Metastatic Spreading of Induced Uterine Sarcomas

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A description is given of the frequency and morphology of metastases of uterine sarcomas induced by dimethylhydrazine combined with estrogen in female CBA mice. The data on the frequency and location of metastases are taken from an experiment carried out to study the effect of ascorbic acid on the induction of uterine sarcomas. Experimental uterine sarcomas in mice metastasize in 20-30% of cases. More than 70% of metastases are to the stomach, and thence to the intestine, whereas metastases in the liver, kidneys, and lungs are less frequent. It is shown that the weight of uterine sarcomas with or without metastases is markedly lower in mice treated with ascorbic acid. Dimethylhydrazine-induced uterine sarcomas may be used as a model to study the effects of different factors on metastatic spreading.

Key Words: *uterine sarcomas; metastases; dimethylhydrazine*

The frequency of uterine sarcomas in women varies from 2 to 9% of all malignant tumors of this organ. Uterine sarcoma is characterized by a malignant course and a poor prognosis. A peculiar feature of this tumor is that it often metastasizes to different organs, including organs of the digestive tract [2,7]. There is an experimental model of uterine sarcoma induced in mice by dimethylhydrazine (DMH) or by a combination of DMH and estrogen [9,10]. This model is easily reproducible, hormone-dependent, and exhibits a peculiar location of metastases. This study describes the peculiarities of metastatic spreading of uterine sarcomas induced by combined administration of DMH and estradiol dipropionate (EP). The data on the frequency and location of metastasis are taken from an experiment where the effect of ascorbic acid (AA) on the induction of uterine sarcomas was studied. The results of this experiment have already been published [3], but the issue of metastatic spreading was not discussed.

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MATERIALS AND METHODS

For the study, female CBA mice aged 2 months were obtained from the *Stolbovaya* nursery of the Russian Academy of Medical Sciences. DMH was administered at 8 mg/kg in distilled water every week for 12 weeks. At the same time EP was injected s.c. at 10 μg per mouse in olive oil once a week one day before carcinogen administration. From the 13th week of the experiment the mice received AA at 0.3, 0.75, and 1.5% added to the drinking water. The 1st group (58 females) received DMH+EP; the 2nd (49 females) DMH+EP+0.3% AA; the 3rd (40 females) DMH+EP+0.75% AA; the 4th (40 females) DMH+EP+1.5% AA. Animals were sacrificed in the 43rd-44th week of the experiment. Internal organs were taken for histological examination, and the uterus was weighed. The material was fixed in 10% formalin, and the sections were stained with hematoxylin-eosin. For electron-microscopic study of two stomach metastases the material was fixed in 4% formaldehyde (from paraformaldehyde) prepared on 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide on the same buffer, and embedded in an Epon-Araldite

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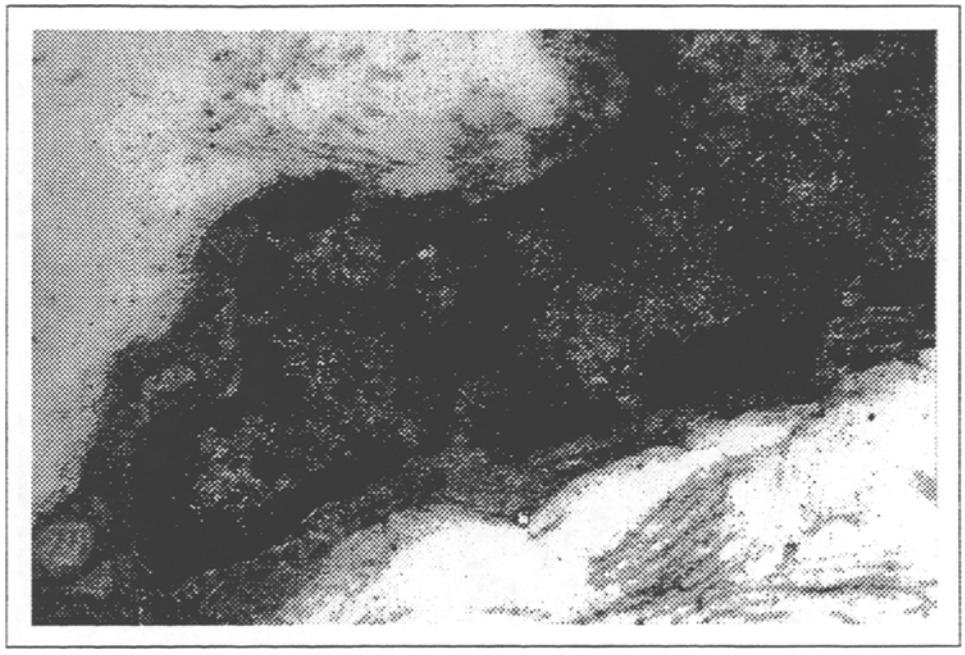

