Prevention and Treatment of Primary Intestinal Tumors in Rats by Piroxicam

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ABSTRACT

Over 90% of methylazoxymethanol acetate-treated male Lobund Sprague-Dawley rats developed intestinal tumors within 20 weeks; the incidence and numbers of tumors remained basically the same at 40 weeks. However, at week 40 the numbers of large tumors (≥0.5 cm in diameter) were increased. At week 40, tumors in methylazoxymethanol acetate-inoculated rats that had been treated with piroxicam (~2.3 mg/diameter) were significantly reduced in numbers, but many large tumors persisted. Rats treated with piroxicam up to 40 weeks carried 0.6 tumor/rat compared with 2.7 tumors/rat among untreated controls (P < 0.0001). Rats at week 20 had developed 2.8 tumors/rat and rats treated with piroxicam from week 20 to week 40 carried 1.4 tumors/rat (P < 0.007). Most of the tumors in the piroxicam-treated rats showed evidence of histological differentiation.

INTRODUCTION

Intestinal tumors have been induced in rats and mice by a variety of chemical agents including dimethylhydrazine, metabolites thereof, and nitrosamines (1–3). In many of the reported experimental procedures, the test animals were given multiple doses of the tumor initiator; however, we have demonstrated that significant numbers of male S-D rats developed intestinal tumors following a single dose of the chemical carcinogen MAM (4). The tumor types that developed in response to the carcinogenic agents ranged from marginally malignant polyps to malignant adenocarcinomas, and metastatic spread was very rare.

High levels of PGs were demonstrated in neoplastic cells of human and animal origins (5–7). While it has been speculated that PGs were involved in the tumorigenic process, the mechanism(s) thereof are as yet unknown. Since NSAIDs are known to interfere with the synthesis of PGs (8), they have been examined for effects on experimentally induced neoplasms. It was demonstrated that administration of NSAIDs interfered with development of experimentally induced tumors in rats and mice; the tumors included myeloma (9) and carcinomas of the breast (10, 11), esophagus (12), and intestine (13–16). Primary intestinal tumors, responding in S-D rats to 4 carcinogenic agents, were prevented or retarded by administration of indomethacin or piroxicam (13–18).

Within 20 weeks, 90% of S-D rats responded to a single s.c. dose of MAM with the development of epithelial tumors in the duodenum and colon. By administering indomethacin in the drinking water (19) or piroxicam in the feed (20), the incidences of intestinal tumors in S-D rats were significantly reduced when they were examined at 20 weeks. Since indomethacin manifested a narrow margin of dosage to toxicity in the intestine, attention was directed at piroxicam which is less damaging to the intestinal tract. While the development of intestinal tumors was prevented in rats by early treatments with indomethacin and with piroxicam, there is no information on the therapeutic effects of NSAIDs on experimentally induced intestinal tumors. In the report that follows, it was demonstrated that piroxicam did manifest demonstrable therapeutic effects on autochthonous intestinal tumors in rats.

MATERIALS AND METHODS

Rats. The S-D rat was random-bred through 56 generations under germfree conditions. At each generation level, groups of rats were conventionalized in a clean isolated animal house in which they were propagated as pathogen-free rats. While the rats are not considered inbred, they do accept reciprocal skin graft transplants. The rats were fed, ad libitum, steam-sterilized natural ingredient diet L-485, which was supplemented with vitamins and minerals (21) and unchlorinated water from deep wells. They were housed in air-conditioned rooms in plastic boxes, on Sanicel bedding, with 12-h light-dark cycles. S-D rats have not developed intestinal tumors spontaneously, but they are unusually susceptible to chemical carcinogenic agents. When a single s.c. inoculation of MAM was administered, neoplasms appeared in the duodenum or in the distal third of the large intestine. The only other lesion noted in all of the MAM-treated rats were occasional small cysts in the livers.

Experimental Design. At age 6 weeks, 85 conventional male S-D rats were inoculated s.c. in the flank area with a single dose of MAM (30 mg/kg body weight) (Schwarz/Mann, Orangeburg, NY). For 1 week thereafter they were housed in plastic isolators from which the effluent filtered air was ducted to the roof, and the contaminated contents of the isolator were removed aseptically and incinerated.

