Eur J Cancer Clin Oncol, Vol. 21, No. 6, pp. 741-744, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

Gastrin has no Promoting Effect on Chemically Induced Colonic Tumors in Wistar Rats

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Abstract—*The effects of prolonged administration of tetragastrin from the beginning of intrarectal instillation of* 1 ml *of* 0.25% N-*methyl*-N'-*nitro*-N-*nitrosoguanidine (MNNG) and after MNNG-treatment on the incidence and histology of colonic tumors were compared in inbred Wistar rats. In week* 35 *prolonged administration of testragastrin in depot form from the beginning of MNNG-treatment resulted in a significant reduction in the incidence of colonic tumors and a significant increase in the incidence of mucinous adenocarcinoma, unlike the well-differentiated adenocarcinoma produced in controls without gastrin. In contrast, prolonged administration of tetragastrin after MNNG-treatment had little or no influence on the incidence, size or histology of colonic tumors. Thus tetragastrin had no promoting effect on colonic tumors.*

INTRODUCTION

GASTRIN exerts a trophic influence on the cells of the oxyntic gland mucosa, duodenal and colonic mucosa and pancreas [1]. Svet-Moldavsky [2], Mukarami and Masui [3] and McGregor *et al.* [4] reported that pentagastrin stimulated the growth of transplanted colonic adenocarcinomas in mice, cultured human colon carcinoma cells and experimentally induced colonic tumors, respectively. These reports suggested that gastrin has a promoting effect on colonic tumors. In contrast, we found that prolonged administration of tetragastrin in depot form to rats from the beginning of intrarectal instillation of MNNG reduced the incidence of colonic tumors [5]. These results suggested that the trophic action of tetragastrin was related to suppression of development of colonic tumors. Therefore, in the present work we compared the effects on the incidences of colonic tumors of tetragastrin injected in depot form into rats from the beginning of intrarectal instillation of MNNG and after MNNG treatment.

Accepted 23 November 1984.
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MATERIALS AND METHODS

In all, 60 young male inbred Wistar rats of about the same age (6 weeks old), initially weighing 100-150 g, were given 1 ml of 0.25% MNNG intrarectally every day for 25 days [6]. A fresh solution of MNNG (Aldrich Chemical Co., Milwaukee, WI) was prepared each week in deionized water and stored in a cool, dark place to avoid denaturation of MNNG by light. Unanesthetized rats were immobilized in a plastic cylinder with an opening at its end to allow intrarectal instillation of the carcinogenic solution. An 8-cm-long vinyl tube connected to a syringe was inserted into the lumen of the large intestine through the anal orifice and the MNNG solution was injected [5]. Groups of rats were treated as follows: group 1 (15 rats) received no further treatment; group 2 (15 rats) were given the vehicle, olive oil, only from the beginning of the experiment; group 3 (15 rats) were given 250 μg/kg body wt of tetragastrin per day in depot form from day 26, after MNNG-treatment; group 4 (15 rats) were given 250 μg/kg body wt of tetragastrin per day in depot form from the beginning of the experiment.

Tetragastrin (Nissui Pharmaceutical Co., Tokyo, Japan) was given as a suspension in olive oil. The rats in groups 3 and 4 were given one injection per day of tetragastrin for the first 25

days, and then one injection every other day until the end of the experiment in week 35. Injections were given subcutaneously in a volume of 2 ml/kg body wt, between 2 and 3 p.m. each day, various sites of injection being chosen. The rats in group 2 received 2 ml/kg body wt of olive oil only per day from day 1 to 50, and then one injection every other day until the end of the experiment.

The experimental groups were kept in different cages in the same room under otherwise identical conditions throughout the experiment, and tap water and a diet of rat chow pellets (Oriental Yeast Co., Tokyo, Japan) were provided *ad libitum*.

Rats that survived for more than 25 experimental weeks were included in effective numbers. Animals were killed when they became moribund and surviving rats were killed at the end of week 35. Rats that died or were killed during the experimental period were autopsied and the large intestine and other organs were examined carefully. The large intestine was opened, pinned flat on a cork mat and fixed with 10% neutralized formalin solution for histological examination. Paraffin sections of 5 μm thickness taken perpendicular to the surface of visible tumors and suspicious lesions were prepared, and stained with hematoxylin and eosin. Serial sections were made whenever necessary to expose the central part of the tumors and the stalk, when present. In addition to tumors, flat mucosa of the fixed colon with no visible tumors was cut into 3-mm-wide, 4-cm-long strips and serial sections were examined for microscopic tumor foci. Sections were examined without knowledge of their origin. As previously reported, colonic tumors were classified histologically into two types on the basis of their cellular and structural atypism: adenomas and adenocarcinomas [5].

