PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 166-169 (1975)

# Colon Cancer Induction in Mice by Intrarectal Instillation of *N*-Methylnitrosourea[1] (38498)

TOMIO NARISAWA[2] AND JOHN H. WEISBURGER

*Naylor Dana Institute for Disease Prevention, American Health Foundation, New York, New York 10021*

In recent years, great strides have been made toward the development of animal models for studying colon cancer (1). Induction of colon cancer can be achieved by subcutaneous injection of 1,2-dimethylhydrazine (2) or the intrarectal administration of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine developed by us (3–7). By using this technique, we have been investigating the pathogenesis of colon cancer and the carcinogenic mechanisms with 1,2-dimethylhydrazine and related compounds in the rat (8, 9).

Our purpose in the present study is to establish that intrarectal instillation of direct-acting carcinogenic chemicals to mice is a useful experimental method for the development of animal models on colon carcinogenesis. Of particular interest is a system to test carcinogenic chemicals at several dose levels and regimens and using a sizeable number of animals. Thus, this experimental system might deserve much further investigation since it is easy technically and also economical. In this experiment, *N*-methylnitrosourea (MNU), one of the most potent and versatile carcinogens among nitroso compounds, was selected as a carcinogen on the basis of the results of Leaver, *et al.* (10). They observed multiple tumors in the stomach, small and large intestine of rats after a single large intragastric dose of MNU. This agent is also of interest since it can be formed readily *in vivo* in the stomach from nitrite and methylurea, or more slowly from methylguanidine (11).

*Materials and Methods.* Female ICR/Ha Swiss mice obtained from Sprague–Dawley Breeding Laboratory, Madison, WI, 7- to 8-weeks old, were used. MNU was dissolved in distilled water immediately prior to use. A 5.0 cm gastric feeding metal tube with a bulbous protective tip was inserted about one-half of the way into the lumen of the large intestine through the anal orifice. The solution described below was then instilled, filling the distal large intestine. Occasionally a small amount of the solution was lost from the anus soon after the instillation. There were no pretreatments or preparation of the mice before the administration of the solution.

There were two experimental and one control group of 30 mice each at the start. In Group I, each mouse was given 0.075 ml of a 2% MNU (1.5 mg) in distilled water solution three times weekly for 2 weeks. A preliminary experiment showed that the maximum tolerated single dose by intrarectal administration was 1.5 mg of MNU. In Group II, each mouse was given 0.075 ml of a 0.4% MNU (0.3 mg) solution three times weekly for 10 weeks. The total dose of MNU was 9 mg in both experimental groups. In Group III, 0.075 ml of distilled water was administered as control.

The mice had free access to Purina Laboratory Chow and tap water. Morbid mice were killed. One mouse in Group II and 27 mice in Group III surviving in good condition clinically without overt signs of neoplasm at the 40th experimental week have been kept for further observation. All of the 30 mice of Group I, 28 mice except one cannibalized mouse in Group II, and 3 mice of Group III which died or were killed before the 40th week, were examined upon autopsy and carefully inspected for gross abnormalities. The stomach and intestine were opened to reveal any tumors. All abnormal tissues and organs suspected to contain metastases were examined histologically by standard methods.

[1] Supported in part by Public Health Service contract NOI-CP-43378 and Grant No. CA 15400 from the National Cancer Institute.

[2] Visiting Investigator, Naylor Dana Institute for Disease Prevention, American Health Foundation, New York, N.Y. 10021, on leave of absence from Akita University School of Medicine, Japan.

*Results.* The average body weight of the mice increased at the same rate in Groups II and III. In Group I, it decreased by a maximum of 12% of the initial weight in the first 2 weeks. Thereafter, i.e., after MNU administration was completed, the weight increased at the same rate as in other groups and the mice appeared to recover. The weight depression was a sign of acute toxicity attributable to frequent applications of a high dose of MNU.

The 30 mice of Group I died or were killed *in extremis* between the 11th and 19th week. In Group II, 28 of 29 mice died or were killed until the 40th week. In addition to neoplasms of the large intestine, lung adenomas and leukemias were noted. Figure 1 shows the relation between localization and latent period of neoplasms in each mouse. Two or three types of these neoplasms were found together in almost all of the mice. It was noticed that leukemias, high early in the test, were observed with a lesser incidence in both experimental groups after the 17th week. In Group III, only three mice died or were killed until the 40th week, but no neoplasms were found.

