PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 161–164 (1981)

Treatment of Chemically-Induced Intestinal Cancers with Indomethacin[1] (41142)

MORRIS POLLARD AND PHYLLIS H. LUCKERT

Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556

Abstract. Intestinal tumors were induced in Lobund strain Sprague–Dawley rats by a single administration of 1,2-dimethylhydrazine (DMH) or of methylazoxymethanol acetate (MAM). At 34 days later (DMH), or at 7 and 35 days later (MAM), groups of rats were administered indomethacin (20 mg/liter) in the drinking water. When examined at Week 20, in those that consumed indomethacin there was a significant reduction in numbers of rats with intestinal tumors, compared to the control rats. In view of the interval between exposure to the carcinogen and treatment with indomethacin, the effect is interpreted as therapeutic or as antipromotional.

Investigators have reported that intestinal cancers produce higher levels of prostaglandin (PG) than the normal intestinal mucosa surrounding the tumors (1, 2). High levels of PG have been demonstrated also in patients with carcinomas of the breast (3, 4), of the kidney (5), and of the lung (6). High levels of PG have been demonstrated in mammary carcinomas which were induced in rats by 7, 12-dimethylbenz(α)-anthracene (7). Since indomethacin interferes with the synthesis of PG, it has been examined for effect on PG-producing experimental tumors. The rationale for therapeutic trials with indomethacin is based on the premise (a) that high production of PG may be an essential endogenous metabolite of tumor cells and (b) if the production of PG is blocked, perhaps the multiplication pattern of the tumor cells would be modified. Investigators have demonstrated by *in vitro* and by *in vivo* procedures that administrations of PG-blocking agents resulted in reduced activities of specific transplanted neoplasms. Some antitumor effects of indomethacin have been demonstrated *in vitro* on fibrosarcoma (8), and Balb 3T3 cells (9) of mouse origin. Administrations of indomethacin to mice with transplanted fibrosarcoma (10) and to rats with Yoshida hepatoma cells (11) resulted in suppression of tumor activities.

Indomethacin (7.5 mg/kg body wt) was administered by daily intrarectal inoculations to Donryu rats at advanced stages of methylazoxymethanol acetate (MAM)-induced tumors, which resulted in 23% reduction of rats with tumors (12). An antitumor effect of indomethacin was demonstrated in male Lobund Sprague–Dawley (S–D) rats with autochthonous tumors which had been induced in the intestines of S–D rats by 1,2-dimethylhydrazine (DMH) (13). In that report, S–D rats had been administered five weekly doses of DMH by gavage (30 mg/kg body wt) and then subsequently (at 3, 12, or 35 days) groups of them were given indomethacin continuously in the drinking water (20 mg/liter). At least 50% of the indomethacin-treated rats were tumor free at 20 weeks after exposure to DMH, but all of the control rats had developed intestinal tumors.

In the report presented here, it was demonstrated that a single dose of DMH produced significant tumor responses in male S–D rats. Also, it had been demonstrated that a single dose of MAM produced significant tumor responses in male and in female S–D rats (14). In order to reduce the tumor burden for test purposes, S–D rats were administered a single dose of DMH or of MAM which induced in them significant levels of autochthonous tumors. At intervals thereafter, groups of rats were administered indomethacin in the drinking water. Compared to data from control groups of rats, the indomethacin treatments resulted in highly significant reductions of tumor-bearing rats.

[1] Supported in part by USPHS Grants RR00294 and CA15957, and from the Fannie E. Rippel Foundation.

0037-9727/81/060161-04$01.00/0

Materials and Methods. Three groups of male Lobund S–D rats were administered freshly prepared DMH by gavage (30 mg/kg body wt) at weekly intervals: group I was given 1 dose; group II, 5 doses, and group III, 10 doses of DMH. The DMH was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. At 20 weeks after first exposures to DMH, each rat was weighed, anesthetized with ether, exsanguinated from the heart, and examined for lesions in the intestinal tract and in other organs. The intestinal tract of each rat was excised from the anus to the stomach, opened longitudinally and washed free of contents. The intestine was examined by 3× magnification, fixed in Bouin's solution for 24 hr, and then stored in 70% ethanol. The intestinal tract was examined again and the tumors were recorded as to numbers, location, and sizes. Individual tumors were processed for histological examinations.

Based on data derived from the above assay, S–D rats were each administered a single dose of DMH by gavage; and 34 days later a group was given, *ad libitum*, indomethacin in the drinking water (20 mg/liter). Water with the drug was replaced at 3-day intervals and the amounts consumed were recorded. Control rats were given drug-free water. The rats were killed at Week 20 after first exposure to DMH; and each rat was examined by the procedure noted above.

Male and female S–D rats were found susceptible to the carcinogenic effect of MAM. Two groups of S–D rats were administered a single dose of MAM acetate (Schwartz/Mann, Orange, N.Y.) by subcutaneous route (30 mg/kg body wt). At 7 or at 35 days thereafter, groups of rats were given indomethacin in the drinking water, as noted above; and control rats were given drug-free water. They were examined after 20 weeks by the same procedure noted above.

