

Reduction of Acetoxymethyl-Methylnitrosamine-Induced Large Bowel Cancer in Rats by Indomethacin

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NARISAWA, T., HERMANEK, P., HABST, M. and SCHMÄHL, D. *Reduction of Acetoxymethyl-Methylnitrosamine-Induced Large Bowel Cancer in Rats by Indomethacin*. Tohoku J. exp. Med., 1984, **144** (3), 237-243 — The nonsteroid anti-inflammatory drug indomethacin, a potent prostaglandin synthesis inhibitor, may play a role in preventing chemically-induced large bowel cancer development in rats. 250 male Sprague-Dawley rats were given weekly intrarectal doses of 2 mg/kg body weight of acetoxymethyl-methylnitrosamine (AMMN) in the first 10 weeks of the experiment to induce large bowel tumors. Experimental groups received a 0.001% aqueous solution of indomethacin ad libitum as drinking water for different time intervals. At autopsy in week 21, the indomethacin treatment in the first and second 10-week periods, or only in the second 10-week period significantly reduced the number of large bowel tumors compared to non-treatment control groups, while the treatment in the first 10-week period alone did not affect the tumor development. It was observed at autopsy in week 31 that the 10-week cessation of treatment after the effective treatments permitted the growth of tumors, but the treatment in the first and second 10-week periods was effective enough to suppress tumor appearance compared to other groups. It can be concluded that indomethacin has an antiproliferative activity on large bowel carcinogenesis. ——— indomethacin; cancer prevention; acetoxymethyl-methylnitrosamine (AMMN); rat large bowel cancer

The antitumor activity of nonsteroid anti-inflammatory drugs such as indomethacin, a potent prostaglandin synthesis inhibitor, has been investigated

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with various types of transplantable tumors in mice and rats (Bennett 1979; Goodwin 1981; Alexander 1982). Administration of these drugs resulted in the inhibition of tumor growth or tumor cell proliferation, and effect which has been associated with the suppression of prostaglandin production in tumor tissue or tumor cells. The drugs can also prevent the development of chemically induced tumors in mouse skin, and in rat large bowel and urinary bladder (Levine 1982; Lowe et al. 1982). One of the authors (Narisawa et al. 1981, 1982, 1983) demonstrated that indomethacin given at various dose schedules inhibited the development of large bowel carcinomas in rats induced by intrarectal administration of N-nitrosomethylurea. Indomethacin blocked the initiation stage and the promotion stage in the course of large bowel carcinogenesis. Pollard and Luckert (1981, 1983) also reported that indomethacin given during the promotion stage reduced tumors of the small and large bowels induced by parenterally administered 1, 2-dimethylhydrazine, methylazoxymethanol and acetoxymethyl-methylnitrosamine (AMMN) in rats.

AMMN is a potent direct acting carcinogen and produces carcinomas restricted to the distal half of the large bowel of rats when administered intrarectally (Habs et al. 1978). The experimental results prompted us to investigate whether large bowel carcinogenesis induced by intrarectally administered AMMN could be suppressed by indomethacin in a systematically designed experiment with a large number of rats.

MATERIALS AND METHODS

A total number of 250 male Sprague-Dawley rats (Charles River Wiga, Sulzfeld), 8 weeks old and weighing approximately 290 g at the start of experiment, were assigned to 5 groups. They had free access to Altromin-1320 laboratory chow and tap water throughout the experiment and were weighed once a month. The carcinogen AMMN (Dr. M. Wiessler, Institute of Toxicology and Chemotherapy, German Cancer Research Center) was dissolved in distilled water at a concentration of 2 mg/ml immediately before use. Indomethacin (Sigma Chemical Co., St. Louis, Mo. USA) was dissolved in absolute ethyl alcohol at a concentration of 10 mg/ml, and 0.1 ml of this stock solution was added to 100 ml of tap water. The aqueous 0.001% solution of indomethacin was given *ad libitum* to indomethacin-treated groups of rats as drinking water. The drinking water was exchanged every other day.

