Morphological Studies of Angiosarcomas Induced by 1,2-Dimethylhydrazine Dihydrochloride in Syrian Golden Hamsters

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SUMMARY

Studies were made on the morphological characteristics of blood vessel tumors induced by the p.o. lifetime administration of 0.001% 1,2 dimethylhydrazine dihydrochloride in Syrian golden hamsters. The lesions were classified as angiosarcomas. They occurred in 89% of the females and 82% of the males (with decreasing frequency) in liver, lungs, muscle, heart, and pancreas.

Gross, light, and electron microscopic and histochemical investigations were performed on the tumors. The macroscopic and light microscopic study showed the typical appearance of angiosarcoma composed of round or elongated polygonal neoplastic endothelial cells which formed vascular spaces and clefts. The histochemical work included the determination of collagen, reticulum, ionized iron, polysaccharide-mucopolysaccharide-glycolic fatty acid content, and alkaline phosphatase enzyme activity by the lesion. The formation of reticulum and ionized iron was negligible, while the amounts or the activity of the other three substances were substantial. Electron microscopic examination revealed the ultrastructural details of the various components of the vascular lesion including part of its developmental stage. Hemorrhagic areas, structural characteristics of the angiosarcoma, malignant endothelial cell nucleus with chromatin arrangements, and its cytoplasm with organelles, intraluminal cell surface, and contact between neoplastic endothelial cells are described and illustrated.

INTRODUCTION

Systematic studies on the possible correlation between chemical structure of substituted hydrazines and tumor development at specific organ sites have been conducted in this laboratory since 1968 in Swiss mice and golden hamsters. One of the most notable aspects of these investigations was the appearance of high incidences of malignant vascular tumors in both species after treatment with 1,2-DMH and in mice following the administration of 1,1-dimethylhydrazine, unsymmetrical (Refs. 23 to 25 and 28; B. Toth, 1,1-Dimethylhydrazine (Unsymmetrical) Carcinogenesis in Mice. Light Microscopic and Ultrastructural Studies on Neoplastic Blood Vessels, submitted for publication to J. Natl. Cancer Inst.).

In earlier carcinogenesis investigations conducted by other workers (3, 7, 16, 20, 32) using 1,2-DMH in 3 species, mice, hamsters, and rats, no tumors were observed in the vascular system. In fact, in some of these the compound failed to produce any tumors (7), while in others tumors of the intestines, lungs, and livers were elicited by repeated s.c. administrations of this chemical. Recently, however, it was reported (2) that 1,2-DMH, when given p.o. at low dosages, induced malignant hemangioendotheliomas in rats.

The present writing is essentially concerned with gross, light, and electron microscopic and histochemical studies performed on angiosarcomas of blood vessels induced by the p.o. lifetime administration of 0.001% 1,2-DMH in hamsters. In addition to the rise of multiple angiosarcomas of blood vessels, the treatment induced appreciable incidences of tumors in the cecum and liver. The findings of the carcinogenesis study were published lately (24).

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

The chemical used was 1,2-DMH (M.W., 133.02, m.p., 167–169°), obtained from K and K Laboratories, Inc., Plainview, N.Y. 1,2-DMH was dissolved in the drinking water as a 0.001% solution and was given continuously for life to 50 female and 50 male hamsters that were 7 weeks old at the beginning of the experiment. The solutions were prepared 3 times a week, and the total water consumption containing 1,1-DMH was measured at the same intervals. All the solutions were contained in brown bottles because of the possible light sensitivity of the chemical. 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.

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

For light microscopic examination, the animals were either allowed to die spontaneously or 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 liver, spleen, kidneys, and at least 4 lobes of the lungs of each hamster, as well as on those organs that showed gross pathological changes. Sections from these tissues were stained routinely with hematoxylin and eosin. In addition the following special stains were used: Gömörí's silver oxide solution for reticulum (5); Van Gieson's stain for collagen fibers (12, 14); MacManus' periodic acid-Schiff reaction for polysaccharides, mucopolysaccharides, and glycolic fatty acids (9, 15); Berlin blue stain for ionized iron (19); and azo dye method of Manheimer and Seligman (13) for alkaline phosphatase activity.

For electron microscopic examination, representative samples of the tumor tissues (approximately 1-mm cubes) were fixed in buffered 1% osmium tetroxide at 4° (1, 17). The specimen blocks were dehydrated in ethanol, passed through propylene oxide, and embedded in Araldite 502-epoxy resin mixture (10). Thick sections (1 µm) were cut from each block, and the slides were stained with 0.2% toluidine blue (29) and examined by light microscopy. Thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome and stained with lead citrate (18, 30) and uranyl acetate (31). Finally, the sections on bare grids were examined at 60 kV in a Philips 300 electron microscope.

RESULTS

The survival rates and incidences of all tumors, with their latent periods, in the 1,2-DMH-treated animals were published recently (24). The compound induced appreciable incidences of blood vessel, cecum, and liver tumors. In the following, the detailed descriptions of blood vessel lesions are presented.

