

Inhibitory Effect of (–)-Epigallocatechin Gallate on Carcinogenesis with N-Ethyl-N'-nitro-N-nitrosoguanidine in Mouse Duodenum

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(–)-Epigallocatechin gallate (EGCG) is the main polyphenolic constituent of green tea infusion and inhibits tumor promotion by teleocidin in two-stage carcinogenesis on mouse skin. In this work, EGCG was found to inhibit tumor promotion in the gastrointestinal tract in a model system of mouse duodenal carcinogenesis with N-ethyl-N'-nitro-N-nitrosoguanidine. The duodenal tumors that developed were studied stereomicroscopically and histologically.

Key words: (–)-Epigallocatechin gallate — Anti-tumor promotion — Mouse duodenal carcinogenesis — N-Ethyl-N'-nitro-N-nitrosoguanidine

(–)-Epigallocatechin gallate (EGCG) is the main polyphenolic constituent of green tea infusion (Fig. 1). Recently, we reported that EGCG inhibited tumor promotion by teleocidin in a two-stage carcinogenesis experiment on mouse skin.¹⁾ Although the mechanism of the inhibition was not fully elucidated, EGCG dose-dependently inhibited the activation of protein kinase C.¹⁾

Thus, to examine further whether EGCG actually inhibits tumor promotion in the gastro-intestinal tract, we tested EGCG in a model system of mouse duodenal carcinogenesis with N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) which was developed by Matsuyama *et al.* in 1975.²⁾ This model system has several advantages, i.e. tumors are induced in a shorter time in duodenum of C57BL/6 mice than in other parts of the digestive tract, and the tumors are histologically adenocarcinomas.

The experiment was carried out twice according to the procedure in Fig. 2. Male C57BL/6 mice at 8 weeks of age were purchased from Shimizu Experimental Materials Co. Ltd., Kyoto. ENNG (Sigma Chemical Co., St. Louis, MO) was given orally as a solution at a concentration of 100 mg/liter for the first four weeks. The mice treated with ENNG were divided into two groups one week after ENNG treatment. The experimental group was orally given a solution of 0.005% EGCG (15 mice) and the control group was given tap water (16 mice). This EGCG, which was isolated from Japanese green tea leaves, contained EGCG (85%), (–)-epicatechin (10%) and (–)-epicatechin gallate (5%) as reported previously.¹⁾ All mice were sacrificed in the 16th week

after ENNG treatment. The esophagus, stomach, duodenum and jejunum were removed together. This upper digestive tract was ligated at the esophageal end, then 10% formalin solution was poured into the jejunal end and the sample was fixed for about 10 min. The duodenum was opened longitudinally and stretched out on a cork mat. The duodenal mucosa was examined stereomicroscopically up to 4 cm distal from the pyloric ring, then embedded and sectioned serially for histological examination. Tumors in the duodenum were seen as an enlarged villus or fusion of several villi, in which atypical glands were noted histologically as reported previously.²⁾ The atypical glands consisted of basophilically stained cells with a large number of mitotic figures. The nuclei were large and located at different levels in the cytoplasm (Figs. 3 and 4).

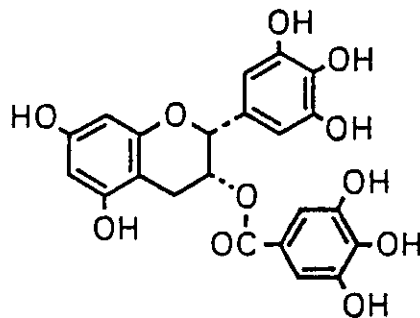


Fig. 1. Structure of (–)-epigallocatechin gallate, EGCG.