The results were analyzed by the *t*-test [7] chi-square test [8] or Fisher's exact probability test [8]. The word 'significant' indicates a calculated *P* value of less than 0.05.

RESULTS

Incidences and numbers of colonic tumors

Table 1 summarizes the incidences and numbers of colonic tumors in each group. In groups 1-4, three, two, five and two rats, respectively, died before week 25, either of respiratory infection or of wasting. No tumors were found in any of these animals, which were excluded from effective numbers.

All rats in groups 1 (no treatment) and 2 (olive oil) had colonic tumors. In group 3 (tetragastrin after MNNG), 9/10 rats (90.0%) had tumors, but the difference from group 1 was not statistically significant. The incidence of rats with colonic tumor in group 4 (tetragastrin from the beginning of the experiment) was significantly less than that in group 1.

There were average numbers of 4.9 ± 0.8 and 4.2 ± 0.3 of colonic tumors per animal in control groups 1 and 2. In group 3 (tetragastrin after MNNG) there were slightly fewer tumors, but the difference from group 1 was not statistically significant. In group 4 (tetragastrin from the beginning of the experiment) there were significantly fewer tumors.

Size, depth and metastases of colonic tumors

Table 2 summarizes the sizes, depths and metastases of colonic tumors in each group. In control groups 1 and 2 (olive oil) the average sizes of colonic tumors were 5.1 ± 0.5 and 5.0 ± 0.4 mm, respectively. In groups 3 (tetragastrin after MNNG) and 4 (tetragastrin from the beginning of the experiment) the average sizes of the tumors were 3.8 ± 0.8 and 6.0 ± 1.5 mm, respectively, but

Table 1. Incidences and numbers of colonic tumors in MNNG-treated rats

Group No.	Treatment*	Effective No. of rats	No. of rats with colonic tumor (%)	No. of colonic tumors per rat
1	MNNG alone	12	12 (100)	4.9 ± 0.8
2	MNNG + olive oil	13	13 (100)	4.2 ± 0.3
3	MNNG + tetragastrin from day 26	10	9 (90.0)†	4.1 ± 1.0†
4	MNNG + tetragastrin from day 0	13	6 (46.2)‡	1.2 ± 0.6§

*MNNG alone: 1 ml of 0.25% MNNG was given intrarectally for 25 days; MNNG + olive oil: 2 ml/kg of olive oil was administered during and after intrarectal instillation of MNNG for 25 days; MNNG + tetragastrin from day 26: 250 μg/kg of tetragastrin was administered after intrarectal instillation of MNNG for 25 days; MNNG + tetragastrin from day 0: 250 μg/kg of tetragastrin was administered during and after intrarectal instillation of MNNG for 25 days.

†Differences between values for groups 1 and 3 and groups 2 and 3 were not statistically significant.

‡Difference between values for groups 1 and 4 was statistically significant ($P < 0.005$).

§Difference between values for groups 1 and 4 was statistically significant ($P < 0.005$).

Table 2. Sizes, depths and metastases of colonic tumors in MNNG-treated rats

Group No.	Treatment†	Effective No. of rats	No. of colonic tumor	Size of colonic tumor (mm)	No. of invasive tumors (%)	No. of rats with metastases (%)
1	MNNG alone	12	59	5.1 ± 0.5	40 (67.8)	3 (25.0)
2	MNNG + olive oil	13	55	5.0 ± 0.4	25 (45.5)	0 (0)
3	MNNG + tetragastrin from day 26	10	41	3.8 ± 0.8†	23 (56.1)†	0 (0)
4	MNNG + tetragastrin from day 0	13	16	6.0 ± 1.5†	7 (47.3)†	0 (0)

*For explanation of treatments see Table 1.
†Differences between values for groups 1 and 3 and groups 1 and 4 were not statistically significant.

the differences from the size of those in group 1 were not statistically significant.

In control groups 1 and 2 the incidences of non-invasive colonic tumors, confined to the mucosal layer, were 67.8 and 62.4%, respectively. In groups 3 (tetragastrin after MNNG) and 4 (tetragastrin from the beginning of the experiment) there were fewer non-invasive tumors, but the differences from the numbers in group 1 were not significant.

Metastases to the mesenteric lymph node were found in two rats, and metastases to the liver in one rat in control group 1, but none in groups 2–4. Thus there were no significant differences in the incidences in different groups.

