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Effects of Sodium Nitrite and Catechol or 3-Methoxycatechol in Combination on Rat Stomach Epithelium

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The effects of sodium nitrite ($NaNO_2$) and catechol or 3-methoxycatechol in combination were examined in male F344 rats. Animals were treated with 0.3% $NaNO_2$ in the drinking water and 0.8% catechol or 2% 3-methoxycatechol in powdered diet for 24 weeks. While catechol or 3-methoxycatechol alone induced low incidences of mild or moderate hyperplasia, simultaneous administration of $NaNO_2$ markedly enhanced the degree of hyperplasia and papilloma formation. In contrast, induction of submucosal hyperplasia and adenomas in the glandular epithelium was reduced. Thus, the results indicate that $NaNO_2$ can modulate the metabolism of antioxidants, so that, possibly via production of new active moieties, targeting of forestomach epithelium is enhanced.

Key words: Antioxidant — Sodium nitrite — Rat stomach

Recently the phenolic antioxidants butylated hydroxyanisole (BHA), caffeic acid, sesamol and catechol have been shown to be carcinogenic for rodent forestomach or glandular stomach epithelium.[1-4] Of these compounds BHA, caffeic acid and catechol all enhanced two-stage rat stomach carcinogenesis initiated with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG),[5-9] 7,12-dimethylbenz[*a*]anthracene,[10] dibutylnitrosamine[11] or methylnitrosourea.[12, 13] However, the same group of phenolic antioxidants is well known to be capable of inhibiting carcinogenesis by reducing active nitrosamine formation. For example, chlorogenic acid, caffeic acid, ferulic acid, gallic acid and syringol have been shown to block *in vivo* or *in vitro* formation of nitrosamines[14-17] or to inhibit *in vivo* tumor induction by $NaNO_2$ and amines.[16] On the other hand, the reaction of phenol with $NaNO_2$ under mildly acidic conditions produces *p*-nitrosophenol and diazoquinones, *p*-diazoquinone being known to be mutagenic to *Salmonella typhimurium* TA 98 and TA 100 without metabolic activation.[18] Similarly, phenol, 3-methoxycatechol and catechol all exhibited direct-acting genotoxicity as evaluated by the SOS chromotest after nitrosation, diazonium compounds being considered to be responsible for the genotoxicity.[19] These results indicate that $NaNO_2$ may modulate catechol-induced stomach lesion development and that combined treatment with $NaNO_2$ and 3-methoxycatechol may induce tumors in the stomach by alternative metabolic pathways. The present paper concerns the effects of $NaNO_2$ and the phenolic antioxidants catechol or 3-methoxycatechol on rat stomach epithelia when given in combination.

Groups of 10 F344 male rats at 10 weeks of age (Charles River Japan Inc., Kanagawa) were treated with 0.3% $NaNO_2$ in the drinking water and simultaneously administered 0.8% catechol (CAS 120-80-90, purity >98%) or 2% 3-methoxycatechol (CAS 934-00-9, purity> 98%) in Oriental MF powdered basal diet (Oriental Yeast Co., Tokyo) for 24 weeks. Additional groups received the 0.3% $NaNO_2$, 0.8% catechol, 2% 3-methoxycatechol or basal diet alone for the same period. All chemicals were obtained from Wako Pure Chemical Industries, Osaka. Animals were kept 5 to a plastic cage in an air-conditioned room at 24±2°C, and food and water were given *ad libitum*. All animals were killed under ether anesthesia at the end of the experimental period, and complete autopsy was performed. Stomachs were removed and injected with 10% buffered formalin solution. After opening along the greater curvature, six strips each were cut from the anterior and posterior walls of the forestomach and 6 strips were cut from the pyloric region of the glandular stomach. Tissues were processed routinely and stained with hematoxylin and eosin. The Fisher exact test and Student's *t* test were used for the statistical analysis of the data.

Final body weights of animals treated with antioxidant or $NaNO_2$ were significantly lower than those of rats receiving basal diet alone. Combined treatment with antioxidants and $NaNO_2$ further reduced the body weights as compared to the antioxidant-alone group values. Food consumption of animals treated with antioxidants and $NaNO_2$ was also slightly lower than that of animals on antioxidant alone (Table I). Grossly, the stomach con-

