Virchows Arch. A Path. Anat. and Histol. 384, 263–267 (1979)

Virchows Archiv A

Carcinogenicity of 1,2-Dimethylhydrazine Dihydrochloride in BALB/c Mice

Influence of the Route of Administration and Dosage

Keisuke Izumi, Hisashi Otsuka, Keizo Furuya, and Akiko Akagi

Second Department of Pathology, School of Medicine, Tokushima University, Kuramoto-cho 3-18-15, Tokushima 770, Japan

Summary. The carcinogenicity of 1,2-dimethylhydrazine dihydrochloride (DMH) by oral, intragastric and subcutaneous administration was examined in 339 BALB/c mice. Subcutaneous injection of DMH induced intestinal tumors in the lower colon of all mice. After oral administration it induced a high incidence of vascular tumors in the liver and soft tissues, but colon tumors were found in only 2 mice when given at a high dosage. On intragastric administration, it induced a fairly high incidence both of colon and vascular tumors. The sites and incidences of vascular tumors and squamous cell carcinomas of the perianal glands were also described.

Key words: Hydrazine – Chemical carcinogenesis – Colonic neoplasm – Hemangioma – Perianal carcinoma.

Introduction

It is well known that 1,2-dimethylhydrazine (DMH) has a strong carcinogenic action on the intestine of rats and mice when administered subcutaneously (Druckrey et al., 1967; Wiebecke, et al., 1969), and that it induces intestinal tumors in rats when given orally (Druckrey, 1970). However, there are few reports on the carcinogenicity of DMH in mice when given orally.

In the present study, the difference in the carcinogenicities of DMH on oral, intragastric and subcutaneous administration was examined in mice.

Material and Methods

In all 215 female and 124 male BALB/c mice of 8–10 weeks old were used. They were divided into 6 groups.

Group I: given 0.001% (I-a), 0.004% (I-b) or 0.008% (I-c) DMH in their drinking water,

For offprints contact: H. Otsuka, M.D., Professor of Pathology

0340-1227/79/0384/0263/$01.00

Table 1. Tumor incidence in various organs in DMH-treated and control mice

Group	Administration of DMH			No. and sex	Observ. period (week)	No. of mice with tumors				
	Route	Dose	Period			Blood vessels	Large intestine	Lungs	Anus	Others
I-a	po	0.001%	until death	37 F	21–40	36 (97%)	0	11 (31%)	0	0
				34 M	16–48	32 (94%)	0	9 (26%)	0	2[a]
I-b	po	0.004%	24	29 F	15–29	22 (76%)	0	4 (14%)	1 (3%)	1[b]
I-c	po	0.008%	10	10 F	17–38	6 (60%)	2 (20%)	5 (50%)	2 (20%)	1[c]
II	–	–	–	30 F	29–88	0	0	1 (3%)	0	2[d]
				28 M	22–88	0	0	1 (4%)	0	0
III-a	ig	weekly 30 mg/kg	24	15 F	30	3 (20%)	10 (67%)	4 (27%)	2 (13%)	0
III-b	ig	weekly 30 mg/kg	24	9 F	40	6 (67%)	9 (100%)	4 (44%)	6 (67%)	0
IV	–	–	–	15 F	40	0	0	0	0	0
V	sc	weekly 30 mg/kg	16–23	47 F	17–29	2 (4%)	47 (100%)	4 (9%)	6 (13%)	0
				38 M	16–26	7 (18%)	38 (100%)	2 (5%)	5 (13%)	0
VI	–	–	–	23 F	22–24	0	0	0	0	0
				24 M	21–24	0	0	0	0	0

po: drinking water; ig: gastric intubation; sc: subcutaneous injection

[a] Kidney adenoma, 2; [b] Breast carcinoma, 1; [c] Squamous cell carcinoma of salivary gland, 1; [d] Breast carcinoma, 1; Malignant lymphoma, 1

Group II: given no treatment, as control for Group I, *Group III:* given DMH by gastric intubation once a week for 24 weeks, killed at week 30 (III-a) or week 40 (III-b), *Group IV:* given 0.1 ml of distilled water by gastric intubation once a week for 24 weeks, killed at week 40, as control for Group III, *Group V:* injected subcutaneously with 30 mg/kg of DMH once a week for 16 or 23 weeks, *Group VI:* given 0.1 ml of distilled water subcutaneously once a week for 16 or 23 weeks, as control for Group V.

The number of animals, route, dose or concentration and period of DMH administration and observation period of each group are shown in Table 1.

Necropsies were performed on all animals. Tissues were fixed in 10% buffered formalin and embedded in paraffin. The stains used were Hematoxylin and Eosin, reticulin, periodic acid-Schiff and Alcian blue.

Results and Discussion

Table 1 shows the incidence of tumors in each group. In the control groups (Groups II, IV and VI) no tumors were observed in the intestine and vascular system.

