Modification by Catechol and Resorcinol of Upper Digestive Tract Carcinogenesis in Rats Treated with Methyl-N-amylnitrosamine

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ABSTRACT

Modifying effects of the environmental contaminant catechol, and its isomers resorcinol and hydroquinone, on methyl-N-amylnitrosamine (MNAN)-induced carcinogenesis were studied in male F344 rats. Groups of 15 rats were given three i.p. injections of 25 mg/kg of body weight of MNAN within the initial 2-wk period, and commencing 1 wk thereafter they were administered 0.8% catechol, 0.8% resorcinol, or 0.8% hydroquinone in powdered basal diet or were given basal diet alone for 49 wk. Additional groups of 10 to 15 rats were similarly treated without prior carcinogen exposure.

Histological examination after sacrifice at wk 52 revealed that the incidences of tongue papillomas and esophageal squamous cell carcinomas in the groups given MNAN followed by catechol (57.1% and 64.3%) or resorcinol (50% and 58.8%) were significantly higher than those in the carcinogen only controls (9.1, and 0%, respectively). Hydroquinone also enhanced the development of esophageal squamous cell carcinomas but was less active than catechol or resorcinol. The incidence of alveolar hyperplasia in the lungs of the group given MNAN followed by catechol (0%) was, in contrast, significantly reduced as compared to the control value (54.5%). Hydroquinone and resorcinol showed a similar but non-significant tendency.

These results indicated that the environmental contaminant, catechol and its isomers, may play a role in the development of human upper gastrointestinal cancer, in addition to exerting modifying effects in other organs.

INTRODUCTION

Catechol (1,2-dihydroxybenzene) is an antioxidant which is present in certain foods, coffee, cigarette smoke, the oxidative type of hair dyes, and film developers (1–5). Human exposure to catechol has been estimated to be more than 30 mg per day because catechol conjugates (1 to 30 mg) are excreted daily in the urine of human volunteers given an unrestricted diet (6). Hydroquinone (1,4-dihydroxybenzene) and resorcinol (1,3-dihydroxybenzene) are also present in the environment as industrial chemicals and as major phenols in cigarette smoke (1, 2).

Annual production of these chemicals in Western Europe is estimated to be 4 to 40 million kg (2). Although these phenols are not mutagenic in the Ames test (7), catechol increased the transformation frequency of Balb/3T3 cells treated with B(a)P3 or β-propionolactone (8). Furthermore, catechol and hydroquinone induce sister chromatid exchanges and delay cell division in human lymphocytes (9–11), and both catechol and resorcinol can induce chromatid breaks and exchanges in Chinese hamster ovary cells (12). Catechol and hydroquinone, but not resorcinol, were also found to cause DNA breakage in vitro, using λ DNA, and in cultured RFL and HeLa mammalian cells (13).

Topical application of catechol, or the weakly acidic fraction of cigarette smoke condensate of which it is a constituent, resulted in cocarcinogenic effects on mouse skin carcinogenesis induced by B(a)P (14, 15), whereas hydroquinone and resorcinol inhibited B(a)P carcinogenicity (16). None of these chemicals exerted any skin-promoting potential (14, 16). Catechol did not demonstrate any cocarcinogenic action for bladder cancer either when applied at a level of 0.05% in the drinking water together with 0.001% BBN, or when 1 to 2% catechol and 1 to 2% BBN were instilled intravesically (17). In other organs, however, catechol has demonstrated pronounced effects. For example, continuous treatment at a dose of 0.2% in the diet significantly increased rat esophageal papilloma multiplicity induced by MNAN (18). Catechol alone but not hydroquinone also resulted in elevated DNA synthesis of esophageal epithelium measured by [3H]thymidine incorporation (19). We recently demonstrated that continuous p.o. administration of 1.5% catechol induced forestomach hyperplasia and increased the labeling index in both the glandular stomach and forestomach epithelium of hamsters (19). Promoting activity in the forestomach and glandular stomach has already been reported for rats initiated with MNNG (20, 21), and it has been suggested that catechol may be involved in tobacco-related cancer induction in humans, which includes tumors of the lung, oral cavity, esophagus, pancreas, and urinary bladder (22).

In the present experiment, the postinitiation modifying potential of catechol, hydroquinone and resorcinol treatments on MNAN-induced carcinogenesis was examined in male F344 rats.