Groups of the MAM-inoculated rats were fed, ad libitum, powdered diet L-485 to which piroxicam (4-hydroxy-2-methyl-Ar-2-pyridinyl-2//-1,2-benzothiazine-3-carboxamide-1.1-dioxide) (22) had been added and mixed (130 mg/kg diet), and control rats were fed drug-free diet. As outlined in Table 1, groups of test rats were maintained for periods of 20 or 40 weeks. At the end of the interval designated for the group, each rat was weighed, anesthetized with ether, and exsanguinated from the exposed heart. The rat was then autopsied and examined for gross and microscopic evidence of disease. The intestines were excised and opened longitudinally, washed free of contents, and examined visually at ×3 magnification, and the tumors were recorded as to location, number, and sizes. Tumors which were over 0.5 cm in diameter were designated "large." The intestines were fixed in Bouin's solution for 18 h and then transferred to 70% ethanol. After a second visual examination at ×3 magnification, individual tumors were excised, weighed, and then processed for histological examinations. Other organs with lesions were similarly fixed and processed for histological examinations.

The recorded data were analyzed for significance by Student's t test.

RESULTS

During the test periods, all of the rats appeared healthy and there were no statistical differences in body weights among rats of the groups noted in Table 1. It was estimated that each treated rat consumed ~2.5 mg piroxicam daily.

The numbers of intestinal tumors that developed in MAM-inoculated rats were basically the same at 20 and 40 weeks (P = 0.9928) except that at 40 weeks more of the tumors were large (Table 1). Tumors that were classified as large weighed an average of 363 mg, and those listed as small weighed an average of 85 mg. The only other lesions observed in the MAM-
inoculated rats were small clear cysts in their livers. The spectrum of tumor types was similar to that described by Ward (23); they ranged in sizes and structures from small superficial polyps to larger polyps on long stalks, to sessile tumors that penetratred through the muscularis mucosa and occasionally to the serosa. There was no evidence of metastasis.

Administration of piroxicam to rats for 39 weeks after treatment with MAM resulted in a significant reduction of intestinal tumors ($P < 0.0001$) compared to control rats on the drug-free diet (Table 1). Also, the number of large tumors (>0.5 cm) was reduced among the piroxicam-treated rats. Rats that were treated with piroxicam from week 20 to week 40 after MAM inoculation had a significant reduction of tumor numbers compared to tumors in rats that had been examined at week 20 ($P < 0.007$), but the number of large tumors per rat was unchanged in the two groups of rats. There was no evidence of intestinal damage in the piroxicam-treated rats.

In the drug-free rats the tumors were adenocarcinomas: the tumor cells were multilayered and disorganized; they lacked polarization; and they showed frequent mitotic figures. Some of the tumors contained cyst-like accumulations of mucus-like material. In contrast, tumors in the piroxicam-treated rats were generally smaller and less active; many of the adenomatous structures showed single layers of cells which were polarized and showed few mitotic figures. Among the latter tumors large cyst-like sacs that were filled with cellular debris and lined by single layers of cuboid cells had developed. The connective tissue stromas were sparse in the tumors from rats on the drug-free diet, while in the drug-treated rats, the stromas were more prominent, loose, and infiltrated with lymphoid cells in addition to connective tissue. One of the piroxicam-treated rats had a large tumor that was similar to the large tumors in the drug-free rats. While many of the tumors in the piroxicam-treated rats were judged generally to be quiescent, there were actually mitotic figures in some areas of individual tumors.

**DISCUSSION**

In assessing the efficacy of a cancer-modulating agent, it is preferable that autochthonous tumor systems be used. In some respects, transplanted tumors are artifacts in that, with passage, the tumor cell population evolves into a clone of the most rapidly multiplying cells in an abnormal environment. Autochthonous organ-related tumors are located in the "normal" anatomical site; they are characterized by cellular heterogeneity; and thus they are more representative of the counterpart tumor in humans. The investigations that were cited in this report involved autochthonous tumors in the intestine, which contribute to the validity of the results.

