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Dose-dependent Promoting Effects of Catechol on Glandular Stomach Carcinogenesis in BALB/c Mice Initiated with *N*-Methyl-*N*-nitrosourea

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The effects of catechol administration in the diet on stomach carcinogenesis in mice after initiation with *N*-methyl-*N*-nitrosourea (MNU) in the drinking water were investigated in a development trial for a new experimental protocol. Male 6-week-old BALB/c mice were given MNU in the drinking water intermittently for a total of three one-week periods, with one-week intervals, at the concentration of 120 ppm (groups 1 and 2). Groups 3 and 4 served as non initiated controls. From week 7, groups 1 and 3 were divided into three subgroups and the mice were fed on diet containing 0.05% (groups 1a and 3a), 0.2% (groups 1b and 3b), 0.8% (groups 1c and 3c) or 0% (groups 2 and 4) catechol for 29 weeks. At week 20, appreciably enhanced development of pepsinogen 1-altered pyloric glands was noted in all catechol-treated groups, in a partially dose-dependent manner (12.8±12.5, 13.8±11.7, and 24.0±12.7/100 pyloric glands respectively, for groups 1, 2 and 3). The incidences of adenomas (groups 1, 2 and 3) were also increased. At week 35, dose-dependent induction of adenocarcinomas in groups 1 (3/19), 2 (3/19) and 3 (14/20) was evident. In addition, the depth of invasion of the adenocarcinomas was enhanced by catechol in a dose-dependent manner, though the histological type was not influenced. Thus, the administration of catechol in the diet strongly enhanced the preneoplastic and neoplastic lesions in mouse glandular stomach induced by MNU in the drinking water, in a dose-dependent manner.

Key words: Catechol — *N*-methyl-*N*-nitrosourea — Pepsinogen 1-altered pyloric gland — Stomach cancer — Mouse

It has been demonstrated that catechol, an important industrial chemical present in a wide variety of materials in our environment,[1-3] strongly enhances cancer development in the glandular stomach of rats initiated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG),[4,5] and increases the development of pepsinogen isozyme 1-altered pyloric glands (PAPG),[6] which are putative preneoplastic lesions.[7-11] However, it has not been clarified whether this effect is species-specific, and a recent long-term *in vivo* study demonstrated that catechol itself can induce adenocarcinoma in rats at high incidence, whereas it only caused adenomas in mice.[12,13]

The glandular stomach of the mouse is generally resistant to carcinogenic action.[14] Several attempts have been made to establish a mouse experimental model of glandular stomach carcinogenesis, but while intragastric instillation of 4-nitroquinoline 1-oxide[15] or 4-hydroxyaminoquinoline 1-oxide[16] has been reported to induce gastric adenocarcinomas, the incidences were only low. Recently, however, Tatematsu *et al.*[17] documented that *N*-methyl-*N*-nitrosourea (MNU) treatment in the drinking water results in a good yield of adenocarcinomas in mice without the induction of squamous cell carcinomas in the forestomach, which caused early animal death in a previously reported intragastric administration study.[18] In the present investigation, the effects of catechol on MNU-initiated glandular stomach carcinogenesis in mice were assessed using a two-stage model.

MATERIALS AND METHODS

Animals Male BALB/c mice (Charles River Japan, Inc., Atsugi), 6 weeks old, were housed in plastic cages with hard wood chips in an air-conditioned room with a 12 h light-12 h dark cycle.

Chemicals MNU (Sigma Chemical Co., St Louis, MO) was dissolved in distilled water and freshly prepared three times per week. Catechol (Wako Pure Chemical Industries, Osaka) was incorporated into Oriental MF basal diet (Oriental Yeast Co., Tokyo), using a cake mixer, at concentrations of 0.05, 0.2, and 0.8%.

Treatment The experimental protocol is shown in Fig. 1. One hundred and ninety animals were divided into four groups. MNU was given intermittently for a total of three one-week periods with one-week intervals in the drinking water *ad libitum* from light-shielded bottles at a concentration of 120 ppm (groups 1 and 2) or 0 ppm (groups 3 and 4). Subsequently, from one week after the last MNU treatment, groups 1 and 3 were divided into three subgroups and the mice were fed on diets contain-

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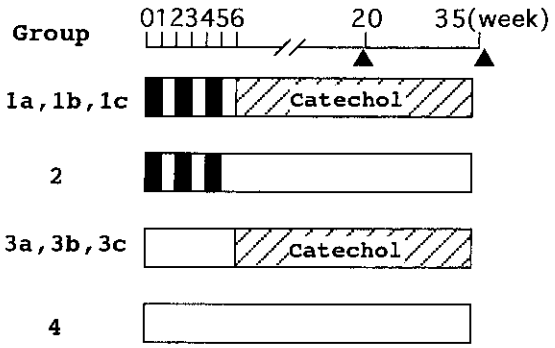

Fig. 1. Schematic representation of the experimental protocol. Closed bars, MNU 120 ppm; open bars, basal diet; hatched bars, basal diet containing 0.05 (groups 1a and 3a), 0.2 (groups 1b and 3b) or 0.8% (groups 1c and 3c) catechol; ▲, animals killed. See "Materials and Methods" for further details.

