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Inhibition of Azoxymethane-induced Colon Carcinogenesis in Rat by Green Tea Polyphenol Fraction

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The effect of green tea polyphenol fraction (GTP) on azoxymethane(AOM)-induced colon carcinogenesis was investigated in male Fischer rats. The rats were given AOM (7.4 mg/kg body weight) s.c. once a week for 10 weeks. A week after the treatment, they were divided into three groups: AOM-control (26 rats), AOM-GTP1 (26 rats) and AOM-GTP2 (25 rats). AOM-GTP1 and AOM-GTP2 groups respectively received 0.01 and 0.1% GTP in drinking water from week 11 to 26. AOM-control group received tap water throughout this experiment. Autopsy on week 26 showed that tumor incidence and average numbers of tumors per rat in the AOM-GTP1 and AOM-GTP2 groups were significantly lower than those of the AOM-control group: 38.1% and 47.6% versus 77.3%; 0.6 and 0.7 versus 1.5. Thus, it was concluded that GTP inhibited the development of AOM-induced colon carcinogenesis. The inhibition by GTP did not show significant dose dependence.

Key words: Colon carcinogenesis — Green tea — Azoxymethane — Polyphenol — (−)-Epigallocatechin gallate

Green tea polyphenols were reported to have anti-mutagenic[1] and anti-tumor activities.[2] Recently, (−)-epigallocatechin gallate (EGCg), one of the main polyphenolic constituents, has been shown to be an anti-tumor promoter in two-stage carcinogenesis experiment on mouse skin.[3] Although the mechanisms underlying these activities remain to be investigated, the above findings on green tea are of much interest from the standpoint of cancer chemoprevention.

Previously, we reported that EGCg inhibited promotion stage of duodenal carcinogenesis induced by *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine,[4] suggesting its preventive effect on carcinogenesis in the alimentary tract. In addition, green tea polyphenols were found *in vitro* to inhibit the growth of intestinal clostridia,[5] which are considered to participate in the biotransformation of a variety of ingested or endogenously formed compounds to carcinogenic products such as N-nitroso compounds and aromatic steroids. The evidence prompted us to study the influence of GTP on colon carcinogenesis. This paper deals with the inhibitory effect of GTP on colon carcinogenesis induced by AOM in rat.

This study was carried out according to the experimental protocol in Fig. 1. Eight-week-old male Fischer rats (Japan SLC Inc., Shizuoka) were given free access to drinking water and standard laboratory chow MF (Oriental Yeast Co., Tokyo). AOM (Sigma Chemical Co., St. Louis, MO), kept at −80°C before use, was dissolved in 0.9% NaCl solution, and injected s.c. at a dosage of 7.4 mg/kg body weight once a week for the first 10 weeks. A week after the last AOM treatment, the treated rats were divided into three groups: AOM-control (26 rats), AOM-GTP1 (26 rats) and AOM-GTP2 (25 rats). The AOM-control group received tap water throughout the experiment. The AOM-GTP1 and AOM-GTP2 groups respectively received 0.01 and 0.1% GTP dissolved in tap water as drinking water from week 11 to 26. GTP used in this study was Sunphenon, a product of Taiyo Kagaku Co., Ltd. (Yokkaichi). It is composed mainly of polyphenolic compounds: (+)-catechin (3.5%), (−)-epicatechin (7.0%), (+)-gallocatechin (14.8%), (−)-epigallocatechin (15.0%), (−)-epicatechin gallate (4.6%), (−)-gallocatechin gallate (11.6%) and EGCg (18.0%). The residue of GTP includes caffeine, sugars, amino acids and moisture. In addition, three groups without the AOM treatment, i.e., control, GTP1 and GTP2 alone (each 10 rats), were prepared as the counterparts of the above AOM-treated groups. Rats in these groups were also injected s.c. for the first 10 weeks, but with 0.9% NaCl solution containing no AOM. All rats were killed on week 26. Their esophaguses, stomachs, small intestines and large intestines were removed, and dissected longitudinally. The location, shape, size and number of tumors were recorded.