*Large bowel neoplasms.* Neoplasms of the large intestine were found in 14 of 30 mice (47%) in Group I and in 18 of 28 (64%) in Group II. The first tumors were seen at the 12th week in Group I and at the 17th week in Group II. Counting only the mice alive after the 17th week in Group II, 18 of 23 mice had large bowel neoplasms, i.e., 78% incidence. Blood stools, prolapse of tumors or ulcerated anal tumors appeared in some cases. The lesions formed sessile polyps, polypoid tumors or ulcerating type neoplasms like the human type. The tumors were observed in the distal colon, rectum and anus in which MNU solution was instilled. There were no tumors in the proximal colon and the upper gastrointestinal tract. The latent period was longer in Group II compared to Group I (Fig. 1). The incidence of squamous cell carcinomas developed at the anus was significantly higher in Group II compared with that of Group I (Table 1). The average number of adenocarcinomas and adenomas per large bowel tumor-bearing mouse was the same, 1.3 in Group I and 1.2 in Group II, but for the squamous cell

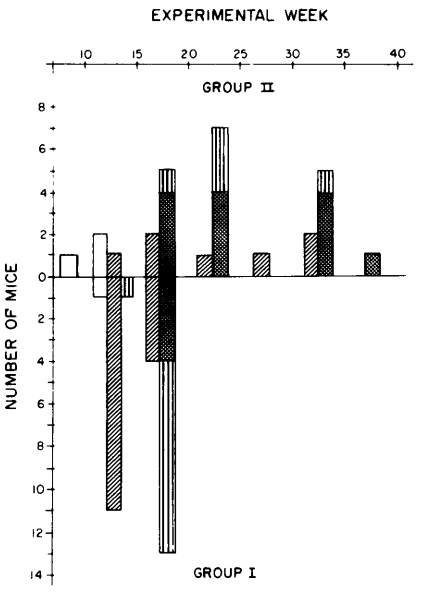


FIG. 1. Localization, incidence and latent period of induced tumors in ICR/Ha Swiss Mice by intrarectal instillation of MNU. Group I was given 1.5 mg MNU three times weekly for 2 weeks, and Group II 0.3 mg MNU three times weekly for 10 weeks. At each time period the length of each bar designated mice with tumors in large bowel and lung ▩, large bowel, lung and leukemia ▥, leukemia and lung, or leukemia ▨, or a mouse without tumor □.

carcinoma it was 0.4 in Group I and 0.9 in Group II. In Group I, four of five mice with squamous cell carcinoma, and in Group II, 11 of 15 also had adenocarcinomas and or adenomas in a large intestine. Thus, there were nine mice in Group I, and three in Group II which presented with adenocarcinomas and/or adenomas alone, and one in Group I, and four in Group II with squamous cell carcinoma alone. The latent period leading to adenocarcinoma and squamous cell carcinoma was similar. The adenocarcinomas were well-differentiated. Both adenocarcinomas and squamous cell carcinomas showed various types of invasion even to the outside of the intestinal wall in advanced cases. The histologic features of these lesions were similar to that of human materials. No metastases were found in the regional lymph nodes and other organs.

*Lung tumors and leukemias.* All mice with large bowel neoplasms also had lung ad-

TABLE I. HISTOLOGIC CLASSIFICATION OF LARGE BOWEL NEOPLASMS IN ICR/Ha SWISS MICE BY INTRARECTAL INSTILLATION OF MNU.

| Groups and treatment | No. of examined mice | No. of mice with the neoplasms | No. of neoplasms | | | |
|---|---|---|---|---|---|---|
| | | | Adeno-carci-noma | Ade-noma | Squamous carci-noma | Papilloma |
| I MNU 1.5 mg × 6 | 30 | 14 | 8 | 10 | 5 | 0 |
| II MNU 0.3 mg × 30 | 28 | 18 | 12 | 9 | 15 | 1 |

enomas which were multiple small nodules on gross observation. Malignant lesions were not found. Leukemia was present in 10 of 14 mice with large bowel neoplasms in Group I and in five of 18 in Group II. Macroscopically, enlargement of the thymus in all of the cases, enlargement of the systemic lymph nodes, especially mesenteric lymph nodes and splenomegaly in some cases were observed. The disease showed the histologic features of lymphocytic leukemia and involved also the lung, kidney, liver, ovary and heart.

*Discussion.* Many of the carcinogenic alkyl nitroso ureido compounds affect the upper digestive organs in experimental animals when they are given orally or intragastrically (12–15). In these compounds MNU probably acts as ultimate carcinogen by heterolysis without intervention of any specific enzyme system (16). Leaver *et al.* (10) reported that a single large intragastric dose of MNU produced tumors not only in the upper gastrointestinal tract, but also in the large intestine. In spite of the chemical unstable characteristics of MNU in a basic environment, it would seem that a sizeable amount of MNU reached the large intestine and acted on the mucosa of the large intestine.

In the present experiment the same total dose of MNU was administered intrarectally to two groups of mice by the combination of different concentration and length of administration of the MNU solution. Distinct results arose in the tumor induction period and the incidence of three kinds of tumors, large bowel neoplasms, lung adenomas and leukemias. The first neoplasm appeared at almost the same time in both groups. Thereafter, in Group II, receiving a lesser dose for a longer time, more than 50% of tumor-bearing mice developed large bowel tumors after the 17th week. This is clearly distinguishable from the results of Group I given a high dose for a short time, in which almost all mice had leukemias, and also large bowel neoplasms and/or lung adenomas, with a brief latency, until the 18th week.