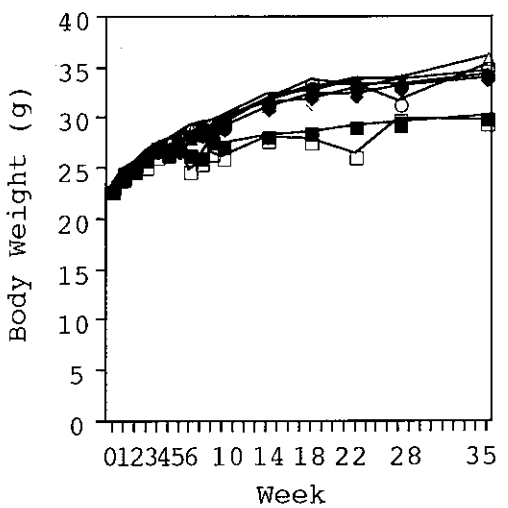

Fig. 2. Body weight curves of mice treated with MNU in the drinking water followed by catechol in the diet. Group: ▲1a, ◆1b, ■1c, ●2, △3a, ◇3b, □3c, ○4.

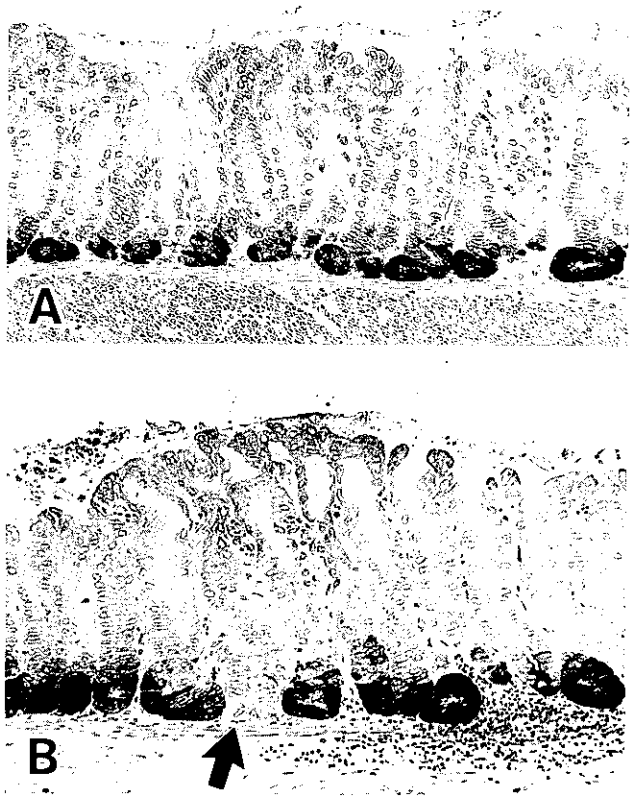

Fig. 3. Pepsinogen isozyme (Pg) 1 immunohistochemistry of pyloric mucosa in a BALB/c mouse in group 3 at week 20. A, Normal pyloric glands. B, Pg 1-altered pyloric glands (PAPG) consisting of cells with low Pg 1 content (arrow). ×200.

ing 0.05% (groups 1a and 3a), 0.2% (groups 1b and 3b) or 0.8% (groups 1c and 3c) catechol or basal diet (groups 3 and 4). The animals were weighed every week and necropsies of subgroups were performed at weeks 20 and 35.

Histopathological and immunohistochemical analyses The excised stomachs were fixed in sublimed formaldehyde and cut into about 6 strips, which were embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (HE) or by an immunohistochemical method for PAPG.[9-11] Neoplastic lesions of the glandular stomach were classified as adenomas and adenocarcinomas. Malignant lesions observed at week 35 in catechol-treated groups were further classified depending on their degree of differentiation (well or poorly differentiated) and the depth of invasion (m, mucosa; sm, submucosa; pm, proper muscle; ss, subserosa; se, serosa). The numbers of PAPG per 100 pyloric glands were also counted. The other organs were carefully checked macroscopically for neoplastic changes.

Statistical analysis The statistical significance of differences in body weights, incidences of lesions, and the numbers of PAPG were evaluated by the use of Fisher's exact probability test or Student's t test.