Indomethacin, 99.7% pure (1-(*p*-chlorobenzyl)-5-methoxy-2-methylindol-3-acetic acid) was a gift from Merck Sharp and Dohme, Rahway, New Jersey. This drug was dissolved in absolute ethanol and then diluted in tap water. The S–D rats were randomly propagated in this laboratory, and maintained in air-conditioned rooms (21°), with 12-hr light–dark intervals. The rats were maintained in isolator systems for 1 week after exposures to DMH or to MAM. They were held in plastic boxes on granulated corn-cob bedding; and fed a steam-sterilized Tek-Lad diet (L-485) and tap water *ad libitum*. All of the data derived from the examinations noted above were recorded and, where indicated, subjected to statistical assessment for significance by Student's *t* test.

Results. The numbers of tumors that developed in male S–D rats, in response to administrations of DMH, were dose related (Table I): one dose of DMH induced average 1 tumor/rat; 5 doses induced 4.8 tumors/rat; and 10 doses induced 14.0 tumors/rat. In addition, tumors were induced in 70, 100 and 100% of the rats, respectively. Thirty-seven of forty-eight (77%) of the rats inoculated with 1 dose of MAM developed intestinal tumors; and following 10 doses of MAM, 10/10 rats (100%) developed significantly increased numbers of tumors in the intestines (Table I). The tumors developed in the colons and in the small intestines, and less frequently in the rectum. The morphological characteristics of the tumors ranged from small superficial polypoid adenomas to large invasive adenocarcinomas, and many of the latter extended through the muscularis to the serosa. They resembled the spectrum of intestinal tumors described by Ward (15). Tumors were not observed in other organs, and a low incidence of metastatic lesions was observed in lymph nodes adjacent to the intestinal tumors. Most of the rats had cystic lesions in the livers.

A single dose of DMH induced intestinal tumors in 9 of 10 (90%) of male rats (Table II). In rats which had consumed indomethacin from 34 days after DMH, there was a significant reduction in (a) numbers of rats with tumors [2 of 9 (22%)]; and (b) in numbers of tumors in the rest of the rats compared to the untreated control rats ($P < 0.05$). A significant difference in tumor incidence was demonstrated in MAM-treated rats: 1 of 7 (14%) rats, which was treated with indomethacin at 7 days after MAM, had tumors; while 7 of 9 (78%) untreated

TABLE I. DOSE RESPONSE OF LOBUND SPRAGUE–DAWLEY RATS TO DMH OR MAM[a]

No. doses	No. rats with tumors/ No. rats inoculated (%)	Total no. tumors	Average no. tumors/ Tumor bearing rat	Average no. tumors/ No. rats inoculated
DMH				
1	21/30 (70)	30	1.4	1.0
5	69/69 (100)	338	4.8	4.8
10	23/23 (100)	323	14.0	14.0
MAM				
1	37/48 (77)	90	2.4	1.8
10	10/10 (100)	204	20.4	20.4

[a] Weanling male Lobund Sprague–Dawley rats were each administered 1,2-dimethylhydrazine by gavage at weekly intervals (30 mg/kg body wt/week), or methylazoxymethanol acetate (MAM), same dosage by subcutaneous inoculation. At Week 20 after the first dose of DMH, each rat was killed and examined for tumors in the intestines.

control rats had tumors ($P < 0.05$). In the second trial, started at 35 days after inoculation of MAM (Table II), none of the 5 indomethacin-treated female rats had a tumor, while 3 of 5 (60%) of the control rats had tumors. There were no significant differences in body weights between the treated and the untreated rats. The estimated daily dose of indomethacin consumed per rat was 3 mg/kg body wt.

The tumors which developed in the DMH-treated rats (without indomethacin) covered the morphological range of tumors described by Ward (15). The DMH-induced tumors which developed in the intestines of the indomethacin-treated rats were small and superficial. Small granulomatous lesions were visible as protrusions ("knobs") on the serosal surface of the small intestines of rats which consumed indomethacin in the drinking water (13).

Discussion. The tumor system used in this report is an excellent model, in that the tumors that developed in response to DMH

TABLE II. EFFECT OF INDOMETHACIN ON INTESTINAL TUMORS INDUCED IN RATS BY 1,2-DIMETHYLHYDRAZINE OR METHYLAZOXYMETHANOL[a]

Interval	Treatment	Rats with tumors/ Rats inoculated	Body weight (Avg/g)	Tumors: Colon	Tumors: Duodenum	Average (tumors/rat)
I. DMH induced						
34 Days	Indomethacin	2/9	403	1	1	0.22
	No drug	9/10	436	11	2	1.30
	significance[b]	0.0019	0.0248			0.0102
II. MAM induced						
7 Days	Indomethacin	1/7	415.4	0	1	0.14
	No drug	7/9	427	4	8	1.3
	Significance[b]	0.0085	0.57			0.0075
35 Days[c]	Indomethacin	0/5	237.2	0	0	0
	No drug	3/5	229.2	4	3	1.4
	Significance[b]		0.6573			

[a] Weanling male Sprague–Dawley rats were administered one dose of DMH by gavage (30 mg/kg body wt), or MAM acetate subcutaneously (30 mg/kg body wt). Thirty-four days later (DMH), and 7 and 35 days later (MAM), groups of rats were given water to which indomethacin was added (20 mg/liter). Control rats received water without the drug. The rats were killed for examinations at 20 weeks after exposures to the carcinogens.