Macroscopic Findings of Blood Vessel Tumors. The vascular lesions usually grew in the form of nodules, ranging from 1 to 25 mm in diameter. Sometimes, however, the tumor grew in an uncircumscribed, diffuse way, invading and destroying the surrounding tissues. In all instances the lesions exhibited soft hemorrhagic consistency and dark reddish coloring. Anemia, enlarged abdomen, and hemoperitoneum were often found together with the development of the tumors.

Light Microscopic Study. All the blood vessel tumors were classified as angiosarcomas. Table 1 shows the location and the frequency of these lesions in the various tissues. They occurred in the following order in both sexes: liver (Fig. 1), lungs (Fig. 2), muscle, heart, and pancreas. Apparently, in the beginning the endothelial cells were activated, became hyperplastic, and grew alongside the liver cell cords. The sinusoids at this stage were somewhat dilated (Fig. 3). Later on, the hyperplastic endothelial cell lines separated from the underlying liver cells.

Also, some of the endothelial cells broke out from the line and moved in various directions in the lumen, which was greatly dilated (Fig. 4). The well-developed tumor was composed of oval or elongated, polygonal-shaped endothelial cells, which formed vascular spaces and clefts. The sizes of these clefts were varied and usually contained blood (Fig. 5). The shapes of the malignant endothelial cells were oval or elongated. Often several layers of these cells were lying on top of each other (Fig. 6). In a few cases, at least part of the lesion was composed of closely bound endothelial cells resembling fibrosarcomas. In a number of instances the lesion involved the lungs and compressed the parenchyma of the organ (Fig. 7), while at the same time the neoplastic growth retained its vascular characteristics (Fig. 8).

Histochemical Work. In the vascular tumor small amounts of reticulum were seen as shown by Gömörí's method. The Van Gieson's collagen stain demonstrated large depots of collagen fibers in the angiosarcomas. The polysaccharides, mucopolysaccharides, and glycolic fatty acids content of the lesion was identified by the MacManus periodic acid-Schiff technique which showed a strong positive reaction. The Berlin blue stain for ionized iron was also used and exhibited only occasional material. Last, the alkaline phosphatase enzyme activity was marked as measured by the Manheimer and Seligman azo dye method.

Electron Microscopic Investigation. The ultrastructural details of the various components of the vascular lesions including part of its developmental stage were studied. Apparently, at the beginning, a hemorrhagic area occurred which exhibited its characteristic components: erythrocytes, fibrinous materials, neutrophilic leukocytes, empty intercellular spaces, etc. (Fig. 9). The well-developed characteristic ultrastructure of angiosarcoma is depicted. The neoplastic endothelial cells are shown with oval, sometimes indented, nuclei. Their cytoplasm is usually small, with few organelles and occasional lipid droplets. In between these cells, empty vascular spaces are visible. Under the basement membrane, substantial amounts of collagen fibers can be seen (Fig. 10). The nucleus of neoplastic endothelial cells is indented, and pale areas in the peripheral chromatin and parts of the nucleoli can be seen. Some of the chromatin is attached to the nuclear surface, and 2 inclusion bodies are also visible (Fig. 11). The
cytoplasm of a typical malignant endothelial cell is shown. The endoplasmic reticulum and the ribosomes are scattered all over. Golgi complex, mitochondria, lysosomes, and multivesicular bodies can be observed (Fig. 12). The intraluminal surface of a neoplastic endothelial cell is also depicted. Several cytoplasmic processes project into the lumen of a blood vessel. In the cytoplasm, numerous filaments can be seen (Fig. 13). Finally, the surface contact between 3 malignant endothelial cells is illustrated. Their plasma membranes run in relatively orderly fashion. Two invaginations and a triangle-shaped extracellular space can be observed (Fig. 14).

Extensive studies on the ultrastructural characteristics of the normal vascular tissues were carried out in several species by a number of investigators. Their findings were summarized recently in 2 monographs (4, 11), which were used throughout this study for comparative purposes.

DISCUSSION

The present study describes the morphological appearance of blood vessel lesions induced by the p.o. lifetime administration of 0.001% 1,2-DMH to Syrian golden hamsters. The tumors were diagnosed as angiosarcomas and were found in 89% of the females and 82% of the males in liver, lungs, muscle, heart, and pancreas. Macroscopic, histochemical, light microscopic, and electron microscopic examinations were performed on the vascular lesions and revealed the typical appearance of multiple angiosarcomas.

It may be of interest to compare here the type of blood vessel tumors induced by identical treatment in the 2 species. In mice these lesions occurred in more organs such as pararenal, parametrial, and paraepididymal tissues, lymph nodes, etc., where they were not observed in hamsters. In both species, light and electron microscopic studies were performed on the cellular ultrastructural characteristics of the vascular lesions. Although they were similar, in mice the neoplastic endothelial cells appeared somewhat more elongated while in hamsters they looked more rounded. The incidences of the tumors were approximately the same in both species, i.e., over 80%. Their latent periods, nevertheless, were slightly shorter in mice than in hamsters (24, 28). From a practical viewpoint it is remarkable that no substantial species difference exists in the carcinogenic response of the vascular systems of mice and hamsters.