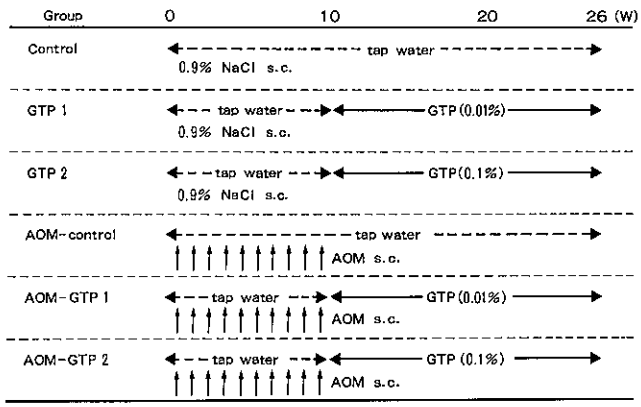

Fig. 1. Experimental schedule for the inhibitory test of green tea polyphenol fraction (GTP) against AOM-induced colon carcinogenesis. AOM was injected s.c. at a dosage of 7.4 mg/kg body weight once a week for the first 10 weeks. Rats received GTP in the drinking water from week 11. The experiment was terminated at week 26. AOM-control, AOM-treated rats without GTP; AOM-GTP1, AOM-treated rats with 0.01% GTP; AOM-GTP2, AOM-treated rats with 0.1% GTP; control, untreated rats (i.e., no AOM treatment) without GTP; GTP1, untreated rats with 0.01% GTP; GTP2, untreated rats with 0.1% GTP.

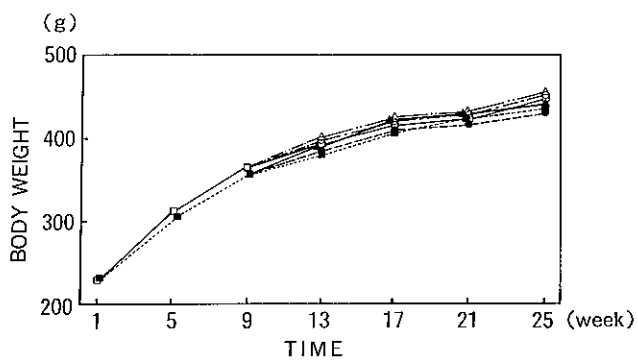

Fig. 2. Body weights during the study. ■, AOM-treated (till week 10) and AOM-control (week 11 to 26); ●, AOM-GTP; ▲, AOM-GTP2; □, untreated (till week 10) and control (week 11 to 26); ○, GTP1; △, GTP2. See the legend to Fig. 1 and the text.

All the tumors were then fixed with 10% formalin for histological examination.

The significance of differences in tumor incidence was analyzed by using the chi-square test, and the remaining data were analyzed by using Student's *t* test.

During the experiment, body weights of rats treated with AOM were generally lower than those without the AOM treatment. However, the reduction of body weight among the AOM-treated groups was much lower in the AOM-GTP2. Statistically significant differences of body weight were observed between the following groups: AOM-treated and untreated on week 9 ($P<0.005$), and AOM-control and AOM-GTP2 on weeks 13, 17 and 21 ($P<0.05$, $P<0.005$ and $P<0.05$, respectively). At autopsy on week 26, the body weights among the six groups were not significantly different (Fig. 2).

Table I. Inhibitory Effects of Green Tea Polyphenol Fraction on Colon Carcinogenesis Induced by AOM

Treatment groups[a]	Tumor incidence ratio[b]	Total number of tumors	Average number of tumors per rat[c]	Diameter of tumors (mm)[d]
AOM-control	17/22 (77.3)	33	1.5±0.2	5.6±3.4
AOM-GTP1	8/21[e] (38.1)	13	0.6±0.2[g]	5.4±2.8
AOM-GTP2	10/21[f] (47.6)	14	0.7±0.2[g]	6.4±3.0

a) See the legend to Fig. 1.
b) Numbers in parentheses are percent tumor-bearing rats.
c) Mean±SE.
d) Mean±SD.
e, f, g) Significantly different from the AOM-control group at $P<0.005$, $P<0.05$ and $P<0.01$, respectively.

The inhibitory effect of GTP is summarized in Table I. Tumor-bearing rats in the AOM-control group amounted to 77.3%. On the other hand, those in the AOM-GTP1 and AOM-GTP2 groups amounted to 38.1% ($P<0.005$) and 47.1% ($P<0.05$), respectively. The two groups, AOM-GTP1 and AOM-GTP2, did not show a significant difference despite the difference in dose. GTP may have an optimum dose to inhibit colon carcinogenesis. Rats without the AOM treatment (i.e., control, GTP1 and GTP2) had no colon tumor. Average number of tumors per rat in the AOM-control group was 1.45, whereas those in the AOM-GTP1 and AOM-GTP2 groups were 0.62 ($P<0.01$) and 0.67 ($P<0.01$), respectively. Mean tumor diameters among the three AOM-treated groups were not significantly different. The localizations of colon tumors were defined by measuring the distances from the anus (mean±SD). They were 10.5±5.5 cm for AOM-control, 7.1±3.9 cm for AOM-GTP1 and 10.4±5.4 cm for AOM-GTP2. The means were not significantly different. Histologically, the colon tumors consisted of atypical glands, and basophilically stained cells with a large number of mitotic bodies were often observed. The nuclei were large, and were located at different sites in the cytoplasm. Tumor cells often invaded the submucosal space, proper muscle layer and subserosal space (Fig. 3).

