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Reversibility of Catechol-induced Rat Glandular Stomach Lesions[1]

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ABSTRACT

The potential reversibility of glandular stomach lesions induced by the clastogen, catechol, was examined in groups of male F344 rats treated continuously with 0.8% catechol in the diet for 12, 24, 48, 72, or 96 weeks. After a return to basal diet for 84, 72, 48, 24, and 0 weeks, respectively, the animals were killed for histopathological examination. Incidences of submucosal hyperplasia, adenomas and adenocarcinomas, average number of tumors per rat, and the size of tumors in rats treated with catechol for 12, 24, 48, 72, and 96 weeks increased time dependently. After cessation of catechol treatment, although average number of tumors per rat slightly decreased, the size of tumors tended to increase. Labeling indices in both tumorous and nontumorous areas decreased after cessation of catechol treatment. The results thus indicate that whereas some submucosal hyperplasias or adenomas may regress, others have the potential to develop into adenomas or adenocarcinomas. However, tumor growth does depend to a certain extert on continued catechol treatment.

INTRODUCTION

Recently carcinogenic potential has been demonstrated for catechol in F344 rats. Thus continuous p.o. treatment with 0.8% catechol for 104 weeks induced glandular stomach adenocarcinomas at incidences of 54 and 43% in male and female animals, respectively (1). Catechol has also been shown to promote rat forestomach and glandular stomach carcinogenesis initiated by MNNG[3] (2) and enhance development of tongue and esophageal lesions after *N*-methyl-*N*-amylnitrosamine initiation (3). It also increased the yields of tumors of the mouse and skin and rat esophagus when coadministered with 7,12-dimethylbenz(a)anthracene (4) and *N*-methyl-*N*-amylnitrosamine (5), respectively. Although catechol proved negative in the Ames assay, several *in vitro* assay systems disclosed genotoxic properties (6). On the other hand, catechol induces ornithine decarboxylase and replicative DNA synthesis but not DNA single strand scission or unscheduled DNA synthesis in rat pyloric mucosa *in vivo* (7). In addition no adduct formation could be detected by the enzymatic ^{32}P-postlabeling assay in rat pyloric mucosa after catechol feeding (8). These data indicate that in spite of its genotoxic properties in *in vitro* assays, a nongenotoxic mechanism might be responsible for the *in vivo* carcinogenicity of catechol. The phenolic antioxidants BHA, caffeic acid, and sesamol are also nongenotoxic and can induce forestomach carcinomas in rats by continuous p.o. treatment for 104 weeks at a dose of 2% in the diet (1, 9). Previous assessment of the reversibility of BHA- and caffeic acid-induced rat forestomach lesions as compared to those caused by the genotoxic carcinogens MNNG, 2-[2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide] and 8-nitroquinoline revealed regression of papillomas and/or hyperplasias after cessation of antioxidant treatment, but not after MNNG, 2-[2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide], and 8-nitroquinoline administration. In the latter case hyperplasias developed into papillomas or carcinomas even after withdrawal of carcinogen treatment (10). Therefore, reversibility may be an important characteristic of nongenotoxic carcinogen-induced lesions. In the present experiment, the potential for regression of catechol-induced glandular stomach lesions was examined in rats using a histopathological approach.

MATERIALS AND METHODS

Animals. A total of 180 male F344 rats, 5 weeks old at commencement of the study, were purchased from Charles River Japan, Inc., Atsugi, Japan. The animals were randomly assigned, 5 or 6 to a plastic cage, and maintained on wood chip bedding in an air-conditioned room at 24 ± 2°C and 55 ± 5% humidity with a 12- light-dark cycle. They were allowed free access to Oriental MF basal diet (Oriental Yeast Co., Tokyo, Japan) and tap water.

Chemical. Catechol (CAS 120–80–9; purity >99%) was obtained from Wako Pure Chemical Industries, Osaka, Japan. The chemical was incorporated into Oriental MF powdered basal diet, using a cake mixer, at a concentration of 0.8% and stored at room temperature in the dark before use. Degradation of catechol was negligible under these conditions as reported previously (2).

