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Carcinogenicity of naturally occurring 1-hydroxyanthraquinone in rats: induction of large bowel, liver and stomach neoplasms

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The carcinogenic potential of 1-hydroxyanthraquinone (HA), a naturally occurring compound, was examined. A total of 60 male ACI/N rats, 1.5 months old at the commencement were divided into two groups. Group 1 (30 rats) were fed the diet containing HA at a concentration of 1% throughout the experiment (480 days). Group 2 (30 rats) served as the control given a basal diet alone. Twenty-five of 29 effective animals in group 1 developed adenomas or adenocarcinomas in the cecum or upper portion of the colon, the mean number of large bowel tumors/tumor bearing rat being 2.3. In addition to these intestinal tumors, liver neoplasms (neoplastic nodules and hepatocellular carcinomas) were observed in 12 rats and benign stomach tumors were obtained in five animals; no rats of group 2 demonstrating development of any of these tumor types. The incidences of the large bowel, liver and stomach neoplasms in group 1 were all significant as compared with group 2 (P < 2 x 10^-13, P < 5 x 10^-5 and P < 3 x 10^-2 respectively) clearly indicating that HA is carcinogenic in rats.

Introduction

In recent years, it has become increasingly apparent that environmental factors play a dominant role in human carcinogenesis (1,2). Naturally occurring carcinogens contained in diets or others have been realized as one of the important factors (3–5), although it is particularly difficult to associate these agents with specific kinds of cancer. Anthraquinones represent the largest group of naturally occurring quinones. Both natural and synthetic anthraquinones have been widely used as colorants in food, drugs, cosmetics, hair dyes and textiles (4,6). However, little information on their hazards to man has yet been accumulated, and although bacterial mutagenicity has been examined and proved positive in some cases (4,6–8), in vivo carcinogenicity or toxicity of these agents has been scarcely investigated. Chrysazin (1,8-dihydroxyanthraquinone), a synthetic anthraquinone which has found use as a popular laxative drug (4,9) is one exception. Mutagenic in Salmonella typhimurium (6,7), it also demonstrated genotoxicity in the hepatocyte primary culture (HPC*)/DNA repair assay (10), and was found to be tumorigenic in the long-term when administered to rats (11) or mice (12). The related 1-hydroxyanthraquinone (HA) is contained in some plants like Tabebuia avellanedae (13) or Cassia occidentalis (14). No clear evidences that the chemical is related to human diets are yet available in literature. However, the plants containing the chemical have been widely used for pharmaceutical purposes such as the treatment of kidney and bladder stones, a laxative mixture and a mild sedative (14,15). In our previous study for genotoxicity in the HPC/DNA repair assay, HA generated stronger DNA repair response than chrysazin (16).

Presently, carcinogenic potential of this phenolic anthraquinone after long-term dietary administration to rats was evaluated. Such evaluation is considered warranted, since it could be possible that humans are exposed to such chemical for a long time mainly through drugs as herbal remedies.

Materials and methods

Two groups of male ACI/N rats, a strain which has been maintained as an inbred line at our laboratory (11), 1.5 months old at the commencement were used in the study.

**Fig. 1.** Growth curves of rats served as controls (group 2) and rats treated with HA (group 1).

**Fig. 2.** Survival curves of rats served as controls (group 2) and rats treated with HA (group 1).
Fig. 3. Gross appearance of the large bowel tumors in a rat of group 1, which died at day 428. A large tumor growing through the gut wall of the colon. A pedunculated junctional polyp of the colon protruding into the cecum. A small polyp in the cecum.

Group 1
Thirty rats were fed a basal diet CE-2 (Japan CLEA Inc., Tokyo, Japan) containing HA at a concentration of 1% throughout the experiment. HA was purchased from Tokyo Chemical Industry (Tokyo, Japan) and confirmed to be pure on TLC using a benzene/acetone (9/1) system (Rf value: 0.53). The mixture of HA (1%) showed no detectable alteration in absorption spectrum even after storage for 1 month at room temperature, indicating analytical purity and stability in the diet.

Group 2
Thirty rats were fed the basal diet without HA and served as controls. Food and water (distilled water) were given ad libitum, and the experiment was terminated 480 days after the start. The animals were inspected and weighed once every 2 weeks for the initial 2 months, and once a month for the subsequent duration of the experiment. They were autopsied at death, after being killed when they became moribund, or after sacrifice under ether anesthesia at the termination of the experiment. Tissues from all major organs were fixed in 10% buffered formalin solution (pH 7.4), sectioned, stained with hematoxylin and eosin, and examined histologically.