All rats were given intrarectal instillation of 2 mg/kg body weight of AMMN once a week from weeks 1 to 10, to induce large bowel tumors according to a method described previously (Habs et al. 1978). The rats in groups Ind-1, Ind-2 and Ind-3 were subjected to indomethacin treatment during weeks 1 to 10 (the first 10-week period of the experiment), weeks 11 to 20 (the second 10-week period) and weeks 1 to 20 (both the first and second 10-week periods), respectively. The rats in the vehicle group received an aqueous 0.1% solution of ethyl alcohol as drinking water during weeks 1 to 20, and the rats in the untreated group received plain tap water throughout the experiment. The last two groups served as controls. During the periods other than the weeks described above, indomethacin- and vehicle-treated rats were given plain tap water without indomethacin or ethyl alcohol. The consumed volume of drinking water was measured periodically.

At week 11, the rats of the untreated group were submitted to endoscopic examination

of the large bowel according to a method reported previously (Narisawa et al. 1975; Merz et al. 1981), to confirm that no grossly detectable large bowel tumors had developed up to this time. The rats were sacrificed and autopsied at weeks 21 or 31. The rats died prior to sacrificing were excluded from the study. At autopsy the large bowel was cut open lengthwise and inspected macroscopically. Location, shape and size of tumors were recorded. Other organs including the stomach and small bowel were also inspected carefully. The large bowel tumors taken from all rats and abnormally changed organs were examined histologically according to standard methods. Data on the number of tumors were analysed using Student's *t*-test.

RESULTS

Indomethacin-treated rats tolerated the multiple doses of AMMN as well as the long-term administration of indomethacin. The mean body weight gain of these rats was similar to that of untreated and vehicle controls. The volume of drinking water consumed was almost identical among the different groups irrespective of indomethacin treatment. The mean dosage of indomethacin consumed did not differ significantly between indomethacin-treated groups (Table 1). No apparent toxicity was observed after long-term indomethacin treatment. Endoscopic examination confirmed that no large bowel tumors had developed in untreated rats until week 11, the week following the administration of the last dose of AMMN.

At autopsy in weeks 21 and 31, it was found that all tumors formed were localized in the distal half of the large bowel. The tumors were plaque-shaped or polypoid, and their diameters ranged from 0.1 to 2.6 cm. Tumor-bearing rats had one or more tumors (16 tumors at the most). It was observed, in general, that the number and size of tumors were smaller in indomethacin-treated rats than in untreated and vehicle controls.

At week 21, the tumor incidence was more than 90% in all groups (Table 2). The number of tumors per animal was significantly smaller in the Ind-2 and Ind-3

TABLE 1. *Dosage of indomethacin computed from the consumed volume of indomethacin solution administered as drinking water*

Treatment groups* (Period of treatment)	Consumed doses of indomethacin (mg/kg body weight/day)†			
	in Week 1	in Week 5	in Week 11	in Week 15
Ind-1 (Weeks 1-10)	1.36 ± 0.02	1.03 ± 0.02		
Ind-2 (Weeks 11-20)			0.92 ± 0.03	0.73 ± 0.02
Ind-3 (Weeks 1-20)	1.38 ± 0.01	0.91 ± 0.02	0.93 ± 0.02	0.83 ± 0.01

* Rats had free access to 0.001% aqueous solution of indomethacin as drinking water during the treatment periods.

† Values represent means ± S.E.M.

TABLE 2. *Inhibition of AMMN-induced large bowel tumors in rats by indomethacin*

Groups* (Period of treatment)	at Week 21			at Week 31		
	Number of rats examined	Number of rats with tumors (%)	Number of tumors per rat†	Number of rats examined	Number of rats with tumors (%)	Number of tumors per rat†
Untreated	29	28 (97%)	4.7±0.63	18	18 (100%)	6.7±0.69
Vehicle (Weeks 1-20)	29	28 (97%)	3.8±0.47	20	20 (100%)	8.1±0.71
Ind-1 (Weeks 1-10)	30	30 (100%)	5.1±0.70	17	17 (100%)	8.2±0.74
Ind-2 (Weeks 11-20)	29	29 (100%)	3.2±0.39‡	18	18 (100%)	8.0±0.76
Ind-3 (Weeks 1-20)	30	27 (90%)	2.1±0.37§	20	20 (100%)	5.5±0.69//

* All rats were administered weekly intrarectal doses of 2 mg/kg body weight of AMMN at weeks 1-10. Rats received 0.001% aqueous solution of indomethacin as drinking water in the Ind groups and 0.1% aqueous solution of ethyl alcohol in the vehicle group during the stated periods.