In man, benign tumors of the blood vessels are the most common lesions while malignant ones are observed infrequently. Cytologically, the induced blood vessel lesions in the current study appeared similar if not identical to those found in man. The causes of human vascular tumors are, however, essentially unknown at present (8).

The incidence of spontaneous vascular tumors is rare in the untreated colony of golden hamsters in this institute. In 100 untreated males kept until natural death, we observed 2 animals with hemangioma of the liver. This lesion was not seen in the 100 corresponding females. Also malignant vascular tumors were not found in either sex (22). Benign and malignant lesions of the vascular system of the hamster have been, however, induced by carcinogenic chemicals in the past.

Among the most notable are those that were evoked in moderate incidences by urethan (27), by dimethylnitrosamine (6), by the concurrent administration of urethan and isonicotinic acid hydrazide (26), and by other compounds (21).

The presently obtained high incidences of angiosarcomas of blood vessels in this species also provide a model for the study of the pathogenesis of this tumor.

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REFERENCES

Morphology of Angiosarcomas in Hamsters


Fig. 1. Angiosarcoma of liver in a 51-week-old female treated with 1,2-DMH. Note the dark hemorrhagic growths in the lobes and the adhesion between the lobes (lower part). Formalin-fixed specimen, X 1.7.

Fig. 2. Angiosarcoma of lungs in a 62-week-old male treated with 1,2-DMH. The dark, roundish multiple neoplastic growths are elevated over the surface. Formalin-fixed specimen, X 1.6.

Fig. 3. Angiosarcoma of lungs in a 60-week-old male treated with 1,2-DMH. The dark, hyperplastic endothelial cells grew alongside the liver cell cords. The sinusoids are somewhat dilated. H & E, X 330.

Fig. 4. Angiosarcoma in the liver of a 53-week-old female treated with 1,2-DMH. The hyperplastic endothelial cells cover the liver cell cords. Often the endothelial cell line was separated from the surface of hepatocytes. Some of these cells are moving freely within the lumen. The sinusoids and blood vessels are greatly dilated. H & E, X 180.

Fig. 5. Angiosarcoma of liver composed of numerous vascular spaces and clefts, which are formed by the neoplastic endothelial cells, in a 32-week-old female treated with 1,2-DMH. The clefts contain mostly red blood cells. H & E, X 150.

Fig. 6. Same as Fig. 5. Observe the oval or elongated neoplastic endothelial cells. Often several layers are lying on top of each other. In the vascular spaces, elements of blood are visible. H & E, X 450.

Fig. 7. Angiosarcoma of lungs in a 42-week-old female treated with 1,2-DMH. Two neoplastic vascular areas in the lungs are visible. They compress the surrounding parenchyma. H & E, X 21.

Fig. 8. Angiosarcoma of lungs in a 57-week-old male treated with 1,2-DMH. The highly invasive neoplastic endothelial cell growths replaced part of the normal lung tissue. Several vascular clefts can be seen. H & E, X 80.

Fig. 9. Hemorrhagic area in the liver of a 45-week-old female treated with 1,2-DMH. Its components include red blood cells (RBC), fibrinous materials (FN), neutrophilic leukocytes (NL), and empty intercellular spaces (IS) X 12,800.

Fig. 10. Angiosarcoma of liver in a 30-week-old male treated with 1,2-DMH showing the typical ultrastructural appearance of the lesion. The neoplastic endothelial cells (E), vascular spaces (VS), and collagen fibers (CF) are distinct. X 12,800.

Fig. 11. Nucleus of a malignant endothelial cell in the liver of a 43-week-old female treated with 1,2-DMH. Note the indentation and the pale areas in the peripheral chromatin, which may be invaginations. Parts of the nuclei (N) are visible. Some of the chromatin (CR) is attached to the nuclear envelope. Two inclusion bodies (arrows) can also be seen. X 27,200.

Fig. 12. Cytoplasm of a neoplastic endothelial cell in the liver of a 51-week-old male treated with 1,2-DMH. Endoplasmic reticulum with ribosomes are abundant. The Golgi complex (G), mitochondria (M), lysosomes (L), and multivesicular body (MB) are also visible. X 34,400.

Fig. 13. Intraluminal surface of a malignant endothelial cell in the liver of a 60-week-old female treated with 1,2-DMH. The cytoplasmic processes (P) or flaps exhibit various shapes and sizes and project into the lumen (L). In the cytoplasm, large amounts of filaments (F) can be seen. X 40,800.

Fig. 14. Surface contact between 2 neoplastic endothelial cells in the liver of a 55-week-old male treated with 1,2-DMH. Observe the triangle-shaped widened extracellular spaces (CS). Surfaces of plasma membranes seem relatively regular. Two invaginations (I, protein pits) are also visible. X 12,800.
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