Tumorigenesis Studies with 1,2-Dimethylhydrazine Dihydrochloride, Hydrazine Sulfate, and Isonicotinic Acid in Golden Hamsters

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SUMMARY

Solutions of 0.012% hydrazine sulfate, 0.001% 1,2-dimethylhydrazine dihydrochloride, and 0.5% isonicotinic acid were given continuously in the drinking water of 9-, 7-, and 5-week-old randomly bred Syrian golden hamsters for the remainder of their lifetime. The consumption of hydrazine sulfate and isonicotinic acid was without any significant carcinogenic action, while 1,2-dimethylhydrazine dihydrochloride induced angiosarcomas of the blood vessels with incidences of 89% in females and 82% in males. In addition, it produced an appreciable number of tumors in the cecum and liver.

These findings clearly show that the carcinogenic effect of hydrazine sulfate is species dependent. Results also indicate that the various organotropic actions of 1,2-dimethylhydrazine dihydrochloride when administered by another route in the same species could possibly be due to different metabolic pathways.

INTRODUCTION

After the discovery of the carcinogenicity of INH in mice, studies have been directed towards 2 main areas. First, investigators wondered whether or not the INH metabolites, including hydrazine, IA, etc., are producing any tumors (3, 15). Second, it appeared promising to investigate the possible tumor-inducing capability of the numerous hydrazine derivatives which are widely used in industry, agriculture, and even medicine.

Hydrazine by itself and as sulfate induces tumors in mice, as reported by a number of studies (3, 6, 14). However, Syrian golden hamsters have also received HS by stomach tube, and their response has been without any apparent carcinogenic action (1).

Strangely enough, at the 1st attempt 1,2-dimethylhydrazine in mice failed to induce neoplasms (7). This study was followed by another that clearly demonstrated its intestinal tumor-inducing capability by s.c. administration (22). When given similarly in golden hamsters, it evoked tumors of the liver, stomach, and intestines (9), and in several strains of rat the compound also elicited the development of various intestinal neoplasias (4, 11). IA given as sodium salt to mice exerted a very low tumor-producing capability, and in subsequent investigations by the same and other workers it was found to be without any carcinogenic effect (2, 3, 15).

The present study, as part of our systematic investigation in this field, was designed to find out the possible tumorigenicity of HS, 1,2-DMH, and IA administered continuously in the drinking water for the life-span of the Syrian golden hamster.

MATERIALS AND METHODS

Syrian golden hamsters from the colony randomly bred by us since 1959 were used. They were housed in plastic cages with granular cellulose bedding, separated according to sex in groups of 5, and given Rockland diet in pellets and the chemicals in tap water ad libitum as described below.

The chemicals used were HS (Fisher certified, ACS, Fisher Scientific Company, Fair Lawn, N. J.); 1,2-DMH, symmetrical (K and K Laboratories, Inc., Plainview, N. Y.); and IA (Eastman Organic Chemicals, Rochester, N. Y.). The solutions were prepared thrice weekly, and the total consumption of water containing the chemicals was measured at the same intervals during the treatment period. All the solutions were contained in brown bottles because of the possible light sensitivity of the chemicals. The experimental groups and the treatments were as follows.

Group 1. HS was dissolved in the drinking water as a 0.012% solution and given continuously for the life-span of 50 female and 50 male hamsters 9 weeks old at the beginning of the experiment. The average daily consumption of water with HS in it per animal was 19.1 ml for the females and 18.8 ml for the males. The average daily intake of HS per hamster, therefore, was 2.3 mg for both sexes.

Group 2. 1,2-DMH was dissolved in the drinking water as a 0.001% solution and given continuously for the life-span of 50 female and 50 male hamsters 7 weeks old at the beginning of the experiment. The average daily consumption of water with 1,2-DMH in it per animal was 15.6 ml for the females and 16.1 ml for the males. The average daily intake of 1,2-DMH, therefore, was 0.156 mg for a female and 0.161 mg for a male.
Group 3. IA was dissolved in the drinking water as a 0.5% solution and given continuously for the life-span of 50 female and 50 male hamsters 5 weeks old at the beginning of the experiment. The average daily consumption of water with IA in it per animal was 15.5 ml for the females and 17.4 ml for the males. The average daily intake of IA, therefore, was 77.5 mg for a female and 87 mg for a male.

All the animals were carefully checked and weighed at weekly intervals, and the gross pathological changes were routinely recorded. The animals were allowed to die or were killed with ether when found to be in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and fixed in 10% buffered formalin. Histological studies were performed on the spleen, liver, kidney, 4 lobes of lungs, selected lymph nodes, and any additional organs that showed gross pathological changes. Sections from these tissues were stained routinely with hematoxylin and eosin and by additional special methods when necessary.