Treatment. At 6 weeks of age, 9 groups of 10–18 rats each were placed on 0.8% catechol diet. Animals in groups 1, 3, 5, 7, and 9 were killed after 12, 24, 48, 72, and 96 weeks of continuous treatment, respectively; and those in groups 2, 4, 6, and 8 similarly receiving the 0.8% catechol diet for 12, 24, 48, and 72 weeks were maintained on basal diet alone until week 96 and then killed. Animals in control groups 10–14 were treated with basal diet alone for 12, 24, 48, 72, and 96 weeks and then sacrificed. Five animals in each group received an i.p. injection of 20 mg of bromodeoxyuridine (Sigma Chemical Co., St. Louis, MO) 1 h before killing. Animals were killed under ether anesthesia and their livers, kidneys, and stomachs were removed; the livers and kidneys were weighed and fixed in 10% buffered formalin solution. Stomachs were injected with formalin solution, opened along the greater curvature, and then further fixed in formalin. Three sections each were cut from the anterior and posterior walls of the forestomach, and six sections each were cut from the glandular stomach. Tissues were processed routinely for hematoxylin and eosin staining and anti-bromodeoxyuridine immunohistochemical staining (11). For the measurement of mucosal thickness in nontumorous regions, an average of six pre-pyloric zone areas from each rat were assessed. The number and size of tumors were determined by macroscopic and microscopic observation. For assessment of labeling indices, counts were made of the numbers of labeled cells per gastric pit in nontumorous regions. Forty gastric pits adjacent to the pylorus were counted per rat. For tumorous lesions including adenoma and adenocarcinoma, counts were made of the numbers of labeled cells per 0.0625 mm^2 and expressed as number per 0.1 mm^2. Student's *t* test and Fisher's exact probability test were used for statistical analyses of the data.

RESULTS

During catechol treatment, a significant reduction in body weight was observed. However, after withdrawal of the compound, body weights returned to control levels. Relative liver and kidney weights were slightly increased during catechol administration but also subsequently returned to control levels.

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[3] The abbreviations used are: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; BHA, butylated hydroxyanisole; i.g., intragastric; Fe-NTA, ferric nitrilotriacetate.

Macrosopically, multiple polypoid lesions were observed in the pyloric region of the glandular stomach of rats treated with catechol continuously, even to a certain extent after cessation of the treatment. These epithelial lesions were classified into hyperplasia, adenoma, and adenocarcinoma categories. Hyperplasia was defined as a focal upward or downward glandular proliferation. Adenoma was defined as a polypoid upward or downward glandular proliferation with compression of surrounding connective tissue. In both these cases of proliferating glands no cellular or structural atypia are evident. Adenocarcinomas in contrast were characterized by less differentiated cells with obvious cellular and structural atypia. However, invasion of the muscle layers was not often seen. Incidences of each type of proliferative lesion observed in the glandular stomach are presented in Table 1. Incidences of hyperplasia, adenomas, and adenocarcinomas time dependently increased in rats treated with catechol continuously for 12 to 96 weeks. The incidence of hyperplasia had significantly decreased 84 weeks after cessation of the 12-week catechol treatment. A similar tendency was observed after the 24-week administration. However, the incidences of hyperplasia, adenoma, and adenocarcinoma in the other groups were not negatively influenced by cessation of catechol treatment. The tumors were divided into 3 diameter categories: less than 1 mm; 1–2 mm; and more than 2 mm. While rats with tumors less than 2 mm in diameter tended to decrease or remain constant in number, those with tumors more than 2 mm tended to increase after withdrawal of catechol exposure in the 12-, 24-, and 48-week groups. The average number of tumors per rat slightly decreased after withdrawal of catechol but this was not statistically significant (Table 2). Mucosal thickness and labeling index data are shown in Table 3. Pyloric gland thickness remained high (0.34–0.45 mm) during catechol treatment but returned to 0.16–0.22 mm thereafter (control levels without catechol treatment were 0.14–0.16 mm; data not shown). Labeling indices in nontumorous pyloric glands paralleled the mucosal thickness data. Labeling indices in adenocarcinomas were very variable (12–67; data not shown) presumably because of the small numbers of adenocarcinomas which could be counted, although those in adenomas significantly decreased after catechol withdrawal in the 24-, 48-, and 72-week groups. Labeling indices in normal areas without catechol treatment (groups 10–14) ranged from 2.52 ± 0.53 (96 weeks old) to 3.61 ± 0.71 (48 weeks old).

