PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 347-349 (1979)

Effects of Methylazoxymethanol Acetate on Inbred Mice: Influence of Genetic Factors on Tumor Induction (40550)

B. A. DIWAN,[1] A. M. DEMPSTER, AND K. E. BLACKMAN

Meloy Laboratories, Inc., 6715 Electronic Drive, Springfield, Virginia 22151

Recent studies from several laboratories have clearly shown that inbred strains of mice (1–4) and rats (5) differ in their response to dimethylhydrazine (DMH)-induced colorectal tumors. In our previous studies (3, 4) using various strains of mice, we observed that strains SWR/J and P/J were most susceptible, strain C57BL/6J was intermediate, whereas strain AKR/J was most resistant to DMH-induced colorectal tumors.

Since MAM, a potent inducer of colon tumors (6–8), is believed to be the proximate carcinogen of DMH (9), we thought it would be worthwhile to investigate its effects in the strains of mice showing differential susceptibility to DMH. The present study was, therefore, performed to investigate the carcinogenic effects of MAM acetate in mice of three inbred strains used in our previous studies (3, 4).

Materials and Methods. Mice of strains AKR/J, SWR/J, and C57BL/6J were obtained from the Production Department of the Jackson Laboratory, Bar Harbor, Maine. MAM acetate (97% pure), purchased from Schwarz-Mann laboratories, Orangeburg, New York was stored at −20° and used without further purification. Mice of each strain, approximately 6 to 8 weeks old, were given SC injections of 20 mg MAM acetate/kg body wt once per week for 10 weeks. To avoid decomposition, MAM acetate was administered within 1 hr of solution in physiological saline. Mice similarly treated with saline alone served as controls.

Animals that became moribund during the experimental period were killed and necropsied. The study was terminated at 35 weeks after the first injection of MAM acetate or saline, at which time all survivors were killed and necropsied. All organs including intestines were examined grossly and histologically for the numbers and types of tumors. Tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin; sections were stained with H&E.

Results. Table I summarizes the average age at the time of autopsy, the incidence, and the types of tumors in different strains of mice. Colorectal tumors occurred in nearly 100% of the SWR/J mice and 77% of the C57BL/6J mice. Most of the control and MAM acetate-treated AKR/J mice died of leukemia, which occurred slightly earlier in experimental mice than those in the control group (32 weeks vs 39 weeks).

Colorectal neoplasms were similar, both in gross appearance and histology, to those induced by DMH and reported earlier (4). The colorectal tumors in SWR/J mice were large (4 to 8 mm) and averaged 12 tumors/mouse. Most of these tumors were carcinomas (Table I) that were microscopically invasive, infiltrating the submucosa and the muscularis; none of them metastasized. The colorectal tumors, were also common in C57BL/6J mice although they were fewer in number (average 5.5 tumors/mouse) and less invasive than were those in the SWR/J mice. Most of the adenomas were sessile polyps or plaque-shaped thickenings of the intestinal mucosa.

Three C57BL/6J mice and 1 SWR/J mouse developed large anal tumors. These tumors were squamous cell carcinomas, originating from the junction of the squamous and columnar cell epithelium of the anal orifice. They were well differentiated and keratinizing.

Leukemias occurred mostly in AKR/J mice (Table I). Leukemia was characterized by thymic enlargement, splenomegaly, and generalized lymphadenopathy; lymphoblasts infiltrated all viscera.

A few lung tumors were observed in SWR/J mice, both in the experimental and control groups (Table I). Histologically these tumors

[1] Please address correspondence and reprint requests to: B. A. Diwan, Meloy Laboratories, Inc., 6715 Electronic Drive, Springfield, Va. 22151.

0037-9727/79/070347-03$01.00/0

TABLE I. EFFECTS OF MAM ACETATE ON THREE INBRED STRAINS OF MICE

Strain and treatment	Tumor incidence N/T[a]	Animals with colorectal tumors No. (%)	Colorectal tumor classification: No. of tumors Total	No. of tumors Per mouse	Adenocarcinomas No. (%)	Adenomas No. (%)	Animals with adenocarcinomas No. (%)	No. of mice with other neoplasms	Average age at the time of autopsy (in weeks)
SWR/J									
Experimental	20/21	20/21 (95)	238	12	135 (57)	103 (43)	17/20 (85)	4[b]	36 ± 4[c]
Control	2/20	0	0	0	0	0	0	2[d]	42 ± 3
C57BL/6J									
Experimental	21/26	20/26 (77)	111	5.5	39 (35)	72 (65)	11/20 (55)	4[e]	38 ± 3.5
Control	0/22	0	0	0	0	0	0	0	43 ± 3
AKR/J									
Experimental	22/28	0	0	0	0	0	0	22[f]	32 ± 6
Control	17/24	0	0	0	0	0	0	17[f]	39 ± 4.5

[a] Number of mice with tumors/total number of mice.
[b] Lung tumors (3) and an anal tumor occurred together with colorectal tumors.
[c] Weeks ± SD.
[d] Lung tumors (2).
[e] Three anal tumors and one leukemia; two anal tumors and one leukemia occurred together with colorectal tumors.
[f] Leukemias.

were benign adenomas. No pulmonary tumors were found in AKR/J or C57BL/6J mice.

MAM acetate produced severe liver damage in C57BL/6J mice; and all C57BL/6J mice killed at 35 weeks following the first injection of MAM acetate had liver lesions. The liver lesions showed cirrhosis, focal necrosis, fibrosis, and biliary cyst formation. Such lesions were also found in six SWR/J mice while none of the MAM acetate-treated AKR/J mice showed any evidence of liver damage.

