Regression of Simple Hyperplasia and Papillomas and Persistence of Basal Cell Hyperplasia in the Forestomach of F344 Rats Treated with Butylated Hydroxyanisole

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ABSTRACT

The reversibility of forestomach lesions induced in rats by butylated hydroxyanisole (BHA) was examined. F344 rats were given a 2% BHA diet for 24, 48, or 72 wk followed by a basal diet for the remainder of the 96-wk experiment. Two other groups of rats were given a 2% BHA diet or basal diet alone for 96 wk. The forestomach lesions at wk 24, 48, 72, or 96 were compared histopathologically. The results showed that exophytic epithelial proliferation (simple hyperplasia or papilloma) induced by BHA was reversible, while endophytic proliferation of basal cells (basal cell hyperplasia) persisted after withdrawal of BHA administration. This suggests that simple hyperplasia and papilloma of the forestomach induced by BHA are not autonomous and need continuous feeding of BHA to develop further.

INTRODUCTION

The antioxidant BHA, which has been widely used as a human food and animal feed additive, consists mainly of 3-tert-butylated hydroxyanisole with 15% or less of 2-tert-butylated hydroxyanisole. Studies in this laboratory showed that BHA is carcinogenic to the forestomach of F344 rats when given at a dose of 2% in the diet for 2 yr (1). While BHA was also shown to be tumorigenic to the forestomach of Syrian golden hamsters (2, 3), its tumorigenic action on hamster forestomach was demonstrated to be due to 3-tert-butylated hydroxyanisole (4). In the dose-response study of the tumorigenic effect of BHA on the rat forestomach, the minimum dose of BHA that gave tumors was 1%, and the dose that gave carcinomas was 2% (5). This carcinogenic dose of BHA is rather high compared with other chemicals, such as 4- or 8-nitroquinoline, N-methyl-N'-nitro-N-nitrosoguanidine, or methylnitrosourea which also induce carcinomas in the forestomach (6–9). BHA is reported to have no mutagenic activity (10–12), and therefore BHA may yield forestomach tumors in a different way from other carcinogens that possess mutagenic activity. In this study we examined the reversibility of the forestomach lesions in F344 rats given 2% BHA diet for various periods.

MATERIALS AND METHODS

Animals. A total of 160 male F344 rats, 5 wk old, was purchased from Charles River Japan, Inc., Atsugi, Japan. Animals were randomly divided, housed 5 to a plastic cage with wood chip bedding, and maintained in an air-conditioned room at controlled temperature (22 ± 2°C) and humidity (55 ± 5%) with a 12-h light-dark cycle. The rats were marked in the forestomach near the limiting ridge. BCH was incorporated into Oriental M powdered basal diet (Oriental Yeast Co., Ltd., Tokyo, Japan) at a concentration of 2.0%, and the mixtures were then made into pellets by the Oriental Yeast Co., Ltd., Tokyo, Japan. Rats were divided into 11 groups and were treated according to the experimental schedule shown in Fig. 1. Rats of each group were killed under ether anesthesia and subjected to postmortem examination. Stomachs were infused with 2 to 3 ml of 10% buffered formalin solution, and after 15 min, they were opened along the greater curvature and fixed in 10% buffered formalin solution, embedded, sectioned, and stained with hematoxylin-eosin.

The forestomach lesions were examined histopathologically and classified into simple hyperplasia, papilloma, basal cell hyperplasia, or squamous cell carcinoma.

Histological changes in the forestomach were diagnosed according to the following criteria: (a) SH, characterized by an absolute increase in the number of epithelial cells of all layers often with hyperkeratosis. The subepithelial border did not show exophytic growth; (b) papilloma, an increase in the number of all layers of the epithelial cells more marked than SH with exophytic arborescent proliferation of the interstitium. In advanced cases, epithelial cells proliferated downward into the subepithelial layer forming keratin pearls. Papillomas were observed as focal lesions near the limiting ridge, often as a continuous lesion to SH; (c) BCH, endophytic proliferation of basal cells in solid or branching forms. BCH was observed at a focal lesion near the limiting ridge; and (d) SCC, invasion of submucosal layers by atypical epithelial foci, the cells of which show various degrees of atypia with various degrees of epithelial pearl formation.

Statistical Analysis. Data on incidences of lesions were analyzed for statistical significance with the χ² test. Other data were analyzed with Student's t test.