MATERIALS AND METHODS

Animals. A total of 115 five-wk-old male F344 rats were obtained from Charles River Japan, Inc., Atsugi, Japan. The animals were housed five to a plastic cage with softwood chips for bedding in an air conditioned room at a temperature 24 ± 2°C and 55% humidity with a 12 h-12 h light-dark cycle. They were maintained on Oriental MF basal diet (Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum before the antioxidant treatment.

Chemicals. MNAN (CAS 13256-07-0) was obtained from Iwai Kagaku Yukuhin Co., Ltd., Kyoto, Japan, and catechol (CAS 120-80-9; purity > 99%), resorcinol (CAS 108-46-3; purity > 99%) and hydroquinone (CAS 123-31-9; purity > 99%) were purchased from Wako Pure Chemical Industries, Osaka, Japan.

Experimental Protocol. At 0, 1, and 2 wk of the experiment, 60 rats were given i.p. injections of MNAN at a dose of 25 mg/kg of body weight. One wk after the last injection, the MNAN-treated animals were divided into 4 groups of 15 animals each and administered Oriental MF powdered diet containing 0.8% catechol, 0.8% resorcinol, or 0.8% hydroquinone, or the basal diet alone for 49 wk. A further 55 rats divided into groups of 10 to 15 received the 0.8% catechol, 0.8% resorcinol, 0.8% hydroquinone, or basal diet alone without MNAN pretreatment. Chemicals were incorporated into powdered basal diet every 4 wk using cake mixer and stored at room temperature in the dark until use. Storage was not associated with any reduction of purity until use.
concentration in the case of 0.8% catechol in the diet after 4 wk, but measurement of hydroquinone and resorcinol at the same time point revealed a drop to 0.73%. The analyses were carried out at Food Research Laboratories, Tokyo, Japan using methanol extraction and high-pressure liquid chromatography.

Animals were weighed, and food intake was measured every 2 to 4 wk. All surviving rats were sacrificed under ether anesthesia at the end of wk 52. The liver, kidneys, tongue, esophagus, stomach, trachea, lung, and nasal cavity were removed, the liver and kidney being weighed and fixed in 10% buffered formalin solution. Esophagus, stomach, trachea, lungs, and nasal cavity were given an injection of formalin solution. The esophagus was slit and the stomach opened via an incision along the greater curvature. Six sections were cut at different levels from the esophagus, three sections from the nasal cavity, and six sections each from the anterior and posterior walls of the forestomach and glandular stomach. One section was cut from each lobe of both right and left lungs, and one sagittal section in addition to grossly abnormal lesions was cut from the tongue. Tissues were processed routinely for histopathological examination. In lung sections, the numbers and area of lesions per square cm of tissue were assessed using a color video image processor (VIP-21C; Olympus-Ikegami Tsushin Co., Tokyo, Japan). Some animals died in the early stage of the experiment, and only those which survived until the final sacrifice were included in the effective numbers. Student's t and Fisher's exact tests were used for statistical evaluation of the data.

RESULTS

Final body weights of rats treated with MNAN followed by catechol, resorcinol, or hydroquinone were significantly lower than those given MNAN alone. The relative liver weight of rats treated with MNAN followed by catechol and the relative kidney weights of rats treated with MNAN followed by resorcinol or hydroquinone were significantly higher than in the MNAN alone group (Table 1).

Tumors or preneoplastic lesions were observed in the tongue, esophagus, lung, and nasal cavity tissues of rats treated with MNAN. Gross size and/or multiplicity of tumors in the esophagus and tongue were clearly greater in the groups treated with MNAN followed by catechol (Fig. 1) or resorcinol than with MNAN alone (Fig. 2). Histopathological findings are summarized in Tables 2 and 3. Lesions in the tongue, esophagus, and nasal cavity were histopathologically classified into PN hyperplasia, papilloma, carcinoma in situ, and squamous cell carcinoma as previously reported (21, 23, 24). PN hyperplasia is defined as a focal upward growing papillary/polypoid, or downward nodular epithelial lesion with fine stromal connective tissue elements. Papilloma is more advanced than PN hyperplasia with multiple connective tissue branching but lacking cellular and structural atypia. In addition to these lesions, alveolar cell hyperplasia and adenomatous hyperplasia were observed in the lung and glandular stomach, respectively. Tumors observed in tongue tissue were mostly papillomas, occurring either as single or double lesions. Although the incidences of PN hyperplasias and carcinomas were not significantly different among the 4 MNAN-treated groups, that of papillomas was significantly increased by subsequent treatment with catechol (57.1%, P < 0.001) and resorcinol (50%, P < 0.05) as compared to the controls (9.1%).