From the data presented in Table 1 and further amplified in the text, a single dose of MAM induced intestinal tumors in 90% of S-D rats within 20 weeks. The numbers of large tumors increased during the subsequent 20 weeks. With this as baseline information, the effect of piroxicam in MAM-inoculated rats was a significant suppression of tumor numbers and sizes at 40 weeks. When piroxicam treatments were started at week 20 (after tumors had already developed among the untreated control rats) the numbers of tumors in the treated groups of rats at week 40 were significantly reduced compared to untreated control rats (Table 1). However, the incidence of "large" tumors was essentially unchanged which suggests that cells, resistant to the effects of piroxicam, had emerged by 20 weeks. The reversal of tumor development cannot be explained at this time because piroxicam is not classified as an oncolytic agent. It is possible that while the antitumor effect of NSAIDs may be interpreted as antipromotional (19, 24), it may also be attributed to immunological enhancement (especially the cell-mediated type) which is associated with a reduction of prostaglandin production (25–27). For example, PGs suppressed the activity of natural killer cells, and this was reversed by indomethacin (28). In addition to blocking the synthesis of PGs, NSAIDs also inhibit the induction of ornithine decarboxylase, an enzyme related to tumor promotion (29). There is evidence that indomethacin reversibly inhibits cell growth in the G1 phase of the cell cycle (30) and that the drug interferes with tumor angiogenesis (31). It is likely that other as yet unidentified tumor-related mechanisms are affected by piroxicam (and possibly other NSAIDs). Dose-dependent effects of piroxicam on autochthonous intestinal tumors have been reported (15, 16).

The histological changes in the tumors from the piroxicam-treated rats suggest that a cellular maturation or differentiation effect had occurred. The tumors had lost some of the physical characteristics of malignancy. Exemplary of this were the reduction in tumor size, increased numbers and arrangements of differentiated cells, and reduced frequency of mitotic figures. It should be emphasized that the effect of piroxicam was not manifested among all of the tumor that had developed; occasional tumors continued to show histological activities that were characteristic of tumors in the rats on the drug-free diet.

It is significant that the anticipated numbers of primary intestinal tumors, induced in rats by two carcinogenic agents (MAM and methyl nitrosourea), were reduced in numbers or were "suppressed" by early treatments with piroxicam (15–17), without evidence of damage to the intestinal mucosa. While there is no evidence that piroxicam effects "cures" of neoplasms, this drug does interfere with the development and growth of autochthonous intestinal tumors, even those that had already developed in MAM-inoculated rats. This was suggested in a report by Reddy et al. (16) on azoxymethane-induced tumors in F344 rats that had been treated with piroxicam. In this respect, a related NSAID (Sulindac) demonstrated some beneficial effects in humans with desmoid tumors and with intestinal polyposis (32, 33). The spectrum of tumor types that are susceptible to NSAIDs is not universal; for example, there were no preventive or therapeutic effects of piroxicam on the development of autochthonous prostate tumors in rats. How-

### Table 1: Effects of piroxicam on numbers and sizes of intestinal tumors induced in rats by MAM

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. positive/no. at risk (%)</th>
<th>No. of tumors (large)</th>
<th>No. of tumors/rat</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. MAM</td>
<td>0–20</td>
<td>19/21 (90)</td>
<td>60 (8)</td>
<td>2.8</td>
</tr>
<tr>
<td>B. MAM</td>
<td>0–40</td>
<td>19/20 (95)</td>
<td>55 (29)</td>
<td>2.7</td>
</tr>
<tr>
<td>C. MAM + piroxicam</td>
<td>0–40</td>
<td>9/21 (43)</td>
<td>12 (6)</td>
<td>0.6</td>
</tr>
<tr>
<td>D. MAM + piroxicam</td>
<td>20–40</td>
<td>17/23 (74)</td>
<td>33 (10)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Weaning male S-D rats were inoculated s.c. once with MAM (30 mg/kg body weight). After 7 days in isolation, groups were fed piroxicam in the feed starting on week 1 or on week 20 after inoculation of MAM. Control groups were fed drug-free diet. Groups of rats were examined for intestinal tumors at week 20 or 40.