Results
Changes in the body weight of rats in the two groups are shown in Figure 1, the administration of HA being associated with decreased weight gain which was particularly marked towards the termination of experiment. In general, no rats from group 1 suffered obvious diarrhea, although their feces were rather soft as compared with those of rats in group 2. Survival curves of rats of both groups are given in Figure 2. One of the 30 rats in group 1 died of pneumonia 243 days after the start of experiment. A second rat died in an unmourished state at day 280, demonstrating a large tumor in the colon. Seven animals of the group died spontaneously or were sacrificed upon becoming moribund between 335 and 462 days. A total of 21 rats of group 1 survived until the end of experiment (mean value of total intake of HA/rat was 76.8 g). Out of 29 effective rats in the group, 25 had intestinal neoplasms. They were all pedunculate or sessile polyps arising in the cecum or the upper third of the colon (Figure 3). The mean number of these tumors/tumor bearing rats was 2.3. Histologically, they were adenomas or adenocarcinomas (Figure 4) (Table I). Besides these tumors, focal hyperplastic lesions of the glandular epithelium of the cecum or colon were frequently encountered in rats with or without neoplasms. In addition, a total of 12 rats in group 1 developed liver neoplasms diagnosed as neoplastic nodules or hepatocellular carcinomas (Figure 5). All rats bearing hepatocellular carcinoma also had neoplastic nodules (Table II) and altered hepatocellular foci were frequent in all of the effective rats of this group. Furthermore, five rats from group 1 had benign stomach neoplasms. They were all solitary tumors (Table II), one being a glandular stomach adenoma and the others being papillomas.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Effective no. of rats</th>
<th>No. of rats with:</th>
<th>No. of rats with large bowel tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cucum</td>
<td>Adenoma</td>
</tr>
<tr>
<td>Group 1 (1% HA)</td>
<td>29</td>
<td>10^5 (15)</td>
<td>5^2 (7)</td>
</tr>
<tr>
<td>Group II (Control)</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Animals surviving >280 days after the start. Numbers in parentheses are of total tumors. P = 3.2 x 10^-4. 3P = 2.4 x 10^-4. 4P = 4.6 x 10^-5. 5P = 1.2 x 10^-4. 6P = 1.6 x 10^-13 (Fisher's exact probability test).
of the forestomach. A Leydig cell tumor was also recognized in one rat of this group. All rats from group 2 survived until the end of the experiment. No tumors or hyperplastic lesions were found with the exception of one Leydig cell tumor and one cortical adenoma of the adrenal gland in another animal. Statistically, the incidences of the large bowel tumors, liver neoplasms and stomach tumors of group 1 were all significant as compared to their absences in group 1 (Tables I and II).

Discussion

It is notable that several anthraquinones demonstrate genotoxic activities especially in bacterial mutagenicity assays (4-7). However, only few studies on the mammalian carcinogenicity of these chemicals have been performed. Although carcinogenicity has been reported for anthraquinones substituted with amino groups (17) or nitro groups (18) or some anthraquinoid compounds such as adriamycin (19) and luteoskyrin (20), the evidence for carcinogenic effects of anthraquinone themselves was limited to the chrysazin case (11,12). In the present study, the majority of rats given HA developed intestinal neoplasms. Furthermore, 40% of the effective animals exposed to the chemical demonstrated hepatocellular neoplasms. The results thus clearly indicate that HA is carcinogenic in rats. The fact that the intestinal tumors were observed in the colon or the upper portion of the colon of animals receiving HA, the same sites being involved in neoplastic development induced by chrysazin (11,12) indicates that both chemicals have similar organotropism. However, the incidence of intestinal neoplasms in the HA case exceeded that observed with chrysazin given to rats of the same strain at the same concentration (11). In addition, HA induced hepatocellular and stomach neoplasms which were not obtained with chrysazin, suggesting that the carcinogenic potential of HA is greater and of wide spectrum.

The present results are basically in agreement with our previous data from genotoxicity assays of anthraquinone in the HPC/DNA repair test system (13). Beyond a clear DNA involvement, however, the mechanism by which HA elicits its tumorigenic effects is unclear. Swanbeck and Zetterberg (21,22) have studied the ability of 20 phenolic anthraquinones to bind to calf thymus DNA in vitro and found that most phenolic anthraquinones and anthracenes exhibit a strong reaction. The related luteoskyrin is known to form a complex with DNA and inhibit DNA-dependent RNA polymerase (23), and our previous result that HA generated unscheduled DNA synthesis in hepatocytes also indicates substantial DNA reactivity. Furthermore, involvement of a relatively non-specific free radical mechanism in the mutagenicity or DNA damage by anthraquinone chemicals has been suggested (6), and photodynamic production of superoxide is recently demonstrated with several anthraquinones (24). Very recently, we performed an analysis of quantum chemical properties of 19 anthraquinones and found that electrophilic frontier densities in the β position may be associated with genotoxic or carcinogenic capability (unpublished results). Our group has also demonstrated that chrysazin is biotransformed selectively by the NADPH-microsome system (25). It is probable that HA is metabolized in hepatocytes, and hepato-intestinal circulation of activated metabolites of the chemical is necessary for the expression of carcinogenicity. In the present study, dietary administration of HA at the concentration of 1% was not associated with obvious diarrhea, in contrast to the chrysazin case. It thus appears that the carcinogenic action in the large bowel of these anthraquinones is not directly related to their laxative effect.

In general, little is known concerning the chronic toxicity or carcinogenicity of anthraquinone chemicals despite their use for many purposes including pharmaceutical preparations. The results of the present study and the earlier demonstration of tumor induction by chrysazin (11,12) strongly suggest that screening of genotoxic anthraquinones and detailed assessment of the human

Fig. 5. Histology of a well differentiated hepatocellular carcinoma in a rat of group 1. H&E, ×110.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Effective no. of rats</th>
<th>No. of rats with:</th>
<th>No. of rats with:</th>
<th>No. of rats with:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>Neoplastic module</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Group I</td>
<td>29</td>
<td>12b (18)</td>
<td>4 (4)</td>
<td>12b (22)</td>
</tr>
<tr>
<td>1% HA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
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*Animals surviving >280 days after the start. Numbers in parentheses are of total tumors. bP = 4.6 × 10^{-5}, cP = 2.4 × 10^{-2} (Fisher's exact probability test).
hazards of these compounds must be pursued. Very recently, we found a clearly synergistic effect of HA with another carcinogen on large bowel carcinogenesis in rats (unpublished results). Such data may be important for evaluation of carcinogenic risk of anthraquinones as well as for elucidation of the mode of actions of their carcinogenicity.

References

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