In the esophagus, the incidence of papillomas was higher in rats treated with MNAN followed by catechol (50%) than in controls (18.2%), but the difference was not significant. Development of squamous cell carcinomas, including early in situ lesions, was, however, significantly increased in rats treated with MNAN followed by catechol (64.3%, P < 0.001) or resorcinol (58.8%, P < 0.01) in comparison to MNAN alone (0%), and a similar trend was observed for hydroquinone (33.3%, difference not significant). Quantitative analysis showed the numbers of squamous cell carcinomas were significantly higher in rats treated with MNAN followed by catechol (P < 0.01), resorcinol (P < 0.01), or hydroquinone (P < 0.05) than with MNAN alone.

While lung alveolar cell hyperplasia developed in 54.5% of the animals treated with MNAN alone, the respective incidences decreased to 0 (P < 0.01), 16.7%, and 16.7% in the groups treated with MNAN followed by catechol, resorcinol, and hydroquinone. The numbers of alveolar cell hyperplasias
and carcinogenic effects on the upper digestive tract remain correlating well with promotion activity.

In the present experiment, both catechol and resorcinol clearly promoted carcinogenesis in the tongue and esophagus, although resorcinol was less active than catechol. Hydroquinone was found to be marginally effective in enhancing esophageal carcinogenesis while itself in the nasal cavity, incidences of PN hyperplasias and papillomas were also lower in rats treated with MNAN followed by catechol (P < 0.02) or hydroquinone (P < 0.05). In the nasal cavity, incidences of PN hyperplasias and papillomas were also lower in rats treated with MNAN followed by catechol, resorcinol, and hydroquinone, or basal diet, respectively. Forestomach hyperplasia was evident in 85.7%, 16.7%, 16.7%, and 0% of rats treated with MNAN followed by catechol, resorcinol, and hydroquinone, or basal diet, respectively, and in 86.7%, 12.5%, 26.7%, and 0% of rats treated with catechol, resorcinol, and hydroquinone alone, or basal diet, respectively. Forestomach papillomas were found in one rat each of the groups receiving MNAN followed by catechol and catechol alone. Adenomatous hyperplasia in the glandular stomach was observed in all rats given MNAN followed by catechol, and in 93.3% of rats given catechol alone.

**DISCUSSION**

In the present experiment, both catechol and resorcinol clearly promoted carcinogenesis in the tongue and esophagus, although resorcinol was less active than catechol. Hydroquinone was found to be marginally effective in enhancing esophageal carcinogenesis. Since we demonstrated in a previous study that catechol also strongly promotes NNG-induced rat forestomach and glandular stomach carcinogenesis while itself inducing hyperplasia in both those organs and even adenocarcinoma in glandular stomach (20, 21), this positive modifying potential appears common to the entire upper digestive tract. Catechol and hydroquinone both induce an increase in the labeling index of rat esophageal epithelium (18), the extent correlating well with promotion activity.

The mechanisms by which antioxidants exert their promoting and carcinogenic effects on the upper digestive tract remain unclear. Catechol itself is a proximate metabolite of benzene which causes myelotoxicity and leukemia in both rodents and humans (25). Peroxide-dependent bioactivation of catechol which produces o-benzoquinone and o-benzosemiquinone radical occurs in rat and human bone marrow cells, as well as in the liver (26–28), and these forms readily react with nucleophilic groups of amino acids and inactivate certain enzymes (26, 29). BHA also has been shown to produce o-benzoquinone and o-semiquinone derivatives which bind to liver microsomal proteins after peroxidative metabolism (30–32). Although such a peroxidative metabolism has not been demonstrated in the forestomach and glandular stomach of rats treated with either BHA or catechol, it cannot be precluded that formation of reactive metabolites in the target organs might be related to the observed promotion of carcinogenesis or enhanced proliferative activity.

Although previous results did not indicate that the lung is a target of MNAN (18, 33), alveolar hyperplasia developed in some animals treated with the carcinogen in the present experiment. This difference could partly be due to differences in the strain of animals used. The finding that catechol and its isomers cannot be precluded that formation of reactive metabolites in the target organs might be related to the observed promotion of carcinogenesis or enhanced proliferative activity.