The most remarkable difference in the incidence of intestinal and vascular tumors was seen between Groups I and V. All the mice in Group V developed colorectal tumors, while in Group I, only 2 mice given a high dosage (I-c)

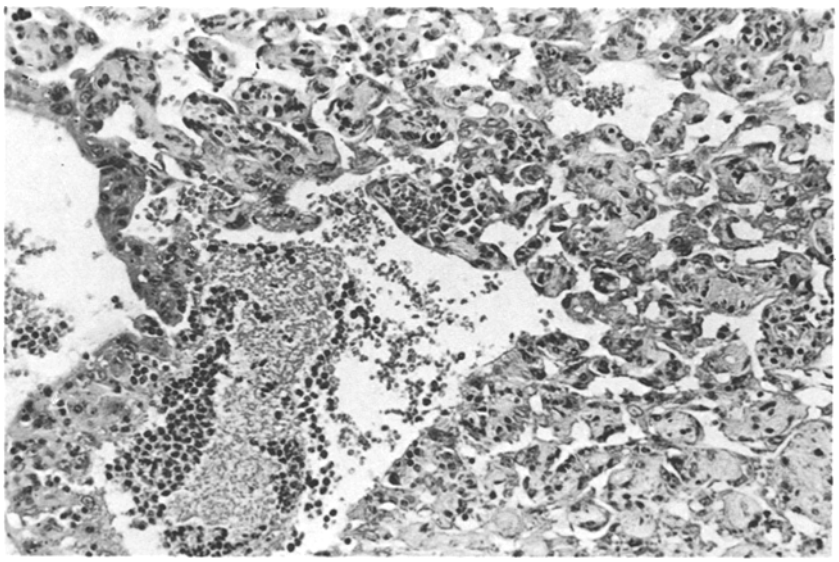

Fig. 1. Hemangioendothelioma developed in pararenal fat tissue. Note the tumor cell proliferation forming narrow, irregular vascular spaces. Group I-a, female. H.E. ×180

had solitary rectal tumor. In contrast, the incidence of vascular tumors was strikingly higher in Group I than in Group V. Tumor incidence in Group III-a was fairly high in the colon and relatively low in vascular system, and the incidence was higher in Group III-b observed 10 weeks longer than Group III-a.

These results clearly show that the carcinogenicity of DMH in mice depends upon the route of DMH administration. There has been no previous report on the induction of intestinal tumors in mice by oral administration of DMH. Toth et al. (1971, 1976) observed vascular and lung tumors in Swiss mice given DMH in their drinking water, but did not find any intestinal tumors. Our study shows that colonic tumors can be induced in mice by intragastric – "oral" in wide sense – administration of DMH.

The intestinal tumors in our experiment on mice were found in a restricted area of the lower colon as mentioned by Haase et al. (1973) and Wiebecke et al. (1971), while this carcinogen induced tumors in a rather wide area of the small and large intestine in rats (Druckrey et al., 1967; Druckrey, 1970). Histologically, early lesions of intestinal tumors were pedunculate or sessile adenomas and adenocarcinomas. Invasive adenocarcinomas were seen in 42% of animals with intestinal tumors in Groups III and V but no metastasis of the tumors was detected in any group.

Vascular tumors were capillary hemangiomas or hemangioendotheliomas usually with dilated vascular lumena, showing a blood cyst-like appearance. The highest incidence of this tumor was seen in the liver (97% in Group I-a, female), followed by fat tissue in the abdominal cavity (53% in Group I-a, male), retroperitoneum (46% in Group I-a, female), subcutaneous and intermuscular tissue (45% in Group I-b). A few tumors were also seen in the intestinal