† Values represent means±S.E.M.

‡ Significantly different from untreated and Ind-1 groups; $p < 0.05$, or 0.02, respectively.

§ Significantly different from untreated, vehicle, Ind-1 and Ind-2 groups; $p < 0.05$, 0.01 or 0.001, respectively.

// Significantly different from vehicle, Ind-1 and Ind-2 groups; $p < 0.05$ or 0.02, respectively.

groups, compared to the untreated, vehicle and Ind-1 groups. The number of tumors in group Ind-3 was significantly smaller than that in group Ind-2. The number of tumors was also reduced in the vehicle group, compared to the untreated control, but the difference was not significant ($0.2 < p < 0.3$). Thus, longer indomethacin treatment over the first and second 10-week periods was much more effective in inhibiting the tumor development than indomethacin treatment given only during the second 10-week period after termination of AMMN administration in the first 10-week period. Indomethacin treatment given only in the first 10-week period, i.e. concomitantly with AMMN administration, however, did not show any effect to inhibit tumor development.

At autopsy in week 31, large bowel tumors had significantly multiplied in all groups, compared to the respective groups of rats examined in week 21 ($p < 0.05$ or better). This indicates that cessation of indomethacin treatment permitted further tumor development even in those groups, in which the effectiveness of indomethacin treatment was demonstrated in week 21. However, the number of tumors was significantly smaller in the high-dose group Ind-3 than in the vehicle, Ind-1 and Ind-2 groups. Thus, long-term indomethacin treatment over the first and second 10-week periods was still effective in suppressing tumor growth, even when followed by a 10-week cessation of treatment, but it did not cure or reject AMMN-induced neoplastic cells in the large bowel.

Histologically, the large bowel tumors were well-differentiated adenocarcinomas involving the mucosa and partly the serosa. There was no distinguishable difference in the microscopic findings among tumors of all groups irrespective of indomethacin treatment. No metastases in lymph nodes or distant organs were found. No tumors had developed in other organs. Ulceration or bleeding of the gastrointestinal tract attributable to the long-term administration of indomethacin were not observed.

DISCUSSION

In the present study it was demonstrated that indomethacin reduces the development of large bowel carcinomas induced by intrarectal administration of AMMN in rats. The result is in agreement with a study by Pollard and Luckert (1981), in which indomethacin given after single intraperitoneal injection of a large dose of AMMN prevented the tumor development in the large bowel as well as in the small bowel. It has been also demonstrated that the drug inhibited the promotion and/or progression of large bowel tumor development induced by intrarectal instillation of N-methylnitrosourea (Narisawa et al. 1981; 1982; 1983). Thus, it can be concluded that indomethacin has an antiproliferative activity on large bowel carcinogenesis. Furthermore, it is evident from the present and a previous study (Narisawa et al. 1983) that the treatment started earlier and continued over a long time results in more effective prevention of tumor development. The treatment given concomitantly with intrarectal administration of

AMMN, however, did not show any effect.

Nonsteroid anti-inflammatory drugs inhibit the phorbol ester TPA-effected promotion in mouse skin carcinogenesis following initiation by 7, 12-dimethylbenz (a) anthracene (DMBA) (Fürstenberger and Marks 1980; Verma et al. 1980; Fischer et al. 1982). It was also reported that the promotion stage of DMBA-induced rat mammary gland carcinogenesis was suppressed by indomethacin (Carter et al. 1983). Furthermore, nonsteroid anti-inflammatory drugs may contribute to block prostaglandin-related co-oxidation in the formation of ultimate carcinogens from carcinogens such as benzo (a) pyrene (Marnett 1981; Robertson et al. 1983) and the urinary bladder carcinogen N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (Cohen et al. 1981). On the other hand, those drugs may reduce the rate of proliferation in transplantable tumors in mice and rats, and also of neoplastic and non-neoplastic cells in vitro (Bennett 1979). The present findings are in agreement with the hypothesis that this effect may be associated with the inhibition of prostaglandin production by those drugs. However, the mechanism of indomethacin-induced inhibition of large bowel carcinogenesis remains to be investigated still because the nonsteroid anti-inflammatory drugs have many pharmacological effects other than the inhibition of prostaglandin synthesis.

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