DISCUSSION

The present experiment clearly showed that incidences of hyperplasia, adenoma, and adenocarcinomas increased depending on the duration of catechol treatment. In addition, while the number of tumors tended to decrease after cessation of catechol treatment, this was accompanied by an increase in the size of individual lesions. The results therefore indicated that some catechol-induced lesions have the potential to develop into adenomas and adenocarcinomas without the necessity of continued compound exposure. Judging from the labeling index data, however, tumor growth rate is likely to depend to a large extent on the presence of catechol.

It is generally considered that nonneoplastic proliferative lesions induced by nongenotoxic chemicals regress after withdrawal of the compound. For example, while continuous p.o. treatment with 3% uracil induced papillomas and later even carcinomas in the rat urinary bladder epithelium, the papillomas regressed when the animals were returned to basal diet (12, 13). Similarly, hyperplastic nodules induced in rats by continuous oral treatment with the peroxisomal proliferator clofibrate clearly decreased after its withdrawal (14). In forestomach epithelium, nongenotoxic BHA- and caffeic acid-induced rat forestomach hyperplasias are also known to be reversible (10, 15). In addition, in the BHA case, the incidence of papillomas also clearly decreased after treatment was stopped (16). In the present experiment, only a portion of the catechol-induced glandular stomach lesions regressed, some hyperplasias demonstrating continued autonomous growth to form adenomas and/or adenocarcinomas. This lack of reversibility of catechol-

Table 1 *Histopathological findings in the glandular stomach: incidence data*

Group	Treatment (wk)		No. of rats	No. of rats with (%)		
	Catechol	Basal diet		Hyperplasia	Adenoma	Adenocarcinoma
1	12	0	10	9 (90)	2 (20)	0
2	12	84	17	6 (35.3)[a]	2 (11.8)	0
3	24	0	10	10 (100)	10 (100)	0
4	24	72	16	10 (62.5)	12 (75)	1 (6.3)
5	48	0	10	10 (100)	10 (100)	1 (10)
6	48	48	14	14 (100)	14 (100)	3 (21.4)
7	72	0	10	10 (100)	10 (100)	4 (40)
8	72	24	18	18 (100)	18 (100)	9 (50)
9	96	0	15	15 (100)	15 (100)	11 (73.3)
14	0	96	12	0	0	0

[a] Significantly different at $P < 0.02$ *versus* group 1.

Table 2 *Histopathological findings of the glandular stomach: tumor size data*

Group	Treatment (wk)		No. of rats	No. of rats (%) with tumors of sizes			No. of tumors (no./rat)
	Catechol	Basal diet		≤1 mm	1–2 mm	>2 mm	
	12	0	10	2 (20)	0	0	0.3 ± 0.6[a]
2	12	84	17	1 (5.9)	0	1 (5.9)	0.2 ± 0.5
3	24	0	10	7 (70)	3 (30)	0	2.0 ± 1.1
4	24	72	16	11 (68.8)	1 (6.3)	2 (12.5)	1.9 ± 2.2
5	48	0	10	10 (100)	8 (80)	3 (30)	7.6 ± 4.1
6	48	48	14	13 (92.9)	9 (64.3)	7 (50)	7.6 ± 3.4
7	72	0	10	10 (100)	10 (100)	1 (10)	11.4 ± 1.8
8	72	24	18	18 (100)	17 (94.4)	8 (44.4)	9.3 ± 3.6
9	96	0	15	14 (93.3)	15 (100)	14 (93.3)	10.7 ± 2.3
14	0	96	12	0	0	0	

[a] Mean ± SD.