*Tumors designated "large" exceeded 0.5 cm in diameter.*
ever, those tumor types that have been suppressed by NSAIDs outnumber those that have not been suppressed.

REFERENCES

1. Lacquer, G. L. Carcinogenic effects of cycad meal and cycasin methlyazo-
  methanol glycoside in rats and effects of cycasin in germfree rats. Fed.
2. Druckrey, H. Organospecific carcinogenesis in the digestive tract. In: W.
  Nakahara et al. (eds.), Topics in Chemical Carcinogenesis, pp. 73–101.
3. Pozharisski, K. M., Likkachev, A. J., Klemashewski, V. F., and Shaposhni-
  kov, D. Experimental intestinal cancer research with special reference to
4. Pollard, M. Antitumor effect of indomethacin in rats with autochthonous
  intestinal tumors. In: B. Levin and R. H. Riddell (eds.), Frontiers in Gas-
  461, 1974.
  plasmacytoma development in BALB/c mice by indomethacin. J. Exp. Med.,
10. Carter, C. A., Milholland, R. J., Shear, W., and Ip, M. M. Effect of
    prostaglandin synthetase inhibitor indomethacin on 7,12-dimethyl-
    benz(a)anthracine-induced mammary tumorigenesis in rats fed different lev-
11. McCornick, D. L., and Moon, R. C. Inhibition of mammary carcinogenesis
    by flubiprofen, a nonsteroidal anti-inflammatory agent. Br. J. Cancer, 48:
12. Rubio, C. A. Further studies on the therapeutic effect of indomethacin on
13. Pollard, M. and Luckert, P. H. Indomethacin treatment of rats with di-
    1327, 1980.
14. Kudo, T., Narisawa, T., and Abo, S. Antitumor activity of indomethacin on
    methyloxazemethanol-induced large bowel tumors in rats. Gann, 71: 260–
    264, 1980.
15. Nigro, N. D., Bull, A. W., and Boyd, M. E. Inhibition of intestinal carcino-
    genesis in rats: effect of difluoromethylformamide with piroxicam in fish oil.
    carcinogenesis by dietary piroxicam, a nonsteroidal anti-inflammatory drug,
    during different stages of rat colon tumor development. Cancer Res., 47:
17. Pollard, M., and Luckert, P. H. Effect of piroxicam on primary intestinal
    1984.
18. Pollard, M., and Luckert, P. H. Effect of indomethacin on intestinal tumors
    induced in rats by the acetate derivative of dimethylnitrosamine. Science
    (Wash. DC), 214: 558–559, 1981.
    1105, 1983.
20. Pollard, M., Luckert, P. H., and Schmidt, M. A. The suppressive effect of
    piroxicam on autochthonous intestinal tumors in the rat. Cancer Lett., 21:
    57–61, 1983.
21. Kellogg, T. F., and Wostmann, B. S. Stock diet for colony production of
22. Lombardino, J. G., and Wiseman, E. H. Piroxicam and other anti-inflam-
23. Ward, J. M. Morphogenesis of chemically induced neoplasms of the colon
    and promotion on N-methyl-nitrosourea-induced colon carcinogenesis in rats
    by nonsteroid anti-inflammatory agent indomethacin. Carcinogenesis
    (Lond.), 4: 1225–1227, 1983.
26. Droller, M., Perlmann, P., and Schneider, M. Enhancement of natural and
    antibody-dependent lymphocyte cytotoxicity by drugs which inhibit prosta-
27. Goodwin, J. S., and Ceuppens, J. Regulation of the immune response by pro-
    natural killer cell activity by prostaglandins. J. Immunol., 124: 2682–2690,
    1980.
    tumor promotion: inhibition of tumor promoter-induced ornithine decarbox-
    ylase activity in epidermis by inhibitors of prostaglandin synthesis. Biochem.
30. Bayer, B. M., and Beaven, M. A. Evidence that indomethacin reversibly
    inhibits cell growth in the G2 phase of the cell cycle. Biochem. Pharmacol.,
31. Ziche, M., Jones, J., and GUllino, P. M. Role of prostaglandin E and copper
32. Waddell, W. R., and Gerner, R. E. Indomethacin and ascorbate inhibit
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