Histological types of colonic tumors and adenocarcinomas

The distribution of histological types of colonic tumors and adenocarcinomas in each group is summarized in Table 3.

Histologically, the colonic tumors induced in rats were chiefly adenocarcinomas. Adenocarcinomas induced in control group 1 and in group 2 (olive oil) were all well-differentiated: the neoplastic cells occurred in acinar clusters, simulating glandular crypts of normal colon mucosa. A mucinous adenocarcinoma was found in group 3 (tetragastrin after MNNG), but the difference in incidences in groups 3 and 1 was not significant. In group 4 (tetragastrin from the beginning of the experiment) mucinous adenocarcinomas were significantly more frequent than in group 1.

DISCUSSION

The antral hormone gastrin has recently been shown to exert a trophic effect on the colon mucosa [1]. It seems reasonable to suspect that if gastrin has a trophic effect on the colon mucosa, it may exert a similar effect on tumors of the mucosa [4]. In fact, Svet-Moldavsky [2] reported the dependence of colon cancer on gastrin. He administered 250 or 600 μg/kg body wt of pentagastrin several times to mice with transplantable hepatoma, sarcoma, colon adenocarcinoma or adenocarcinoma of the small intestine. These injections of gastrin stimulated the growth of colon adenocarcinomas, but not of the other tumors. He concluded that this hormone-dependence could be used in treatment and prophylaxis of some tumors of the gastrointestinal tract. Murakami and Masui [3] reported similar results. A human colon carcinoma cell line has been established in culture from a tumor line transplantable in nude mice. Gastrin, like insulin, glucagon and epidermal growth factor,

Table 3. Histological types of colonic tumors and adenocarcinomas in MNNG-treated rats

Group No.	Treatment*	No. of colonic tumors	Adenoma (%)	Adenocarcinoma Total	Adenocarcinoma Well-differentiated type (%)	Adenocarcinoma Mucinous type (%)
1	MNNG alone	59	6 (10.2)	53	53 (100)	0 (0.0)
2	MNNG + olive oil	55	9 (16.4)	46	46 (100)	0 (0.0)
3	MNNG + tetragastrin from day 26	41	10 (24.4)	31	30 (96.8)	1 (3.2)†
4	MNNG + tetragastrin from day 0	16	1 (6.3)	15	12 (80.0)	3 (20.0)‡

*For explanation of treatments see Table 1.
†Difference between the incidences of mucinous adenocarcinomas in groups 1 and 3 was not statistically significant.
‡Difference between the incidences of mucinous adenocarcinomas in groups 1 and 4 was statistically significant ($P < 0.01$).

was found to stimulate growth of these cells in a serum-free medium. But the dependence of gastrin upon spontaneous carcinogenesis of the colon is not clear. In 1982 McGregor *et al.* [4] examined the effect of an endogenous hypergastrinemic state induced by antral exclusion and short-term administration of exogenous pentagastrin on colon carcinogenesis induced by methylazoxymethanol. They found that both chronic endogenous hypergastrinemia and short-term injections of exogenous pentagastrin significantly increased the syntheses and concentrations of DNA, RNA and protein in colonic tumors. But the number of tumors per rat, and the size and distribution of tumors within the colon did not differ from those in sham-operated rats. In 1982, Oscarson *et al.* [9] tested the potential trophic effect of gastrin in promoting carcinogenesis in rats with 6- to 16-fold variation in endogenous gastrin resulting from antrectomy or fundectomy, followed by injection of 1,2-dimethylhydrazine. The incidence and grade of differentiation of the resulting tumors, as well as the thickness, content of nucleic acids and specific activity of DNA in the colonic mucosa, were similar in the high-gastrin and low-gastrin animals and were not different from those in unoperated control animals. They concluded that endogenous gastrin at a high concentration does not act as a cofactor in dimethylhydrazine-induced carcinogenesis, and thus that endogenous gastrin is not a physiological trophic hormone for the large bowel [9, 10], although intraperitoneal injections and intraluminal infusions of gastrin have a trophic effect on the large bowel mucosa [1, 11]. Trophic effects distal to the duodenum produced by exogenous gastrin may result from a supranormal level [10]. In the present work we found that prolonged administration of tetragastrin at 250 μg/kg body wt after MNNG-treatment had little or no influence on the incidence, size or histology of colonic tumors. The doses of tetragastrin that we used probably produced serum levels of more than one order of magnitude above physiological levels. Therefore we conclude that exogenous tetragastrin, like endogenous gastrin, has no promoting effect on colonic tumors.

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