Fig. 1. Metastasis of mouse malignant fibrous histiocytoma in the stomach wall. ×30,000. Fibroblastlike tumor cell. Cell surface has no processes. Cytoplasm contains widened channels of granular endoplasmic reticulum. Bundles of fibrillar structures are seen near the cell.

mixture. Ultrathin sections were viewed in a JEM 100CX electron microscope.

RESULTS

Uterine sarcomas and a moderate number of tumors of other locations (anal region, epithelial intestinal tumors, mammary gland tumors) were found in all animals treated with DMH+EP [3]. Small uterine tumors (weight less than 1 g) macroscopically looked like a diffuse thickening of the body and horns of this organ. Large tumors consisted of tuberous formations (weighing less than 6 g) occupying the whole organ and spreading to the parametrium. The mean weight of the normal uterus is 0.24±0.03 g. Two histological types of tumor were isolated: stromal sarcomas and malignant fibrous histiocytomas. Elongate fibroblastlike cells and areas of immature mesenchymal cells comprised the first type, whereas the malignant fibrous histiocytomas were characterized by histiocytelike cells, and giant cells with diastase-resistant PAS-positive granules in the cytoplasm. Table 1 shows that 20-33% of uterine sarcomas metastasize to different organs, primarily the stomach and then to the large intestine. Sometimes metastases are found in the liver, lymph nodes (mesenteric), kidneys, and lungs. The mean weight of metastasizing uterine sarcomas is twice as high as the weight of sarcomas without metastases. The differences are reliable in the 1st, 3rd, and 4th groups and are border-line significant in the 2nd group (Table 2). It is to be noticed that sarcomatous changes in the stomach and other organs specified as metastases were found only in mice with uterine sarcomas. During many years of studies, DMH has never been found to cause any sarcomatous changes in the stomach, intestine, or other organs without causing simultaneous damage to the uterus. Stomach metastases looked like white structures protruding into the cavity of the prestomach. There were

TABLE 1. Frequency and Location of Uterine Sarcoma Metastases

Index	Group			
	1st	2nd	3rd	4th
Effective number of animals 58	49	40	40	
Number of animals with metastases (in % as related to those with uterine sarcomas)	40 (69%)	23 (47%)	24 (60%)	20 (50%)
Of these, with metastases in: 12 (30%)	6 (26%)	8 (33%)	4 (20%)	
prestomach	12	6	7	3
large intestine	7	4	2	-
liver	1	-	-	2
lymph nodes	1	-	-	2
kidneys	-	-	1	-
lungs	1	-	-	1

Fig. 2. Metastasis of mouse malignant fibrous histiocytoma in the stomach wall. ×15,000. Histiocytelike cell with lysosomes. Cell fragment with distinct cell processes.

