



## Inhibitory effect of sulindac against chemically-induced primary colonic tumors by N-methyl-N-nitrosourea in mice

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### Abstract

**AIM:** To investigate the chemopreventive effect of sulindac, a nonsteroidal anti-inflammatory drug (NSAID), on the growth of N-methyl-N-nitrosourea (MNU)-induced mouse colonic tumors.

**METHODS:** The experimental colonic tumor model induced by intrarectal instillation of MNU in mice was used in the present study. In the first experiment, MNU intrarectal was instilled and sulindac administered concurrently to a group of mice for a period of 18 wk, while a control group of animals received MNU only for the same period. In the second experiment, two groups of mice that had already been treated with MNU for 12 wk received sulindac or not for another 18 wk.

**RESULTS:** The tumors induced in mice were all located in the distal part of the large intestine. There were no significant differences in the location and the gross appearance of the tumors in the MNU-induced group and control group in both experiments. In the first experiment, sulindac caused a significant reduction in both the number of mice with colonic tumors and the number of tumors per mouse. Sulindac had a significant inhibitory effect on the growth of the MNU-induced tumors. However, in the second experiment, the inhibitory effect of sulindac was less or disappeared.

**CONCLUSION:** Sulindac has a protective effect against the chemical induction of colonic tumors by MNU in mice. The chemopreventive effect is more significant in the initial stage of the tumor, while in the promotion stage this effect is less or disappeared. Sulindac can not cause the regression of established tumors.

**Key words:** