Methylhydrazine Tumorigenesis in Syrian Golden Hamsters and the Morphology of Malignant Histiocytomas

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SUMMARY

A 0.01% solution of methylhydrazine was administered daily in the drinking water of 6-week-old randomly bred Syrian golden hamsters for the remainder of their lifetime. The treatment gave rise to malignant histiocytomas of liver and tumors of the cecum. Thirty-two % of the females and 54% of the males developed malignant histiocytomas whereas among the controls no such lesions were seen. The incidence of tumors of cecum was 18% in females and 14% in males, compared to 1% in the controls.

Macroscopic, light and electron microscopic, and histochemical investigations of the liver lesions showed the characteristic appearance of malignant histiocytomas. The histological involvement of the tissues by the tumors is presented. The fine structure of the malignant histiocytoma, the nuclei, and cytoplasms with organelles of the tumor cells are described and illustrated in detail.

Methylhydrazine is a rocket fuel component, and some of its derivatives have been proposed for use in treating certain human cancers. Because it has been shown to produce tumors in mice and now in hamsters, precautions should be taken concerning its use.

INTRODUCTION

Systematic studies on the carcinogenic action of substituted hydrazines in mice and hamsters have been in progress in this laboratory since 1968. These studies have sought to determine (a) whether a relationship exists between chemical structure and tumor development at specific organ sites, (b) whether neoplastic reaction is species dependent, and (c) whether these chemicals represent major environmental hazards. In mice, hydrazine, MH, 1,2-dimethylhydrazine, 1,1-dimethylhydrazine, and benzoylhydrazine induced lung tumors. In addition, 1,2-dimethylhydrazine and 1,1-dimethylhydrazine produced blood vessel tumors. Furthermore, 1,1-dimethylhydrazine also caused kidney and liver tumors and benzoylhydrazine produced malignant lymphomas (33, 35, 38, 40, 42). In hamsters, hydrazine had no carcinogenic effect, but 1,2-dimethylhydrazine gave rise to tumors of blood vessels, cecum, and liver (36, 37). Thus, it appears that tumor type is somewhat dependent on chemical structure.

Earlier, MH and MH sulfate were studied in mice for tumorigenic effect but failed to produce tumors (14, 29). More recently, however, it was shown that these chemicals enhanced the development of lung tumors when administered for life to mice (35).

This report describes the carcinogenic effect of MH in Syrian golden hamsters and the morphology of malignant histiocytomas produced by the treatment.

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 and were separated into groups of 5 according to sex. Wayne Lab-Blox regular diet (Allied Mills, Inc., Chicago, Ill.) and tap water were given ad libitum.

The chemical used was MH (M.W. 46.07, b.p. 87-88°) obtained from Eastman Organic Chemicals, Rochester, N. Y.; its chemical structure is:

\[ \text{CH}_3\text{NHNH}_2 \]

The treated groups and the controls were as follows.

The toxicity study was carried out prior to the chronic experiment. Five dose levels of MH, such as, 1, 0.1, 0.01, 0.001, and 0.0001%, were administered in the drinking water for 35 days to golden hamsters. Each group consisted of 8 animals, 4 females and 4 males. By taking into account 4 parameters: survival rates, body weights, chemical consumption figures, and histological changes, the 0.01% dose was found to be suitable for the lifelong treatment. This toxicity technique for chronic carcinogenesis experiment was developed in this laboratory and described recently in detail (39).

Group 1. MH was dissolved in the drinking water as a 0.01% solution and was given continuously for life to 50 female and 50 male hamsters 6 weeks old (44 days) at the beginning of the experiment. The solution was prepared 3 times weekly and the total water consumption containing MH was measured at the same intervals. The average daily consumption of MH-containing water per animal was 14.8 ml for females and 12.9 ml for males. Therefore, the average daily intake of MH was 1.3 mg for a female and 1.1 mg for a male.


Group 2. As a control, 100 females and 100 males were kept untreated.

The experimental and control animals were carefully checked and weighed weekly, and the gross observable changes were recorded at the same time.

For the light microscopic examination, the animals either were allowed to die spontaneously or were killed with ether when they were found to be in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histological studies were done on the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testis, brain, nasal turbinale, and at least 4 lobes of the lungs of each hamster as well as on those organs showing gross pathological changes. Sections from these tissues were stained routinely with hematoxylin and eosin. In addition, the following special stains were used: Snook’s silver method for reticulum (32); McManus’ periodic acid-Schiff reaction for polysaccharides, mucopolysaccharides, and glycolic fatty acids (21); Berlin blue stain for ionized iron (30); and Sudan black B method for fat (16, 18).

For electron microscopic examination, tumor tissues of 10 different animals were taken (approximately 1-mm cubes) and were fixed in buffered 1% osmium tetroxide at 4°C (5, 25). From each tumor 5 specimen blocks were dehydrated in ethanol and passed through propylene oxide and embedded in an Araldite (502) epoxy resin mixture (17). Thick sections (1 μ) were cut from each block and the slides were stained with 0.2% toluidine blue (43) and examined by light microscopy. Five thin sections from each block were cut with glass knives on a Porter-Blum MT-1 ultramicrotome and stained with lead citrate (28, 45) and uranyl acetate (46). Finally, the sections on bare grids were examined at 60 kV in a Philips 300 electron microscope.

RESULTS

The survival rate at 10-week intervals is recorded in Table 1. It is clear from this that MH treatment substantially reduced the survival when compared with the corresponding controls.

Table 2 presents the numbers of various types of tumors, their percentages, and latent periods. The following 2 types of neoplasms are described below in detail.

Malignant Histiocytoma (Kupffer’s Cell Sarcoma) of Liver. In the MH-treated females, 16 hamsters (32%) developed such tumors. The average latent period was 70 weeks, the first was found at the 46th week and the last at the 92nd week of age. In the MH-treated males, 27 animals (54%) developed malignant histiocytomas. Their average latent period was 78 weeks, the first was observed at 47 weeks and the last at 103 weeks of age.

The tumor in all instances was found in the liver. In addition, the tumor growth was observed in 6 lungs, 2 lymph nodes, and 2 spleens.

Grossly, the tumor usually grew in diffuse manner, infiltrating the various lobes of liver and exhibiting yellowish color (Fig. 1). In a few instances, however, the lesion exhibited nodular forms which were well circumscribed (Fig. 2).

The light microscopic examination revealed the typical appearance of the malignant histiocytoma. Apparently, at the beginning, the histiocytes grew along the sinusoidal walls and seemed darker than the surrounding cells. Also, many sinusoids were dilated. This stage perhaps represented the starting of malignant transformation. The well-developed histiocytoma sometimes exhibited nodular appearance. The malignant cells replaced the normal architecture of liver and spread in the sinusoids at the periphery of the nodule (Fig. 3). The cytoplasm of malignant histiocytes were abundant, pale, and vacuolated, which seemingly represented the place of fat droplets. Their nuclei were usually small, round or oval, darkly stained, and relatively regularly in size, and the nuclear membranes were distinct (Figs. 4 and 5). It seemed that the tumor disseminated to other organs through vascular channels. In a number of cases the entire lumen of blood vessel in the liver was obliterated by the neoplastic histiocytes (Fig. 6). In 6 animals, the tumors metastasized into the lungs. The blood vessel was filled with the neoplastic cells (Fig. 7). One of the features of this tumor was the presence of the bizarre, giant multinucleated cells. These cells had large cytoplasm which were usually vacuolated. They contained numerous nuclei that were located at the periphery (Fig. 8).

Histochemical work was performed on 10 tumor growths. In the lesion, moderate amounts of reticulum were shown by Snook’s method. The Berlin blue stain for ionized iron has demonstrated large amounts of deposits. The polysaccharides, mucopolysaccharides, and glycolic fatty acids content of the tumor were identified by the McManus periodic acid-Schiff technique which showed definite reaction. Finally, the Sudan black B method for fat was used, which revealed high amounts in the tumor cells.

Study on the ultrastructural characteristics of the tumor revealed that they were composed of neoplastic histiocytes which formed solid network. The cells were rich in organelles and their cytoplasm contained large amounts of lysosomes. Many irregular vacuoles were observed, some of which had membranes that may represent endoplasmic reticulums. Other droplets were associated with dense material assumed to be part of the lysosome structure (Fig. 9). The neoplastic cells were relatively closely attached to each other and to the normal surrounding hepatocytes. In between them the Disse spaces were observable. The cytoplasm of histiocytes contained many organelles; large amounts of lysosomes, as well as mitochondria, endoplasmic reticula, vesicles, and ribosomes, were visible. The nuclei were usually round or oval and sometimes indented. In the nuclei, the chromatin was often marginalized. In addition it was also observed in the central areas in clusters. Inclusion-like bodies were also seen (Fig. 10). The surface area of the neoplastic histiocyte had several irregular projections of various sizes. The peripheral area of the cytoplasm contained pinocytotic vesicles, free ribosomes, and other organelles (Fig. 11). In the cytoplasm of these cells, many lysosomes, mitochondria, free ribosomes, endoplasmic reticulum, Golgi complex, etc., were seen. In the multinucleated giant cell, the nuclei were closely packed and were essentially similar to that of the mononucleated histiocytes. Their cytoplasm was abundant and was loaded with lyso-
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Table 1

Treatment and survival rate in MH-treated and control golden hamsters

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial no. and sex of hamsters</th>
<th>No. of survivors at Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01% MH in drinking water daily for life</td>
<td>50 F 49 48 48 47</td>
<td>39 27 16 4 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 M 50 49 48 48</td>
<td>43 39 30 18 8 2</td>
</tr>
<tr>
<td>2</td>
<td>Untreated Control</td>
<td>100 F 100 100 100 92</td>
<td>74 61 46 31 20 7 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 M 96 93 90 87</td>
<td>80 74 57 42 32 22 15 10</td>
</tr>
</tbody>
</table>

somes, granular bodies that appeared to be membrane bound and presumably part of the lysosomal systems, mitochondria, vesicles, etc.

Tumors of Cecum. In the MH-treated females, 9 hamsters (18%) developed 13 such lesions. Of these, 7 animals had 9 polypoid adenomas, 1 had a polypoid adenoma and an adenocarcinoma and 1 had 2 adenocarcinomas. The average latent period was 64 weeks, the first was observed at the 50th week and the last at the 76th week of age. In the MH-treated males, 7 animals (14%) developed 9 such tumors. Of these, 5 hamsters had 6 polypoid adenomas, 1 had 1 polypoid adenoma and an adenocarcinoma, and 1 had an adenocarcinoma. Their average latent period was 77 weeks, the first was found at the 64th week while the last at the 94th week of age.

In the untreated controls in 2 hamsters, 2 polypoid adenomas were observed, a female at the 53rd week and a male at the 84th week of age.

The diagnostic criteria and the morphological illustration of cecal tumors were described and shown in a previous publication (34).

Other Tumors. Table 2 also shows the various other types of tumors that were seen in the treated and control animals. Since they occurred in low incidences, they cannot be attributed to the treatment.

DISCUSSION

The present study reports the induction of malignant histiocytomas of the liver and tumors of the cecum in Syrian golden hamsters treated with a 0.01% solution of MH in the drinking water daily for life. Thirty-two % of the females and 54% of the males developed malignant histiocytomas; this type of tumors was not seen in the untreated controls. In addition, the percentage of tumors of the cecum rose from 1 to 18% in the females and from 1 to 14% in the males compared to the controls.

The morphological characteristics of malignant histiocytomas were studied by light and electron microscopy and histochemistry. Forty-three tumors were observed in the liver and, in addition, tumor growths were found in 6 lungs, 2 lymph nodes, and 2 spleens. Histologically, they were malignant histiocytomas or Kupffer's cell sarcomas. Ionized iron, fat, reticulum, and polysaccharides, mucopolysaccharides, and glycolic fatty acids contents were studied histochemically. Large amounts of ionized iron and reticulum were observed, while the other 2 substances exhibited lesser reactions. The electron microscopic examination showed the fine structure of the well-developed lesion, the cell nuclei with chromatin arrangement, the cytoplasms with organelles including the prominent lysosomes and lipid droplets, and the cell surfaces with the various projections.