Table I. Final Body and Organ Weights, and Food Consumption Data

Treatment	No. of rats	Body wt. (g)	Relative organ wt. (g/100 g body wt.)		Food consumption (g/rat/day)
			Liver	Kidney	
Catechol + $NaNO_2$	10	$279.2 \pm 18.1^{a)}$	$2.46 \pm 0.05^{a)}$	$0.74 \pm 0.04^{a)}$	13.1
Catechol	10	$356.7 \pm 18.2^{b)}$	$2.72 \pm 0.09^{b)}$	$0.64 \pm 0.06^{b)}$	14.1
3-Methoxycatechol + $NaNO_2$	10	$292.6 \pm 15.6^{a)}$	$2.42 \pm 0.11^{a)}$	$0.68 \pm 0.03^{a)}$	14.1
3-Methoxycatechol	10	379.7 ± 18.9	$2.64 \pm 0.13^{b)}$	$0.60 \pm 0.04^{b)}$	14.5
$NaNO_2$	10	342.3 ± 14.3	2.20 ± 0.07	$0.60 \pm 0.02^{b)}$	12.9
Basal diet	10	395.3 ± 20.5	2.27 ± 0.08	0.55 ± 0.02	15.0

a) $P < 0.01$ vs. catechol, 3-methoxycatechol, or $NaNO_2$ alone group.
b) $P < 0.01$ vs. basal diet group.

Fig. 1. Stomach of a rat treated with 3-methoxycatechol and $NaNO_2$ for 24 weeks. Note prominent thickening of the blackened forestomach epithelium with papillary projections.

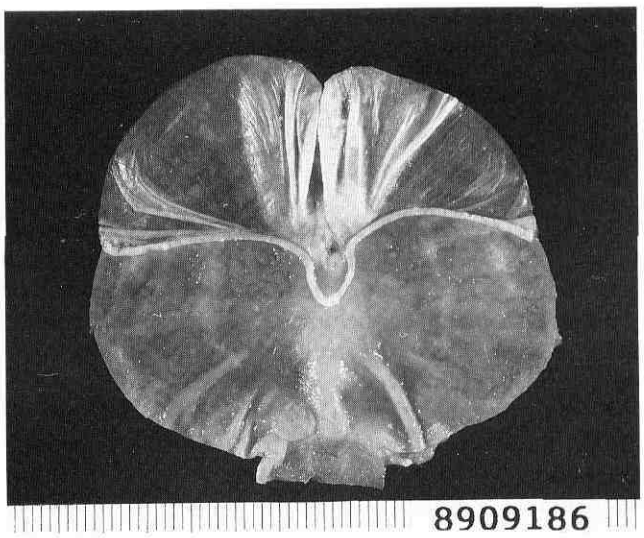

Fig. 2. Stomach of a rat treated with 3-methoxycatechol alone for 24 weeks. Small nodular lesions are evident in the pyloric glandular region.

tents and forestomach epithelia of rats treated with $NaNO_2$ plus antioxidants demonstrated blackening and marked papillary projections with dense keratin-like material being evident scattered throughout the epithelium (Fig. 1). No such abnormalities were observed in the forestomach of rats treated with catechol or 3-methoxycatechol alone (Fig. 2). The pyloric region of the glandular stomach of rats treated with catechol or 3-methoxycatechol was thickened nodularly, but the changes were much less in animals treated with $NaNO_2$ plus catechol or $NaNO_2$ plus 3-methoxycatechol. Histological changes in the forestomach were classified into hyperplasias and papillomas, hyperplasias being further divided into mild, moderate, severe and marked categories depending on the thickness of the mucosa, as previously reported.[20] As listed in Table II, one rat and 5 rats, respectively, treated with $NaNO_2$ plus catechol, and $NaNO_2$ plus 3-methoxycatechol, had papillomas. Moderate hyperplasia was not found in rats receiving catechol alone, and only 20% of rats had moderate hyperplasia in those receiving 3-methoxycatechol alone. However, simultaneous treatment with $NaNO_2$ markedly enhanced the incidences of moderate, severe and marked hyperplasia, i.e., all rats treated with catechol plus $NaNO_2$ had moderate, 80% had severe and 2% had marked hyperplasia; all rats treated with 3-methoxycatechol plus $NaNO_2$ had moderate and severe hyperplasia and 50% demonstrated marked hyperplasia. In the glandular stomach, lesions

Table II. Histopathological Findings in the Forestomach

Treatment	No. of rats	No. of rats with				
		Hyperplasia				Papilloma
		Mild	Moderate	Severe	Marked	
Catechol+$NaNO_2$	10	$10^{b,c)}$	$10^{a,c)}$	$8^{a,c)}$	2	1
Catechol	10	$5^{f)}$	0	0	0	0
3-Methoxycatechol+$NaNO_2$	10	$10^{e)}$	$10^{a,e)}$	$10^{a,e)}$	$5^{b,d)}$	$5^{b)}$
3-Methoxycatechol	10	$7^{e)}$	2	0	0	0
$NaNO_2$	10	4	3	0	0	0
Basal diet	10	0	0	0	0	0

a) $P<0.01$, *b*) $P<0.05$ vs. catechol or 3-methoxycatechol alone group.
c) $P<0.05$, *d*) $P<0.05$ vs. $NaNO_2$ alone group.
e) $P<0.01$, *f*) $P<0.05$ vs. basal diet group.