[b] Students' t test.

[c] Female rats.

and to MAM are autochthonous, of multiple types, and located in the appropriate organ system. They manifest a low level of metastatic spread. By reducing the dosage of DMH and MAM, the resulting tumor burden was reduced (Table 1). This protocol, modified from that used in the previous report with DMH (13), resulted in more significant benefits to the indomethacin-treated rats.

The effects of chemical carcinogenesis can be modified by several procedures: (a) specific genetically defined strains of mice and of rats are resistant to the carcinogenic effects of DMH (14, 16); (b) chemical agents inhibit the action of DMH, and metabolites thereof, when administered prior to and/or simultaneously with the carcinogen (17); and (c) chemical agents produce an anti-promotional or a therapeutic effect after exposures of the host to the carcinogen. In this respect, it has been determined that DMH is metabolized and excreted within 24–48 hr after administration to rats; and that the *in vivo* metabolism of MAM is even more rapid (18, 19). The designated intervals between exposures to DMH or to MAM and the administrations of indomethacin suggest that the effects of the drug were directed not so much at the carcinogenic agent as at the transformed cells resulting thereof. The beneficial effect of indomethacin in rats which had been injected with MAM would indicate that the effect was directed at the stigma induced in cells by the ultimate carcinogenic metabolite (MAM).

The effects of indomethacin on rats with DMH-induced intestinal tumors may be attributed to three possible actions: (a) the tumors produce essential endogenous prostaglandins which are associated with cell propagation; or (b) prostaglandins are immunosuppressive (20–22), and when their production is blocked the immune mechanisms of the host function more effectively; or (c) the results of treatments have no relationship to prostaglandin production. At this time these propositions, in relation to the DMH and the MAM-induced intestinal tumors, are hypothetical.

1. Bennett, A., and del Tacca, M. Gut **16**, 409 (1975).
2. Bennett, A., del Tacca, M., Stamford, I. F., and Zebro, T., Brit. J. Cancer **34**, 881 (1977).
3. Bennett, A., Charlier, E. M., McDonald, A. M., Simpson, J. S., Stamford, I. F., and Zebro, T., Lancet **2**, 624 (1977).
4. Powles, T. J., Coombes, R. C., Neville, A. M., Ford, H. T., Gazet, J. C., and Levine, L., Lancet **2**, 138 (1977).
5. Cummings, K. B., and Robertson, R. P., J. Urol. **118**, 720 (1977).
6. Seyberth, H. W., Segre, G. V., Morgan, J. L., Sweetman, B. J., Potts, J. T., and Oates, J. A., New Engl. J. Med. **293**, 1278 (1975).
7. Tan, W. C., Privett, O. S., and Goldyne, M. E., Cancer Res. **34**, 3229 (1974).
8. Levine, L., Hinkle, P. M., Voelkel, E. F., and Tashjean, A. H., Biophys. Res. Commun. **47**, 888 (1972).
9. Hong, G., Wheless, C., and Levine, L., Prostaglandin **13**, 271 (1977).
10. Lynch, N. R., and Salomon, J.-C. J. Nat. Cancer Inst. **62**, 117 (1979).
11. Trevisani, A., Ferretti, E., Capuzzo, A., and Tomasi, V., Brit. J. Cancer **41**, 341 (1980).
12. Kudo, T., Narisawa, T., and Abo, S., Gann **71**, 260 (1980).
13. Pollard, M., and Luckert, P. H., Cancer Treatment Reports, in press, 1980.
14. Pollard, M., and Zedeck, M., J. Nat. Cancer Inst. **61**, 493 (1978).
15. Ward, J. M., Lab. Invest. **30**, 505 (1974).
16. Evans, J. T., Shows, T. B., Sproul, E. E., Paolini, N. S., Mittelman, A., and Hauschka, T., Cancer Res. **37**, 134 (1977).
17. Wattenberg, L. W. J. Environ. Pathol. Toxicol. **3**, 35 (1980).
18. Fiala, E. S., Bobotas, G., Kulakis, C., Wattenberg, L. W., and Weisburger, J. H., Biochem. Pharmacol. **26**, 1763 (1977).
19. Fiala, E., Personal communication, 1980.
20. Grinwich, K. D., and Plescia, O. J. Prostaglandins **14**, 1175 (1977).
21. Droller, M. J., Perlmann, P., and Schneider, M. U. Cell. Immunol. **39**, 154 (1978).
22. Fulton, A. M., and Levy, J. G., Cell. Immunol. **52**, 29 (1980).

Received December 22, 1980. P.S.E.B.M. 1981, Vol. 167.