In our control groups, mice did not have lung tumors or leukemias to the 40th experimental week, when they were 47- to 48-weeks old. Thus, the rapid development of leukemias and lung adenomas was the result of MNU administration. It seems that this chemical was absorbed quickly from the large intestine, and reached sensitive target organs. This was unexpected considering the fairly polar nature of MNU and its ready decomposition at the pH prevailing in the colon. Although not demonstrated, there may be also a reaction mediated by bacterial enzymes from the gut microflora. MNU also affected directly the epithelium of the large intestine. In the present study, squamous cell carcinomas in the anal region were found in relatively high incidence. These were observed also in mice injected subcutaneously 1,2-dimethylhydrazine (17–20). However, there is no recorded squamous cell carcinoma induced in rats with the same carcinogen. It seems a species or strain-specific phenomenon, not a chemical or technical problem. Moreover, MNU given intrarectally produced colorectal carcinomas in all tested rats, but no squamous cell carcinomas were found (our unpublished data).

The present study leads to the conclusion that frequent applications of a small dose of MNU seems superior for the production

of colon cancer in mice by the intrarectal instillation procedure, compared with a less frequent application of a large dose. Also, intrarectal instillation in mice is a simpler and more natural method to detect the oncogenicity, not only to the large intestine but also other organs, of carcinogenic substances in the environment and of such materials formed from dietary components or digestive secretions by the bacterial flora in the gut. Such experiments are now under way.

*Summary.* The same total dose of 9 mg of *N*-methylnitrosourea (MNU) was given intrarectally to two groups of ICR/Ha mice by varying the dose rate and frequency. Thirty individual doses of 0.3 mg each three times per week induced large bowel neoplasms with high incidence after the 17th week, compared with six doses of 1.5 mg each three times per week which also led to leukemia in all mice until the 18th week. Small adenomatous nodules of the lung appeared in almost all mice of both groups. In the large intestine, adenocarcinomas and adenomas were found in the distal colon and rectum, and squamous cell carcinomas at the anal canal. Intrarectal instillation of carcinogenic chemicals such as MNU to mice is a good method to develop animal models for colon carcinogenesis, and also other target organs.

We thank Dr. Napoleon E. Magadia, Mr. Ching-Quo Wong, and Miss Alzbeta Hulanova for excellent technical assistance. Also, Dr. Elizabeth K. Weisburger and Dr. Harry B. Wood, Jr., National Cancer Institute, for providing *N*-methylnitrosourea.

1. Weisburger, J. H., Dis. Col. Rect. **16,** 431 (1973).
2. Druckrey, H., *in* "Carcinoma of the Colon and Antecedent Epithelium" (W. J. Burdette, ed.), p. 267. Charles C Thomas, Springfield, IL. (1970).
3. Narisawa, T., Sato, T., Hayakawa, M., Sakuma, A., and Nakano, H., Gann **62,** 231 (1971).
4. Narisawa, T., Nakano, H., Hayakawa, M., Sato, T., and Sakuma, A., *in* "Topics in Chemical Carcinogenesis" (W. Nakahara, S. Takayama, T. Sugimura and S. Odashima, eds.), p. 145. University Tokyo Press, Tokyo (1972).
5. So, B. T., Magadia, N. E., and Wynder, E. L., J. Nat. Cancer Inst. **50,** 927 (1973).
6. Nakano, H., Tohoku J. Exp. Med. **110,** 7 (1973).
7. Narisawa, T., Magadia, N. E., Weisburger, J. H., and Wynder, E. L., J. Nat. Cancer Inst. **53,** 1093 (1974).
8. Narisawa, T., and Nakano, H., Gann **64,** 93 (1973).
9. Reddy, B. S., Weisburger, J. H., Narisawa, T., and Wynder, E. L., Cancer Res. 34, 2368 (1974).
10. Leaver, D. D., Swann, P. F., and Magee, P. N., Brit. J. Cancer **23,** 177 (1969).
11. Mirvish, S. S., J. Nat. Cancer Inst. **46,** 1183 (1971).
12. Sugimura, T., Fujimura, S., Kogure, K., Baba, T., Saito, T., Nagao, M., Hoshi, H., Shimosato, Y., and Yokoshima, T., Gann Monograph **8,** 157 (1967).
13. Magee, P. N., Fd. Cosmet. Toxicol. **9,** 207 (1971).
14. Druckrey, H., *in* "Topics in Chemical Carcinogenesis" (W. Nakahara, S. Takayama, T. Sugimura and S. Odashima, eds.), p. 73. University Tokyo Press, Tokyo (1972).
15. Weisburger, J. H., 7th Natl. Cancer Conf. Proc., 465 (1973).
16. Schoental, R., and Rive, D. J., Biochem. J. **97,** 466 (1965).
17. Wiebecke, B., Löhrs, U., Gimmy, J., and Eder, M., Zeitschr. Ges. Exp. Med. **149,** 277 (1968).
18. Pegg, A. E. and Hawks, A., Biochem. J. **122,** 121 (1971).
19. Thurnherr, N., Deschner, E. E., Stonehill, E. H., and Lipkin, M., Cancer Res. **33,** 940 (1973).
20. Evans, J. T., Hauschka, T. S., and Mittelman, A., J. Nat. Cancer Inst. **52,** 999 (1974).

Received July 29, 1974. P.S.E.B.M. 1975, Vol. 148.