucosa of rats administered tea catechins. Further studies focusing on the ileal epithelium will assist in further elucidation of the mechanism of catechin effects.

In summary, we present two important research findings regarding tea catechins. First, oral administration of green tea extract at in vivo doses caused no significant changes in gastric mucin in the rat stomach. Second, administration of tea catechins caused a remarkable increase in the rat ileal mucin content, particularly sulfomucin derived from goblet cells.

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