Table 3 *Mucosal thickness and labeling index data for glandular stomach epithelia of rats treated with catechol*

Group	Treatment (wk)		Mucosal thickness (mm)	Labeling index	
	Catechol	Basal diet		Nontumorous (no. of labeled cells/crypt)	Adenoma (no. of labeled cells/0.1 mm²)
1	12	0	0.34 ± 0.03[a]	7.64 ± 1.64	26.4 (2)[b]
2	12	84	0.16 ± 0.03[c]	2.41 ± 1.29[c]	12.8 (2)
3	24	0	0.41 ± 0.02	7.11 ± 1.06	54.6 ± 28.3 (15)
4	24	72	0.18 ± 0.04[c]	3.26 ± 1.23[c]	29.0 ± 16.3 (7)[d]
5	48	0	0.42 ± 0.03	8.75 ± 1.27	76.0 ± 40.6(42)
6	48	48	0.22 ± 0.06[c]	1.64 ± 0.45[c]	38.9 ± 22.1 (29)[c]
7	72	0	0.43 ± 0.03	6.71 ± 1.90	61.4 ± 29.1 (50)
8	72	24	0.21 ± 0.05[c]	2.21 ± 0.82[c]	25.4 ± 19.9 (18)[c]
9	96	0	0.45 ± 0.07	4.50 ± 2.28	28.6 ± 16.8 (34)
14	0	96	0.15 ± 0.02	2.52 ± 0.53	

[a] Mean ± SD.
[b] Numbers in parentheses, number of adenomas examined.
[c] Significantly different at $P < 0.001$ *versus* respective catechol alone groups.
[d] Significantly different at $P < 0.05$ *versus* respective catechol alone groups.

induced glandular stomach lesions may thus indicate true genotoxic properties for this compound. In support of this, catechol has been shown to be positive in the HeLa DNA synthesis and mouse bone marrow micronucleus tests, while also inducing chromatid breaks and exchanges in Chinese hamster ovary cells as well as sister chromatid exchange in human lymphocytes (6). However, no evidence that catechol can exert genotoxicity in gastric mucosa *in vivo* is available since DNA adducts are not formed in the glandular stomach epithelium of rats treated with catechol for 2 weeks as evaluated by the ^{32}P-postlabeling assay (8, 17). Furthermore unscheduled DNA synthesis and DNA single strand scission, which also represent initiating activity, were both negative in rat glandular stomach epithelium after a single i.g. administration of catechol (7). On the other hand replicative DNA synthesis and ornithine decarboxylase assays which indicate promoting activity were positive for the glandular stomach epithelium of rats treated with catechol (7).

Administration of the nonmutagen Fe-NTA i.p. results in high incidences of renal tumors in rats and mice long after cessation of the Fe-NTA exposure (18, 19). Thus irreversible proliferative lesions are presumably generated in the proximal tubules of the kidney by Fe-NTA. This chemical induces lipid peroxidation and produces 8-hydroxydeoxyguanosine as well as tubular necrosis in the target tissue (20–22) within 1 week after a single i.p. injection. Therefore oxidative DNA damage might be a factor underlying its carcinogenic and/or toxic effects. However, formation of 8-hydroxydeoxyguanosine, a DNA damage product associated with active oxygen species, could not be demonstrated in rat pyloric mucosa treated with catechol for 2–48 weeks (23). Therefore, there is no direct analogy between the catechol and Fe-NTA cases. Whether the lack of reversibility is indeed a reflection of catechol genotoxicity therefore remains open to question.

It has been shown that simple partial incision of the glandular stomach epithelium can cause submucosal hyperplasia. In addition, and more surprisingly, adenoma development in the pyloric mucosa has been reported to become apparent 12 weeks after gastrojejunal anastomosis, mucinous carcinomas being found after only 24 weeks without carcinogen treatment (24). Chronic epithelial damage due to bile acids has been considered an important factor in the induction of such lesions (25–27). Catechol administration is associated with similar damage and ulceration in the pyloric mucosa adjacent to pyloric ring, and although not confirmed at present, the possibility that reverse flow of bile acids might continuously stimulate epithelial proliferation even after withdrawal of catechol cannot be ruled out. Experiments are under way to elucidate whether this or other factors are involved in the carcinogenicity of this environmentally important agent.

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