Table III. Histopathological Findings in the Glandular Stomach

Treatment	No. of rats	No. of rats with	
		Submucosal hyperplasia	Adenoma
Catechol+$NaNO_2$	10	$3^{a)}$	$1^{a)}$
Catechol	10	$10^{b)}$	$10^{b)}$
3-Methoxycatechol +$NaNO_2$	10	$1^{a)}$	2
3-Methoxycatechol	10	$9^{b)}$	$7^{b)}$
$NaNO_2$	10	0	0
Basal diet	10	0	0

a) $P<0.01$ vs. catechol or 3-methoxycatechol group.
b) $P<0.01$ vs. basal diet group.

were classified into submucosal hyperplasia (submucosal growth) and adenoma (adenomatous hyperplasia) categories as previously reported.[4,20] All rats treated with catechol had submucosal hyperplasia and adenomas, and 9 and 7 rats treated with 3-methoxycatechol had submucosal hyperplasia and adenomas, respectively. The incidences of these lesions were remarkably reduced by the combined treatment with $NaNO_2$ (Table III).

Phenolic compounds have been shown to catalyze or inhibit nitrosamine formation by the interaction of amines and $NaNO_2$.[14-17] However, the present experiment clearly demonstrated that the target organ for induction of cell proliferation by phenolic antioxidants can be altered by treatment in combination with $NaNO_2$. Thus, in the present study catechol and 3-methoxycatechol induced strong cell proliferation in the glandular stomach epithelium as evidenced by the induction of submucosal hyperplasia and adenomas, but combined administration of $NaNO_2$ strongly reduced their activity. On the other hand $NaNO_2$ remarkably enhanced the development of proliferation-associated lesions in the forestomach of rats treated with catechol or 3-methoxycatechol.

Although the decreased incidences of adenomas and submucosal hyperplasia in rats treated with antioxidants and $NaNO_2$ could be partly due to reduced food consumption and therefore decreased antioxidant intake, the observed shift in target site favors the interpretation that alternative reactive metabolites exist. Thus, in place of those which induce glandular stomach cell proliferation, other metabolites, targeting the forestomach epithelium and associated with blackening, appeared to be formed by the reaction with $NaNO_2$. It has been shown that phenol reacts with nitrite to produce nitrosophenol,[21] *p*-diazoquinone and *o*-diazoquinone.[18] Interestingly, *p*-diazoquinone proved to be mutagenic in *S. typhimurium* TA 98 and TA 100 without metabolic activation.[18] Similarly, Ohshima *et al.* reported that 3-methoxycatechol and catechol could produce direct-acting mutagenic diazonium compounds after nitrosation under acidic conditions *in vitro*.[19] Although it is not known whether nitrosated phenolic compounds or diazoquinone metabolites are indeed formed *in vivo* in the rat stomach treated with antioxidants and $NaNO_2$, it is not likely that such mutagenic compounds are responsible for the development of catechol-induced glandular stomach lesions because catechol treatment-associated DNA-adducts could not be demonstrated by the enzymic ^{32}P-postlabeling method.[22] Furthermore catechol did not induce single-strand DNA breaks[23] in the rat stomach epithelium while inducing cell proliferation and cancers in the glandular stomach.[4]

It is of interest that a high incidence of adenomas in the rat was found after treatment with 3-methoxycatechol alone for 24 weeks in the present study. We earlier demonstrated glandular stomach carcinogenicity and/or promotion of glandular stomach carcinogenesis for a

number of *o*-dihydroxybenzene derivatives, i.e. catechol, *p*-methylcatechol and *p-t*-butylcatechol.[7,20] Therefore 3-methoxycatechol, which has been identified in wood smoke condensates, appears to be a new phenolic compound targeting the glandular stomach epithelium. Our present results are supported by those of Ohshima *et al.*,[24] who showed that hickory smoke condensates (HSC) which contain catechol and *o*-methoxycatechol induced ornithine decarboxylase (ODC), replicative DNA synthesis (RDS) and DNA single-strand breaks in the rat pyloric mucosa, but the administration of HSC with $NaNO_2$ decreased the induction of ODC, RDS and DNA single-strand breaks. These results indicate that tumor-initiating and/or tumor-promoting activities of HSC would be diminished by simultaneous treatment with $NaNO_2$. The present results further suggest that catechol and 4-methoxycatechol may be strongly carcinogenic to rat forestomach in the presence of $NaNO_2$, considering the strong correlation between cell proliferation and carcinogenicity[5] and the marked hyperplasia and papillomas observed in the present case.

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