RESULTS

During the treatment with BHA diet, rats had a 10 to 15% decrease in body weight gain as compared to control rats. After withdrawal of the BHA diet, rats gained weight, and average body weights increased in proportion with the duration of basal diet administration (Fig. 2). However, final average body weights of the rats given the BHA diet were all significantly less than that of the control group.

Forestomach lesions observed in each group are summarized in Table 1. In Group 10, in which rats were given the 2% BHA diet for 96 wk, incidences of SH, papillomas, or BCH were all 100%, and that of SCC was 17%. These findings are similar to those in our previous reports (1, 3, 5), and the carcinogenic effect of BHA was reconfirmed; thus, forestomach lesions seen in Groups 1 to 9 should be indicative of the development of carcinoma or of reversible lesions.

In Groups 1 to 4, reversibility of the forestomach lesions induced by administration of BHA diet for 24 wk was examined (some of the results were reported in Ref. 13 as a rapid communication). SH, papillomas, and BCH were observed in all rats treated with the BHA diet continuously for 24 wk (Group 1). As reported in previous studies (1–5, 14–18), these lesions were marked in the forestomach near the limiting ridge. BCH...
REVERSIBILITY OF FORESTOMACH LESIONS INDUCED BY BHA

Table 1 Incidence of forestomach lesions in F344 rats exposed to BHA

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of rats with lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SH</td>
</tr>
<tr>
<td>1</td>
<td>24 wk BHA</td>
<td>10</td>
<td>10 (100)*</td>
</tr>
<tr>
<td>2</td>
<td>24 wk BHA → 24 wk BD</td>
<td>9</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>3</td>
<td>24 wk BHA → 48 wk BD</td>
<td>9</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>24 wk BHA → 72 wk BD</td>
<td>18</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5</td>
<td>48 wk BHA</td>
<td>10</td>
<td>10 (100)</td>
</tr>
<tr>
<td>6</td>
<td>48 wk BHA → 24 wk BD</td>
<td>10</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>7</td>
<td>48 wk BHA → 48 wk BD</td>
<td>19</td>
<td>0 (0)</td>
</tr>
<tr>
<td>8</td>
<td>72 wk BHA</td>
<td>10</td>
<td>10 (100)</td>
</tr>
<tr>
<td>9</td>
<td>72 wk BHA → 24 wk BD</td>
<td>18</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>10</td>
<td>96 wk BHA</td>
<td>18</td>
<td>18 (100)</td>
</tr>
<tr>
<td>11</td>
<td>96 wk BD</td>
<td>18</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* Number of rats surviving at the time of sacrifice.
† Numbers in parentheses, percentage.
§ BD, basal diet.
¶ Significantly different from Group 1 (P < 0.001).
‖ Significantly different from Group 1 (P < 0.05).
# Significantly different from Group 5 (P < 0.001).
* Significantly different from Group 8 (P < 0.001).

was observed with papillomas (Fig. 3). Subsequent feeding without BHA for 24 wk (Group 2) resulted in complete regression of SH and papilloma. However, BCH was observed in 44% of the rats, although the decrease of incidences was significant. In Group 3, 48 wk after cessation of BHA administration, BCH persisted. BCH was still observed (Fig. 4) at an incidence of 28% in rats given the basal diet for 72 wk after 24-wk administration of the BHA diet (Group 4). In groups given BHA for 48 wk (Groups 5 to 7), similar forestomach lesions were observed; that is, just after the administration of the BHA diet (Group 5), both marked SH and papillomas with BCH were observed in all rats, while further feedings of diet without BHA for 24 or 48 wk (Groups 6 and 7) resulted in a complete regression of these changes and persistence of BCH.

In the forestomach of rats given the BHA diet continuously for 72 wk (Group 8), grayish-white, wart-like, or villous nodules were observed grossly, focally in the area near the limiting ridge. Histologically, these lesions were composed of marked

Fig. 1. Experimental design. Animals: male F344 rats, 6 wk old. 2% BHA in pellet diet; BD, pellet diet without BHA. S, sacrifice.

Fig. 2. Sequential changes of mean body weight of rats administered diet with BHA (●) or without BHA (○).

Fig. 3. Papillomas with basal cell hyperplasia in the forestomach of a rat given BHA for 24 wk. H & E, × 85.