In man, a variant of the presently induced lesion occurs, described as malignant histiocytosis. It is a relatively rare, systemic neoplastic disease occurring in adults and children. It has 2 clinical forms, cutaneous and visceral. So far, the etiological agent is unknown (27).

In mice, the lesion has been described by a number of investigators. Dunn (9) proposed the term reticulum cell neoplasm type A, while previously used terms included monocytoma or monocytosis (10), monocyctic leukemia (15), reticuloendothelioma (6), and malignant histiocytoma (11, 41). Some of these lesions occurred spontaneously; others, however, were induced chemically.

MH has been shown to be embryotoxic and teratogenic in mice (22). It also impaired renal function in dogs (44) and caused convulsions in mice, rats, rabbits, and dogs (24, 48). Its vapor produced hemolysis, elevated temperatures, and pulmonary edema in various species (12). MH has also been shown to break DNA strand in mice (23), form complexes with nickel (1), cause behavioral stimulations in monkeys (47), curtail the mobilization of fat from adipose tissue, and decrease respiratory quotient (2). Also, it was absorbed when applied on the skin of dogs (31). In rats, MH is metabolized to carbon dioxide and methane (8, 26) and is oxidatively demethylated to yield formaldehyde by rat liver microsomes (49). It also inhibited some drug metabolizing liver microsomal enzyme systems (13). Furthermore, it was demonstrated that pyridoxine and pyridoxamine are effective antidotes for the toxicity of MH (7).

MH is used in industry as a rocket propellant and some of its derivatives, 1-methyl-2-p-(isopropyl-carbamoyl)benzyl-hydrazine and N-isopropyl-α-(methylhydrazino)-p-toluamide HCl (3, 4, 19, 20), are used as carcinostatic agents in the treatment of Hodgkin's disease, malignant melanoma, and various forms of hematological abnormalities. In view of the reported carcinogenicity of MH in mice (35) and now in hamsters, its hazardousness should receive attention.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Henry Rappaport for diagnostic help, Dr. Richard Wilson for advice on the interpretation of ultrastructure, Tim

Table 2
Tumor distribution in MH-treated and control golden hamsters

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Effective no. and sex of animals</th>
<th>Malignant histiocytomas</th>
<th>Tumors of cecum</th>
<th>Other tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. %</td>
<td>Latent periods (age in wk)</td>
<td>No. %</td>
</tr>
<tr>
<td>1</td>
<td>0.01% MH in drinking water daily for life</td>
<td>49 F</td>
<td>16 32 70 (46-92)</td>
<td>9 18 64 (50-76)</td>
<td>3 papillomas of forestomach (51, 67, 76, 76, 90, 103) 6 papillomas of forestomach (51, 67, 76, 76, 90, 103) 2 adrenocarcinomas of glandular stomach (51, 76) 2 leiomyosarcomas of glandular stomach (76, 80) 2 adrenal cortical carcinomas (81, 83) 1 angiomata of spleen (65) 1 polyoid adenoma of colon (90) 1 carcinoma of salivary gland (100) 1 adrenal cortical adenoma (78) 1 anisokaryotic cell sarcoma of heart (63) 1 squamous cell carcinoma of nasal cavity (47)</td>
</tr>
<tr>
<td>50 M</td>
<td></td>
<td>27 54 78 (47-103)</td>
<td>7 14 77 (64-94)</td>
<td></td>
<td>2 adrenocarcinomas of glandular stomach (51, 76) 2 leiomyosarcomas of glandular stomach (76, 80) 2 adrenal cortical carcinomas (81, 83) 1 angiomata of spleen (65) 1 polyoid adenoma of colon (90) 1 carcinoma of salivary gland (100) 1 adrenal cortical adenoma (78) 1 anisokaryotic cell sarcoma of heart (63) 1 squamous cell carcinoma of nasal cavity (47)</td>
</tr>
<tr>
<td>2</td>
<td>Untreated control</td>
<td>99 F</td>
<td>1 1 53</td>
<td></td>
<td>7 malignant lymphomas (74, 79, 81, 93, 94, 99, 110) 3 adrenal cortical carcinomas (79, 94, 110) 3 leiomyosarcomas of uterus (35, 92, 100) 3 dermal melanocytomas (57, 66, 73) 2 papillomas of forestomach (80, 92) 2 adrenocarcinomas of uterus (115) 1 adrenal cortical adenoma (100) 1 adrenocarcinoma of ovary (80) 1 adenoma of Langerhans islands (99) 1 adrenocarcinoma of kidney (64) 1 adenoma of thyroid (84) 1 sarcoma, s.c. (74)</td>
</tr>
<tr>
<td>97 M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7 adrenal cortical carcinomas (80, 101, 111, 114, 121, 121, 126) 6 papillomas of forestomach (66, 81, 89, 121, 121, 124) 4 malignant lymphomas (73, 98, 94, 98) 3 adrenal cortical adenomas (74, 101, 123) 1 dermal melanocytoma (116) 1 carcinoma of forestomach (82) 1 papilloma of gallbladder (123) 1 leiomyosarcoma, abdominal (81) 1 hepatoma (82)</td>
</tr>
</tbody>
</table>