no cases of metastatic spreading in the glandular part of the stomach. As a rule, microscopically the metastases had the structure of a fibroblastic sarcoma, in which sometimes giant cells with diastase-resistant PAS-positive granules in the cytoplasm were noted. On the submicroscopic level, two cell types are found in stomach metastases. Some cells were fusiform, mainly with a smooth surface and elongate nucleus. Channels of granular endoplasmic reticulum and a moderate number of mitochondria were found in the cytoplasm (Fig. 1). Bundles of narrow fibers without pronounced striation adhere close to these fibroblastlike cells. The second type of cells consisted of histiocytelike tumor cells with one nucleus, were often of irregular shape, and had numerous cell processes. The cytoplasm contained lysosomes, myelinlike bodies, and a developed granular endoplasmic reticulum (Fig. 2). Such cells have been described in the malignant fibrous human histiocytoma [6,8], showing that the metastases studied here belong to this kind of tumor. Intestinal metastases looked like polyps. Spreading of fibrosarcomatous tissue was detected histologically near intestinal lymph follicles, occupying the muscle and mucous layers. In addition to sarcomatous lesions, DMH induced the growth of glandular intestinal polyps [3]. Sarcomatous lesions of the intestine without simultaneous involvement of the uterus was not found. Liver and kidney metastases were small sarcomatous nodes in the organ parenchyma. One of the lung metastases had the histological structure of a hemangioendothelioma, like the primary uterine tumor. Another metastasis looked like the primary uterine tumor identified as a stromal sarcoma. Thus, uterine sarcomas, being estrogen-dependent tumors inhibited by progesterone [9], develop in DMH-treated CBA mice with a high frequency. The malignancy of the tumors is attested to by the ease of their transfer to another organism and their rapid rate of growth after transplantation [1]. Recent investigations isolated two types of sarcomas, namely stromal and malignant fibrous histiocytomas [4,10], which were also found in our study. Uterine sarcomas in women are of a different structure: leiomyosarcomas, endometrial stromal sarcomas, and mixed Müller tumors [2]. In the present experiment sarcomas metastasized in 20-30% of cases. Since the sarcomatous process was absent in the stomach, intestine, and liver of mice without uterine sarcomas, the primary nature of these lesions can be ruled out. More than 70% of all metastases were to the stomach. According to Rose *et al.* [7], only 3

TABLE 2. Mean Weight of Uterine Sarcomas with and without Metastases, g ($M \pm m$)

Uterine sarcoma	Group			
	1st	2nd	3rd	4th
With metastases	5.3±2.0	3.5±1.8	3.2±1.8	2.8±2.5
Without metastases	2.6±1.8	1.7±2.0	1.6±1.4	1.0±0.6
p	0.008	0.064	0.035	0.011

out of 79 uterine sarcomas in women metastasized to the stomach, whereas 27.4 and 26% were to the small and large intestine, respectively. In our study the first target organ in mice is the prestomach and the second the intestine (nearly half of all metastases) (Table 1). Many authorities note that most frequently uterine sarcomas metastasize to the lungs [2,5,7]. We found only two cases of metastatic involvement of the lungs in mice. Lazareva [2] points out that the topography of uterine sarcoma metastases in women depend on the histological type of the tumor. According to her data, leiomyosarcomas often metastasized to the lungs (90%), endometrial stromal sarcomas metastasized to regional lymph nodes (42.9%), and mixed Müller tumors induced multiple involvement of organs (30.8%). The topography of metastatic spreading of experimental uterine sarcomas is for the moment hard to explain.

According to our data, experimental uterine sarcomas can metastasize both through the bloodstream and via the lymphatics, the latter probably being the route of metastases to the stomach and intestine. The metastatic nodule in the stomach protruded into the stomach cavity but the serous membrane remained intact. The metastatic process in the intestine starts from the lymph follicle and spreads over the muscle layer. Lazareva notes [2] that tumor size has proved to be a prognostic criterion in uterine sarcomas. The larger the experimental uterine sarcomas, the more frequent was metastatic spreading (Table 2). The mean weight of metastasizing tumors was twice as high as that of tumors without metastases. As noted above, in this experiment we were studying the inhibiting effect of AA on DMH-induced sarcomogenesis of the uterus. A clear tendency for the weight of tumors to decrease was found when AA was administered [3]. Table 2 shows that the weight of uterine sarcomas with or without metastases in mice treated with AA is significantly lower (p=0.01 and 0.03, respectively) than in animals which received no AA. A correlation is found between the AA concentration and tumor weight.

Thus, DMH-induced uterine sarcomas may be used as a model for studying the effects of different factors on metastatic spreading.

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