It is of interest that, among the dihydroxybenzenes tested, catechol was the most active, followed by resorcinol, while hydroquinone demonstrated only marginal activity in promotion of upper digestive tract carcinogenesis. With regard to

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**Table 2: Incidences of lesions in rats treated with MNAN followed by chemicals**

<table>
<thead>
<tr>
<th>Organ and lesions</th>
<th>MNAN → catechol (n = 14)</th>
<th>MNAN → resorcinol (n = 12)</th>
<th>MNAN → hydroquinone (n = 12)</th>
<th>MNAN → basal diet (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN hyperplasia</td>
<td>14 (100)</td>
<td>12 (100)</td>
<td>11 (91.7)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Papilloma</td>
<td>7 (50)</td>
<td>1 (8.3)</td>
<td>1 (8.3)</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>9 (64.3)</td>
<td>7 (58.8)</td>
<td>4 (33.3)</td>
<td>0</td>
</tr>
<tr>
<td>Nasal cavity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN hyperplasia</td>
<td>10 (71.4)</td>
<td>11 (91.7)</td>
<td>2 (100)</td>
<td>10 (90.1)</td>
</tr>
<tr>
<td>Papilloma</td>
<td>0</td>
<td>3 (25)</td>
<td>4 (33.3)</td>
<td>3 (27.3)</td>
</tr>
</tbody>
</table>

* Effective numbers.
* Numbers in parentheses, percentage.
* Significantly different at P < 0.02 versus MNAN → basal diet.
* P < 0.05.
* P < 0.001.
* P < 0.001.

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**Table 3: Quantitative analysis of esophagus and lung lesions**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of rats/No. of rats</th>
<th>No. of rats/Area (mm²/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNAN → catechol</td>
<td>14</td>
<td>15</td>
<td>0.07 ± 0.06*</td>
</tr>
<tr>
<td>MNAN → resorcinol</td>
<td>12</td>
<td>8</td>
<td>0.67 ± 0.62*</td>
</tr>
<tr>
<td>MNAN → hydroquinone</td>
<td>12</td>
<td>4</td>
<td>0.33 ± 0.47*</td>
</tr>
<tr>
<td>MNAN → basal diet</td>
<td>12</td>
<td>0</td>
<td>0.42 ± 0.44</td>
</tr>
</tbody>
</table>

* Significantly different at P < 0.01 versus MNAN → basal diet.
* Mean ± SD.
* P < 0.02.
* P < 0.05.
structure-activity relationships in induction of proliferation in the forestomach epithelium of rats, continuous p.o. treatment with 2% caffeic acid (3,4-di-hydroxycinnamic acid) induced pronounced hyperplasia, whereas 2% ferulic acid (4-hydroxy-3-methoxycinnamic acid) lacked this activity (35). In addition, caffeic acid enhanced forestomach carcinogenesis in rats pre-treated with 7, 12-dimethylbenz(a)anthracene (36), while itself inducing forestomach tumors after continuous p.o. treatment at a dose of 2% in the diet for 60 wk (37). Recently we found that α-methylcatechol, in which one hydroxy substituent of catechol is replaced by a methoxy substituent, did not enhance forestomach and glandular stomach carcinogenesis of rats pre-treated with MNNG, whereas p-methyIcatechol and p-tert-butylcatechol both induced proliferation in these organs and enhanced forestomach and glandular stomach carcinogenesis (38), as with catechol (21). Therefore, these findings indicate that the presence of an α-dihydroxy structure in the benzene ring is an important factor for the induction of proliferation in forestomach or glandular stomach epithelium and, presumably, promotion of upper digestive tract carcinogenesis. Although in mouse 2-stage skin carcinogenesis initiated with DMBA, catechol and hydroquinone did not enhance papilloma formation, while resorcinol demonstrated slight promotion (39), this apparent anomaly might be explained by degradation of catechol by light.

In conclusion, the possibility that dihydroxybenzenes and particularly catechol and resorcinol may play roles in the reported association between tobacco use and carcinogenesis in the upper digestive tract, i.e., oral cavity, esophagus, and stomach of humans, deserves further attention.

REFERENCES

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CATECHOL ISOMERS AND ESOPHAGEAL CARCINOGENESIS

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