RESULTS

The survival rates at 10-week intervals are recorded in Table 1. HS and IA treatments had no effect on survival, while 1,2-DMH significantly reduced it when compared with the corresponding controls (13). The average weekly weight curves of the treated and control hamsters were recorded throughout the experiments, and the treatments with HS and IA somewhat reduced the weights, while 1,2-DMH drastically lowered them, especially after the age of 30 weeks, when compared with the controls (13).

The number, incidences, and latent periods of all obtained tumors in the variously treated groups are summarized in Table 2. 1,2-DMH induced appreciable incidences of tumors of blood vessels, cecum, and liver, while the other 2 chemicals failed to evoke significant incidences of neoplasms.

Tumors of Blood Vessels. In the 1,2-DMH-treated females, 44 hamsters with an incidence of 89% developed such tumors. Their average latent period was 51 weeks; the 1st was found at 29 weeks and the last at the 74th week of age. In the 1,2-DMH-treated males, 41 animals with an incidence of 82% developed tumors of the vascular system. The average latent period of this neoplasm was 52 weeks; the 1st was observed at the 29th week and the last at the 76th week. The location and frequency of these lesions in the various tissues were in the following order: liver, lungs, muscle, heart, and pancreas. The gross and light and electron microscopic examinations of these lesions revealed the typical appearance of angiosarcomas. In general, they were similar to those induced in the blood vessels of mice by this compound (21). Another publication will deal with the morphological characteristics of the tumors.

Tumors of Cecum. In the 1,2-DMH-treated females, 17 animals with an incidence of 34% developed such lesions. Out of these, 8 had polypoid adenomas at 31, 38, 43, 53, 56, 67, 69, and 71 weeks; 3 had leiomyosarcomas at 52, 69, and 69 weeks; and 6 had adenocarcinomas at 32, 52, 54, 56, 69, and 71 weeks of age. In the males of this group, 6 hamsters developed cecal tumors with an incidence of 12%. All were classified as polypoid adenomas. Their average latent period was 61 weeks; the 1st was found at the 44th week and the last at the 76th week.

Macroscopically and histologically, the obtained tumors of cecum were similar to those found and described recently with urethan treatments in this species (16).

Tumors of Liver. In the 1,2-DMH-treated females, 10 hamsters with an incidence of 20% developed such tumors. Out of these, 7 were classified as benign hepatomas and 3 were classified as malignant liver cell carcinomas. In the males of this group, 7 animals with an incidence of 14% developed hepatic lesions. Out of these, 4 were classified as hepatomas and 3 as liver cell carcinomas.

In many 1,2-DMH-treated animals, skin pigmentation increased, with the appearance of black spots and nodules on the back and flanks. Only those 2 mm or larger in their longest diameter were classified as dermal melanocytomas.

In addition to these tumors, a number of other neoplasms occurred in the variously treated groups (Table 2).

The microscopic examinations of the different tissues treated with the other 2 chemicals revealed the following pathological changes. In the HS-treated animals, in the liver there were dilated vessels and sinusoids, vacuolated cells, biliary cyst formations, and rearranged architecture, which in a few instances was accompanied by regenerative nodules. In IA-treated hamsters, there was some biliary cyst formation, and in a few cases regenerative nodules were seen also in the liver.

DISCUSSION

The continuous administration of 0.012% HS and 0.5% IA in the drinking water for the life-span of adult, randomly bred Syrian golden hamsters resulted in no detectable carcinogenic
Table 2