* Values in parentheses, latent periods (age in weeks).
Grinbergs for technical assistance in the experiment, Walter Williams for the photography, and Carol Mackiewicz and Kathy Goergen for help in the electron microscope technique.

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Fig. 1. Malignant histiocytoma, liver. Observe the yellowish diffuse tissue infiltration in all lobes. Female, 77 weeks old. Formalin-fixed specimen, ×
1.5.

Fig. 2. Malignant histiocytoma, liver. The tumor growths are nodular, well circumscribed, and yellowish in appearance. Male, 69 weeks old.
Formalin-fixed specimen, × 1.5.

Fig. 3. Malignant histiocytoma, liver. Observe the nodular appearance of lesion. The pale, vacuolated neoplastic cells spread in the sinusoids at the
periphery of the nodule. Male, 80 weeks old. H & E, × 42.

Fig. 4. Malignant histiocytoma, liver. The neoplastic histiocytes (Kupffer’s cells) replaced the normal architecture and arranged in nodular patterns.
The cells have a large, pale, vacuolated cytoplasms and the nuclei are relatively small, round or oval, and dark. Male, 80 weeks old. H & E, × 150.

Fig. 5. Malignant histiocytoma, liver. The nuclei appear round or oval and relatively regular. The cytoplasms are large, pale, and vacuolated. Female,
55 weeks old. H & E, × 1000.

Fig. 6. Malignant histiocytes in liver. The neoplastic cells obliterate the entire lumen of a vessel. Apparently, the tumor disseminates through such
channels. Female, 75 weeks old. H & E, × 150.

Fig. 7. Malignant histiocytes in the lung. A blood vessel in the center is filled with these histiocytes. Male, 63 weeks old. H & E, × 160.

Fig. 8. Multinucleated giant cells in a malignant histiocytoma, liver. These cells are large and round and contain numerous nuclei that are usually
located at the periphery. Their cytoplasms vacuolated. Empty spaces are visible around these cells. Male, 67 weeks old. H & E, × 300.

Fig. 9. Malignant histiocytoma of liver. The characteristic ultrastructure of the lesion. Observe the numerous neoplastic histiocytes (H) that are rich in
organelles. The nuclei are relatively regular, and in the cytoplasms several vacuoles (V), droplets (D), and lysosomes (L) are visible. Male, 85 weeks
old. Electron micrograph, × 8000.

Fig. 10. Nucleus of a malignant histiocyte. Note its roundish shape. Margination of chromatin (CR) is visible; also it is distributed in the central areas
in clusters. Few inclusion-like bodies (arrows) are also apparent. Male, 85 weeks old. Electron micrograph, × 14,000.

Fig. 11. Surface area of a malignant histiocyte. Several irregular cell projections (P) are distinct. Various sizes of vesicles (VE) (pinocytosis) are
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