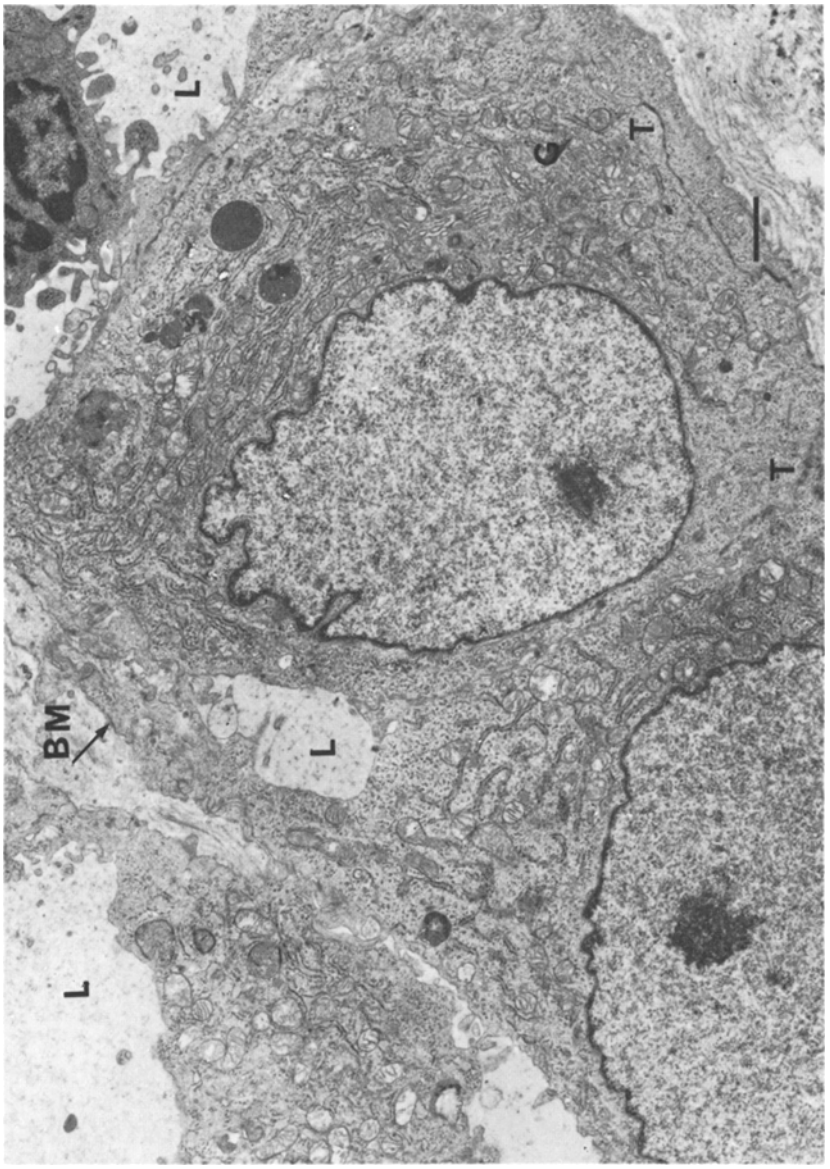

Fig. 2. Neoplastic endothelial cells. Note the characteristic vascular spaces (*L*), fine cytoplasmic processes, rich organella, tight junctions (*T*) and basement membrane (*BM*). ×8,100

wall, mediastinum and lungs. Histologically, round or elongated tumor cells proliferated, usually forming irregular vascular spaces (Fig. 1). These tumors were similar in location and histology to those we observed previously on the treatment of the same strain of mice with dimethylnitrosamine (Otsuka et al., 1971).

Ultrastructure demonstrated vascular channels of varying sizes lined by neoplastic endothelial cells. Numerous fine cytoplasmic processes were observed along the luminal surface. The cytoplasm contained numerous rough endoplasmic reticulum, free ribosomes, well-developed Golgi complexes and very thin filaments. Many tight junctions were observed on the cell borders and basal lamina formed a discontinuous layer around the periphery of the cells (Fig. 2). These findings correspond to the description of the blood vessel tumors by Toth et al. (1971).

All perianal tumors were squamous cell carcinomas originating from the perianal sebaceous glands. Groups of sebocytes were frequently found within the tumors as described by Turusov (1978). In one animal in Group III-b, metastasis of the carcinoma to the lung was observed. Marked hyperkeratosis with squamous metaplasia and various degrees of dysplastic change of the epithelium were observed in a few mice. Perianal carcinomas were observed in animals in the groups that had high incidence of colonic tumors, suggesting that a proximate carcinogen in the faeces may play a role in inducing cancer of the gland.

The incidence of lung tumors was higher in groups received DMH by any route than in control groups, especially in Groups I-c and III-b. Kidney adenoma, carcinomas of the breast and salivary gland and malignant lymphoma were found incidentally in a few mice.

This work was supported in part by a Grant-in Aid for Cancer Research from the Ministry of Education, Japan.

References

Druckrey, H., Preussmann, R., Matszkies, F.U. Ivankovic, S.: Selektive Erzeugung von Darmkrebs bei Ratten durch 1,2-Dimethylhydrazin. Naturwissenschaften **54**, 285–286 (1967)

Druckrey, H.: Carcinoma of the colon and antecedent epithelium. Burdette, W.J. (ed.), pp. 267–279. Springfield: C.C. Thomas 1970

Haase, P., Cowen, D.M., Knowles, J.C., Cooper, E.H.: Evaluation of dimethylhydrazine induced tumors in mice as a model system for colorectal cancer. Br. J. Cancer **28**, 530–543 (1973)

Otsuka, H., Kuwahara, A.: Hemangiomatous lesions of mice treated with dimethylnitrosamine. Gann **62**, 147–156 (1971)

Toth, B., Wilson, R.B.: Blood vessel tumorigenesis by 1,2-dimethylhydrazine dihydrochloride (symmetrical). Am. J. Pathol. **64**, 585–600 (1971)

Toth, B., Malick, L., Shimizu, H.: Production of intestinal and other tumors by 1,2-dimethylhydrazine dihydrochloride in mice. Am J. Pathol. **84**, 69–86 (1976)

Turusov, V.S.: Tumours of the anal region induced in mice by 1,2-dimethylhydrazine. Cancer Lett. **5**, 97–102 (1978)

Received June 9, 1979