Fig. 4. Simple hyperplasia and papillomas have disappeared, and only basal cell hyperplasia is observed in the rat given BHA for 24 wk and then basal diet for 72 wk. H & E, × 225.
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Fig. 5. Macroscopic appearance of the forestomach of a rat given BHA for 72 wk continuously. Large white polyp-like tumor protrudes into the lumen, and the forestomach near the limiting ridge is thickened. Scale, mm.

Fig. 6. Photomicrograph of marked papillomas in the forestomach shown in Fig. 5. The epithelium shows an intricate papillary growth pattern with hyperkeratosis. H & E, × 34.

Fig. 7. Early carcinoma in the forestomach of the rat given BHA for 72 wk and then basal diet for 24 wk. The carcinoma (C) is observed adjacent to an area of basal cell hyperplasia (arrow).

SH and papillomas with BCH, and no carcinoma was seen. Some papillomas showed endophytic proliferation into the submu cosa, forming keratin pearls, but atypia was not seen. Large polyp-like tumor was observed in one rat (Fig. 5). Histologically, the epithelium showed intricate papillary growth (Fig. 6), which was not observed in other rats in the group. However, within 24 wk after withdrawal of BHA (Group 9), the surface of the forestomach was smooth and wart-like, or villous nodules were no longer observed. Histologically, the squamous epithelium was nearly normal, and SH or papillomas were not observed. The submucosal layer was thickened with fibrous connective tissue, which was probably related to previous marked interstitial proliferation. Remnants of intraepithelial keratin pearls were also found. In contrast to the disappearance of SH and papillomas, some BCH still remained although no dysplastic change was observed. One early stage SCC was found, which appeared to arise from the proliferative basal cell layer (Fig. 7), suggesting the importance of BCH in the course of forestomach carcinogenesis by BHA. In controls (Group 10), no proliferative lesions were observed.

DISCUSSION

In this work, reversibility of the forestomach lesions of F344 rats administered 2% BHA diet for 24, 48, or 72 wk was examined. Results show that SH and papillomas are completely reversible within 24 wk after withdrawal of BHA. On the other hand, BCH induced during the treatment with BHA regressed much slower than SH or papillomas, and its persistence is associated with the duration of BHA exposure.

In previous studies, SH of the forestomach of rats administered BHA for up to 13 wk was shown to be reversible (15, 16). In the present study, SH and papillomas were shown to be reversible. Most importantly marked SH and papillomas induced by the continuous administration of BHA for 72 wk disappeared in 24 wk after withdrawal of BHA, leaving only submucosal fibrosis and remnants of keratin pearls. This means that SH and papillomas induced by the administration of BHA even for 72 wk are not autonomously growing lesions, but that continuous feeding of BHA is necessary for them to grow in size or to progress to carcinoma. Therefore, it can be said that BHA exerts the carcinogenic activity through the long period of continuous marked proliferation of the forestomach epithelium. This might be one possible mechanism of carcinogenesis by nonmutagenic chemicals. As the exact mechanism of development of marked hyperplasia or papilloma in the forestomach by BHA is not understood yet (19, 20), further study is necessary in this respect.

In contrast to the rapid disappearance of SH or papillomas (within 24 wk), BCH persisted for a long period (over 72 wk). It is not clear through this experiment whether BCH is neoplastic or not, but BCH resembles basal cell carcinoma in its appearance, especially when BCH is observed after a long period of BHA administration. The morphological type of basal cell carcinomas includes solid, keratotic, cystic, or adenoid forms. Some BCH observed with papillomas often resembled solid or keratotic basal cell carcinoma. In human cases, there are also mixed carcinomas which reveal SCC contiguous to a basal cell carcinoma. It is interesting that histologically, SCCs in the forestomach of BHA-treated rats often resemble the human mixed carcinomas. This might indirectly indicate the importance of BCH in forestomach carcinogenesis by BHA. In the present experiment, BCH was shown to be persistent for more than 72 wk after withdrawal of BHA treatment, and the...
longer the duration of treatment with BHA, the greater the extent of BCH. Furthermore, one early carcinoma was observed at the site of BCH in the rat given the BHA diet for 72 wk followed by the basal diet. However, BCH usually did not show atypia, and the incidence of BCH decreased after withdrawal of BHA treatment, so it might be said that BCH is simply very slow in regress after withdrawal of the causative agent. Further study is necessary to determine the meaning of BCH in forestomach carcinogenesis.

REFERENCES


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