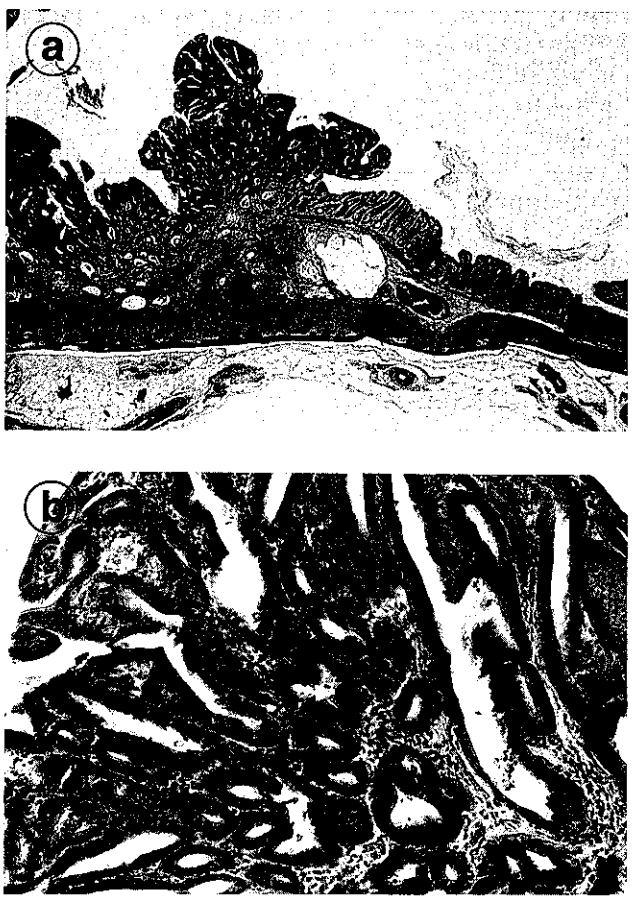

Fig. 3. Histology of colon tumors in Fischer rats treated with AOM. a: Tumors consisted of atypical glands, often seen invading the submucosal space. H-E, ×100. b: Atypical glands were composed of basophilic large cells with a large number of mitotic bodies. The nuclei were large and hyperchromatic. H-E, ×400.

As mentioned above, EGCg was shown to be an antitumor promoter in a two-step carcinogenesis experiment on mouse skin[3] and in an *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine-induced mouse duodenal carcinogenesis experiment.[4] In the present study, GTP was administered to the AOM-treated rats one week after the last AOM treatment. Therefore, it might have inhibited the promotion stage of the AOM-induced colon carcinogenesis. The reduction of tumor incidence and average number of tumors per rat in this experiment was consistent with those found in the above experiments. GTP contained 74.5% polyphenolic compounds, including EGCg. These polyphenols are closely similar in their structure and biological activity. Although the inhibition of AOM-induced colon carcinogenesis could be due to components other than the polyphenols, from circumstantial evidence,[3,4] it is reasonable to conclude that the polyphenols exerted the antitumor promoting activity against colon carcinogenesis. The effects of oral administration of GTP on colon mucosal ornithine decarboxylase activity and other biomarkers of colon carcinogenesis are under investigation.

It is widely accepted that changes in intestinal microflora may be related to colon carcinogenesis. Fecal profiles of intestinal microflora in patients with colon carcinoma and patients with nonhereditary large bowel polyps are clinically different.[6] Recently, it was suggested that tumor growth or malignant transformation was related to the increase of *Clostridium* spp. except *C. perfringens*.[7] Since green tea polyphenols have selective inhibitory activity against the growth of intestinal clostridia *in vitro*,[5] it is tempting to suggest that GTP inhibits AOM-induced colon carcinogenesis at least partially through its inhibitory effect on the intestinal microflora.

In this experiment, GTP was administered to rats in the drinking water at concentrations of 0.01 and 0.1%. In terms of mg/kg body weight, the daily GTP intakes by these rats were comparable to those taken by average Japanese people. A recent epidemiological study showed a lower risk of gastric cancer among those with a high consumption of green tea (10 or more cups per day).[8] These findings, coupled with those previously reported,[4] led to the suggestion that green tea polyphenols are useful in preventing carcinogenesis in the alimentary tract.

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