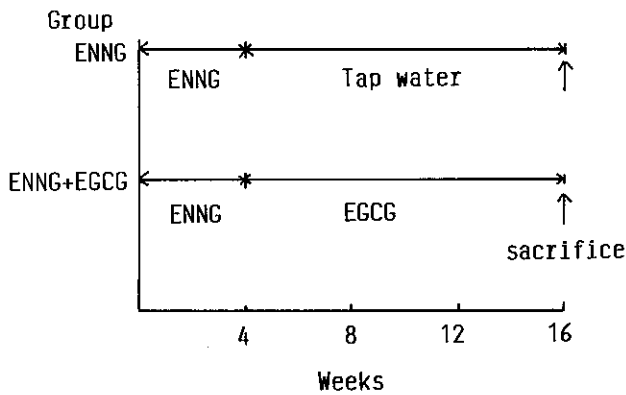
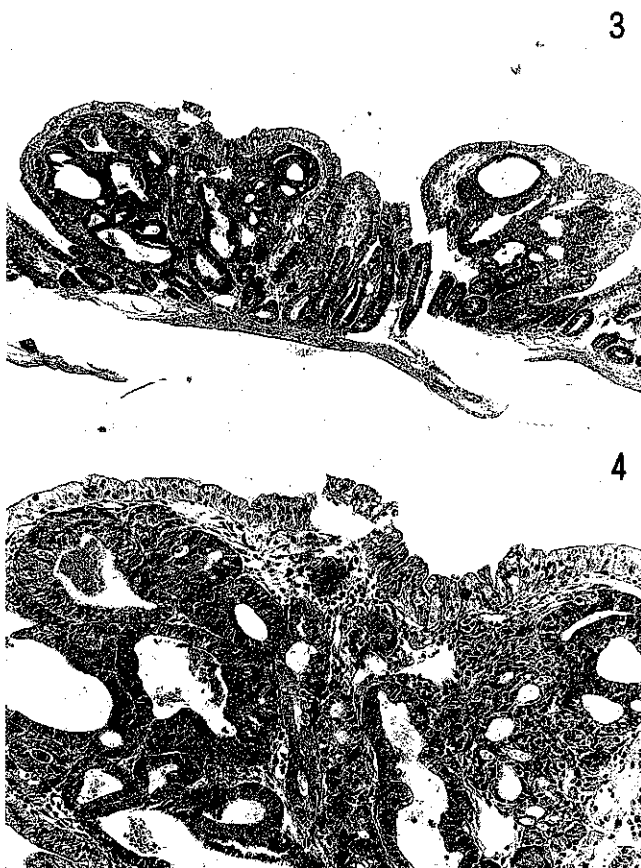


Fig. 2. Experimental schedule. ENNG, 100 mg/liter po; EGCG, 0.005% po.



Figs. 3 and 4. Micrographs of a duodenal lesion in C57BL/6 mouse given ENNG (100 mg/liter) for 16 weeks. Fig. 3: The fused villi are occupied by atypical glands. H-E, $\times 100$. Fig. 4: The atypical glands revealed basophilic cells with a large number of mitotic figures. The nuclei were large and located at different levels in the cytoplasm. H-E, $\times 400$.

The inhibitory effects of EGCG were examined in terms of tumor incidence (percent of tumor-bearing mice), total number of tumors and average number of tumors per mouse (Table I). The percentage of tumor-bearing mice of the group treated with ENNG plus EGCG was 20% at week 16 after ENNG treatment, while that of the control group was 63%, in both Exps. 1 and 2 ($P < 0.001$). Average number of tumors per mouse in the group treated with ENNG plus EGCG was 0.3 in Exps. 1 and 2, while that in the control group was 1.2 in Exp. 1 and 0.8 in Exp. 2. These results strongly suggested that EGCG inhibited carcinogenesis with ENNG in the duodenum.

EGCG is known to have anti-mutagenic activity against benzo[*a*]pyrene diol epoxide in *Salmonella typhimurium* TA 98 and TA 100³⁾ and also anti-tumor promoting activity on mouse skin treated with 7,12-dimethylbenz[*a*]anthracene plus teleocidin.¹⁾ Furthermore, naturally occurring dietary plant phenols including tannic acid were evaluated as possible anticarcinogens in an initiation and promotion protocol and a complete skin tumorigenesis protocol.⁴⁾ In our experiments, EGCG was given in the drinking water one week after ENNG treatment. Therefore, EGCG might inhibit the development of carcinogenesis, the so-called promotion stage of duodenal carcinogenesis with ENNG. The reductions of tumor incidence and of average number of tumors per mouse which were observed in these two experiments were consistent with those found in the experiment on anti-tumor promotion in mouse skin with EGCG.¹⁾

Table I. Inhibitory Effects of EGCG on Duodenal Carcinogenesis with ENNG

	ENNG+EGCG	ENNG
Experiment 1		
Tumor incidence (percent of tumor-bearing mice)	3/15 (20%)*	10/16 (63%)*
Total number of tumors	5	19
Average number of tumors per mouse (mean \pm SE)	0.3 \pm 0.2	1.2 \pm 0.4
Experiment 2		
Tumor incidence (percent of tumor-bearing mice)	3/15 (20%)*	10/16 (63%)*
Total number of tumors	4	12
Average number of tumors per mouse (mean \pm SE)	0.3 \pm 0.2	0.8 \pm 0.2

* $P < 0.001$

The amount of EGCG which was taken in the drinking water (0.005% EGCG solution) roughly corresponded to 0.15 mg/mouse/day. Japanese tea-lovers may take as much as about 1 g of EGCG per day in green tea. Therefore, the experimental dose of EGCG is relevant to the human situation. In addition to EGCG, green tea contains several similar compounds, which might also have EGCG-like actions. Therefore, we can assume that green tea *per se* has an inhibitory effect on duodenal carcinogenesis with ENNG. Since duodenal carcino-

genesis is rare in humans, it will be necessary to study the inhibitory effects on stomach or colon carcinogenesis. The results will provide us with important information about whether green tea is useful to humans for the purpose of cancer chemoprevention.

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