Discussion. It is evident from this report that strains of mice vary in their response to MAM acetate treatment. As it is clear from Table I that SWR/J mice are most susceptible to colorectal tumors induced by MAM acetate. The incidence of colorectal tumor is also high (77%) in mice of strain C57BL/6J whereas AKR/J mice were most resistant to MAM acetate-induced colorectal tumors (Table I).

In our previous studies (3, 4), we showed that different strains of mice vary in their response to the carcinogenic effects produced by DMH. It is not surprising that SWR/J mice, which were most susceptible to colorectal tumors induced by DMH, are also susceptible to MAM acetate. However, the incidence of colorectal tumors is much higher in MAM acetate-treated C57BL/6J mice than in those treated with DMH (77 vs 48%, respectively, by t test $P < 0.05$). It is also interesting that AKR/J mice, resistant to colorectal tumors induced by DMH (3, 4), are equally resistant to the similar tumors or preneoplastic lesions induced by MAM acetate.

MAM is a metabolite of DMH and is formed when the latter undergoes oxidative metabolism (10). It was, therefore, assumed that the difference in the susceptibility to DMH in different strains of mice could be due to the inability of some strains to metabolize DMH. This assumption finds some support from the recent studies by Moria *et al.* (11) and Copper *et al.* (12). Moria *et al.* showed that DMH is metabolized to a potent mutagen *in vivo* and the mutation induction was lower in colon tumor-resistant C57BL/6 mice than in susceptible ICR mice. Cooper *et al.* determined the formation and persistance of methylated purines in the DNA of different organs of mice with genetically different susceptibility to DMH. From this study, they concluded that the low carcinogenic response of C57BL/Ha mice was due to the smaller extent of initial alkylation of colon DNA, which probably reflects differences in the enzymatic metabolism of the parent carcinogen.

The higher susceptibility of C57BL/6J mice to the induction of colorectal tumors by MAM acetate as compared to DMH (4) might be partially related to their inability to metabolize DMH. The incidence of colorectal tumors in C57BL/6J mice was, however,

much lower than that of SWR/J mice (Table I); also, the tumors were smaller in size and less invasive. Clearly, further work is required to understand the mechanism of total resistance of AKR/J mice to colorectal tumors induced by MAM acetate. The absence of colorectal tumors in AKR/J mice implies that either tumorigenesis had not occurred or that the latency period in AKR/J mice is especially long. Had these mice not died early of leukemia, colorectal tumors might have developed during the average life span of these mice.

The present data are similar to the finding of Pollard and Zedeck (13) who showed that different stocks of rats differ in their response to MAM-induced colorectal tumors. Our results suggest, however, that the mechanism of resistance might be different in mice than in rats. The resistant rats did suffer liver damage, indicating that the carcinogen could be activated in these animals. No such liver damage was observed in MAM acetate-treated AKR/J mice, suggesting that these mice may be lacking the dehydrogenase system needed to activate MAM (14, 15).

Summary. Three inbred strains of mice, AKR/J, SWR/J, and C57BL/6J, showing differential response to dimethylhydrazine (DMH)-induced colorectal tumors, were given SC injections once a week for 10 weeks with 20 mg methylazoxymethanol (MAM) acetate/kg body wt. Colorectal tumors occurred in nearly 100% of the SWR/J mice and 77% of C57BL/6J mice that survived 20 or more weeks after the first injection. Three C57BL/6J mice and 1 SWR/J mouse developed anal tumors. None of the AKR/J mice developed colorectal or anal tumors; a possible factor here could have been the earlier deaths of animals, mostly from leukemia. The influence of genetic background on the susceptibility to MAM acetate-induced colorectal tumors is discussed.

1. Evans, J. T., Hauschka, T. S., and Mittelman, A., J. Nat. Cancer Inst. **52,** 999 (1974).
2. Evans, J. T., Shows, T. B., Sproul, E. E., Parolini, N. S., Mittelman, A., and Hauschka, T. S., Cancer Res. **37,** 134 (1977).
3. Diwan, B. A., Meier, H., Proc. Amer. Ass. Cancer Res. **17,** 100 (1976).
4. Diwan, B. A., Meier, H., and Blackman, K. E., J. Nat. Cancer Inst. **59,** 455 (1977).
5. Asano, T., and Pollard, M., Proc. Soc. Exp. Biol. Med. **158,** 89 (1978).
6. Laquer, G. L., McDaniel, E. G., and Matsumoto, H., J. Nat. Cancer Inst. **39,** 355 (1967).
7. Zedeck, M. S., and Sternberg, S. S., J. Nat. Cancer Inst. **53,** 1419 (1974).
8. Reuber, M. D., Digestin **14,** 311 (1976).
9. Weisburger, J. H., Reddy, B. S., and Joftes, D. L. (eds.), Union Int. Cancer Tech. Ser. **19,** 34 (1975).
10. Fiala, E. S., Bobotos, A., Kulakis, D., Wattenberg, L. W., and Weisburger, J. H., Biochem. Pharmacol. **26,** 1763 (1977).
11. Moria, M., Kato, K., Ohta, T., Watanabe, K., Watanabe, Y., and Shirasu, Y., J. Nat. Cancer Inst. **61,** 457 (1978).
12. Cooper, H. K., Buecheler, J., and Kleihues, P., Cancer Res. **38,** 3063 (1978).
13. Pollard, M. and Zedeck, M. S., J. Nat. Cancer Inst. **61,** 493 (1978).
14. Grab, D. J., and Zedeck, M. S., Fed. Proc. **36,** 348 (1977).
15. Grab, D. J., and Zedeck, M. S., Cancer Res. **37,** 4182 (1977).

Received November 15, 1978. P.S.E.B.M. 1979, Vol. 161.