RESULTS

A statistically significant reduction of body weights was observed in groups 1c and 3c from weeks 7 to 35 ($P<0.05$ at each time point) (Fig. 2).

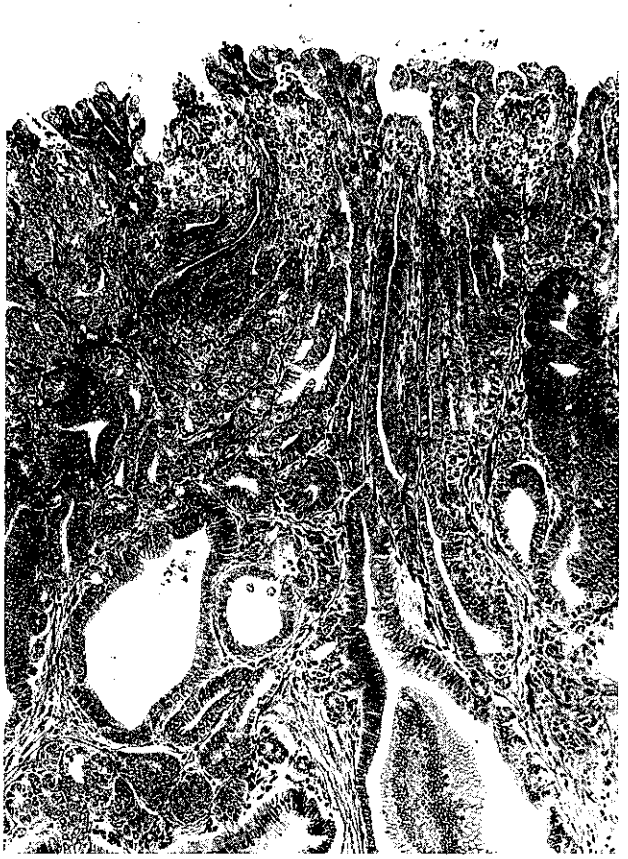

Fig. 4. Differentiated adenocarcinoma in group 3 at week 35. HE ×100.

Fig. 5. Undifferentiated adenocarcinoma in group 3 at week 35. HE ×100.

At week 20, adenomas were observed in 1 (group 1a), 1 (group 1b) and 2 (group 1c) of 10 mice and adenocarcinomas in 1 (group 1a) and 2 (group 1c) of 10 mice. Increased numbers of PAPG (Fig. 3) were evident in all catechol-treated groups as compared with the corresponding basal diet control group, in a partially dose-dependent manner (12.8±12.5, 13.8±11.7 and 24.0± 12.7 PAPGs/100 pyloric glands respectively, in groups 1a, 1b and 1c). The difference in numbers of PAPG between groups 1c and 2 was statistically significant ($P<0.05$).

At week 35, dose-dependent induction of adenocarcinomas (Figs. 4 and 5) was noted in groups 1a (3/19), 1b (3/19) and 1c (14/20). The incidence of adenocarcinomas in group 1c was significantly higher than those in groups 1a, 1b, and 2 ($P<0.001$ in each case). Adenomas were found in 6 of 19 (group 1a), 7 of 19 (group 1b), 4 of 20 (group 1c), 1 of 10 (group 3b) and 3 of 10 (group 3c) mice. Because of the development of neoplastic lesions in the glandular stomach of the mice at week 35, PAPG could not be evaluated at this time point. The histopathological findings and the data for the numbers of PAPG are summarized in Table I. The histological types of adenocarcinomas found at week 35 did not vary with catechol treatment (Table II), but the depth of invasion was clearly correlated with the catechol dose (Table III). No other neoplastic lesions were macroscopically apparent in any animals throughout the experiment.

DISCUSSION

The present study clearly demonstrated that dietary catechol strongly enhances the development of precancerous lesions and cancers in the glandular stomach of mice pretreated with MNU. The yield was dose-dependent and the tumors were found after a relatively short period, without other neoplastic changes to complicate the experimental system and influence the histopathological analysis. Moreover, catechol alone caused PAPG and adenomas even in animals not initiated with MNU.