Tumor distribution in HS-, 1,2-DMH-, and IA-treated golden hamsters

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Effective no. and sex of hamsters</th>
<th>Tumors of vessels</th>
<th>Tumors of cecum</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>Latent period (age in wk)</td>
</tr>
</tbody>
</table>
| 1     | 0.012% HS in drinking water daily for life | 45F  | 4   | 8   | 65 (56–77) | 4 adenomas of thyroid (47, 84, 107, 114)
|       |           |                                   | 49M  | 3   | 6   | 94 (71–119) | 3 papillomas of forestomach (62, 66, 104)
| 2     | 0.001% 1,2-DMH in drinking water daily for life | 49F  | 44  | 89  | 51 (29–74) | 5 papillomas of forestomach (32, 78, 91, 91, 98)
|       |           |                                   | 50M  | 41  | 82  | 52 (29–76) | 3 adenocortical carcinomas (98, 126, 126)
| 3     | 0.5% IA in drinking water daily for life   | 44F  | 2   | 4   | 83 (79–88) | 7 hepatomas (51, 53, 54, 59, 59, 60, 69)
|       |           |                                   | 45M  | 2   | 4   | 104 (85–123) | 3 liver cell carcinomas (56, 59, 63)
|       |           |                                   |       |     |     |             | 1 neuroblastoma (51)
|       |           |                                   |       |     |     |             | 1 adenocarcinoma of glandular stomach (53)
|       |           |                                   |       |     |     |             | 1 adenocarcinoma of breast (74)
|       |           |                                   |       |     |     |             | 2 dermal melanocytomas (52, 54, 54)
|       |           |                                   |       |     |     |             | 4 hepatomas (41, 41, 41, 64)
|       |           |                                   |       |     |     |             | 3 liver cell carcinomas (48, 56, 76)
|       |           |                                   |       |     |     |             | 1 papilloma of forestomach (38)
|       |           |                                   |       |     |     |             | 1 polyploid adenoma of colon (66)
|       |           |                                   |       |     |     |             | 1 malignant lymphoma (56)
|       |           |                                   |       |     |     |             | 2 papillomas of forestomach (95, 110)
|       |           |                                   |       |     |     |             | 1 carcinoma of ovary (112)
|       |           |                                   |       |     |     |             | 1 fibromyosarcoma of uterus (91)
|       |           |                                   |       |     |     |             | 2 adenomas of thyroid (76, 123)
|       |           |                                   |       |     |     |             | 1 liver cell carcinoma (102)
|       |           |                                   |       |     |     |             | 1 adenocortical adenoma (85)
|       |           |                                   |       |     |     |             | 1 adenocortical carcinoma (101)
|       |           |                                   |       |     |     |             | 1 papilloma of trachea (123)
|       |           |                                   |       |     |     |             | 1 papilloma of forestomach (112)
|       |           |                                   |       |     |     |             | 1 malignant lymphoma (107)

a Values in parentheses, latent periods (age in weeks).

effect. Although it appears that the number of polypoid adenomas of cecum was somewhat higher in the HS-treated than in the untreated hamsters, statistically the difference was not significant. In addition, similar administration of 0.001% 1,2-DMH, symmetrical, induced angiosarcomas of blood vessels, with incidences of 89% in females and 82% in males. It also produced an appreciable number of tumors in the cecum and liver.

The current observation concerning the effect of HS on golden hamsters corroborates the findings of a previous study done in another laboratory. Accordingly, no tumor induction was observed when the compound was given daily by stomach tube for 60 and 100 days (1). The main difference between the 2 investigations was the length of the treatments, i.e., in the present one the chemical was administered continuously for the entire life-span. With regard to the effect of IA, no tumors of any kind were induced under the present experimental conditions in golden hamsters.

In the 1st study, when IA compound was administered p.o. as sodium salt to BALB/c mice, it was claimed to have induced multiple lung adenomas with an incidence of 19%. From this the authors concluded that the compound is a very low-tumor-producing chemical. In their subsequent study, they gave the same chemical to CBA/Cb/Se mice and found it to be without any tumorigenic action (2, 3). Finally, IA has been given continuously in the drinking water for the life-span of Swiss, AKR, and C3H mice in this laboratory without producing any tumors (15).

Earlier, 1,2-dimethylhydrazine given by repeated s.c. injections to golden hamsters by other workers induced liver, stomach, and intestinal tumors (9). Strangely enough, no tumors of the blood vessels were seen by this investigator. In contrast, in the current study the induction of angiosarcomas of blood vessels was the main neoplastic response. No proper explanation can be provided for the difference; nevertheless, it could very well be that the metabolism of the compound when
given s.c. and p.o. is different and could account for its tissue-specific carcinogenicity. Interestingly, in mice and rats the chemical induced intestinal tumors in other laboratories (4, 11, 22) and again mainly blood vessel neoplasm in the former species when tested by us (21).

The carcinogenicity of INH in mice has been shown unequivocally (3, 5, 8, 10, 12, 18, 20). One of its metabolites, hydrazine, has also been demonstrated to be a carcinogen in mice (6, 14). In view of the fact that INH is not carcinogenic in hamsters (10, 17, 19), it was thought that this might be explainable on the basis of metabolism, i.e., a carcinogenic metabolite which may be absent in the hamsters could be responsible for tumor induction in mice. The current investigation thus clearly demonstrated that if hydrazine and/or IA are produced from INH in hamsters, they cannot be considered as tumor-producing substances.

Studies with HS and 1,2-DMH are part of the systematic investigation aimed at determining the possible relationship between the chemical structure of substituted hydrazines and tumor development at specific organ sites. With identical methods of treatment, i.e., p.o. ad libitum drinking water administration and 2 hosts, Swiss mice and golden hamsters, the available results indicate that there exists a clear-cut variation in the carcinogenic responses of different organs to hydrazine derivatives which is, up to a certain extent, species dependent.

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REFERENCES


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