Table I. Sequential Changes in Quantitative Data for Neoplastic Lesions and PAPG in the Glandular Stomach of Mice Treated with MNU Followed by Catechol

Groups	Treatment	Effective no. of mice	Adenoma (%)	Adenocarcinoma (%)	PAPG (no./100 pyloric glands)
20 weeks					
1a	MNU→Catechol (0.05%)	10	1 (10)	1 (10)	12.8±12.5
1b	MNU→Catechol (0.2%)	10	1 (10)	0 (0)	13.8±11.7
1c	MNU→Catechol (0.8%)	10	2 (20)	2 (20)	24.0±12.7[a]
2	MNU→Basal diet	10	0 (0)	0 (0)	5.3±3.0
3a	Catechol (0.05%)	10	0 (0)	0 (0)	4.9±4.3
3b	Catechol (0.2%)	10	0 (0)	0 (0)	9.0±9.1
3c	Catechol (0.8%)	10	0 (0)	0 (0)	9.0±4.9
4	Basal diet	5	0 (0)	0 (0)	2.2±1.2
35 weeks					
1a	MNU→Catechol (0.05%)	19	6 (32)[a]	3 (16)	N[f]
1b	MNU→Catechol (0.2%)	19	7 (37)[b]	3 (16)	N
1c	MNU→Catechol (0.8%)	20	4 (20)[a]	14 (70)[c, d, e]	N
2	MNU→Basal diet	20	0 (0)	0 (0)	N
3a	Catechol (0.05%)	10	0 (0)	0 (0)	N
3b	Catechol (0.2%)	10	1 (10)	0 (0)	N
3c	Catechol (0.8%)	9	3 (33)	0 (0)	N
4	Basal diet	5	0 (0)	0 (0)	N

a) Significantly different from group 4 at $P<0.05$.
b) Significantly different from group 4 at $P<0.01$.
c) Significantly different from group 4 at $P<0.001$.
d) Significantly different from group 1 at $P<0.001$.
e) Significantly different from group 2 at $P<0.001$.
f) Not examined.

Table II. Histopathological Classification of Adenocarcinomas Observed at Week 35

Group	Treatment	Total no. of lesions	Well differentiated	Poorly differentiated
1a	MNU→Catechol (0.05%)	3	2	1
1b	MNU→Catechol (0.2%)	3	2	1
1c	MNU→Catechol (0.8%)	16	12	4
2	MNU→Basal diet	0	0	0

Table III. Depth of Invasion of Adenocarcinomas Observed at Week 35

Group	Treatment	Total no. of lesions	Depth of invasion				
			m	sm	pm	ss	se
1a	MNU→Catechol (0.05%)	3	2	1	0	0	0
1b	MNU→Catechol (0.2%)	3	2	1	0	2	0
1c	MNU→Catechol (0.8%)	16	11	4	2	8	1
2	MNU→Basal diet	0	0	0	0	0	0

These results are in line with earlier reports[4, 6, 19] describing the induction of PAPG in rats and strong promoting effects on second-stage rat glandular stomach carcinogenesis after initiation with MNNG. In the present study, the depth of invasion of adenocarcinomas was also enhanced by catechol with clear dose-dependency, while the degree of differentiation was not affected.

Catechol is not only an industrial chemical but is also present in certain foods, such as onions, crude beet sugar, coffee and smoked fish, as well as in cigarette smoke.[1-3] Estimated on the basis of urinary excretion data,[20] the dose levels of catechol applied in the present study are about 100–1000 times the probable level of human exposure. However, since mixtures of low-dose carcinogens or promoters may exert combined effects,[21, 22] possible additive or synergistic action of catechol with other carcinogens or promoters can not be ignored. Dietary catechol has been shown to act as a cocarcinogen for *N*-methyl-*N*-amylnitrosamine-induced rat stomach carcinogenesis.[23]

A second important finding of the present investigation was the clear catechol dose-related induction of PAPG at week 20. This preneoplastic lesion has been extensively characterized in rat glandular stomach,[7-11] and has been described in the pyloric glands of FVB/N and MT100

mice given MNU by intragastric intubation.[24] The time difference in dose-related induction of PAPG and adenocarcinomas in the present study implies that PAPG may be a useful endpoint lesion, as suggested recently by Yamamoto *et al.*[25]

We previously reported that ten weekly intragastric MNU intubations of 0.5 mg/mouse induced adenocarcinomas in the glandular stomach at high incidence 20 to 40 weeks after treatment, but that squamous cell carcinomas in the forestomach also frequently occurred and many animals died as a result.[18] More recently, we found that MNU induced glandular stomach adenocarcinomas without the development of forestomach squamous cell carcinomas when given in the drinking water for 42 to 54 weeks.[17, 25] These experimental systems, however, still suffer from the disadvantage of high yields of hemangioendothelial sarcomas in the spleen.[17]

Human gastric cancers have been classified into differentiated (papillary and tubular adenocarcinomas) and undifferentiated (poorly differentiated adenocarcinomas and signet ring cell carcinoma) types.[26] Thus, the ideal experimental animal model should feature both of these types. This was the case in the present study, in clear contrast to the finding of mostly differentiated glandular stomach cancers in rats treated with MNNG.[27, 28]

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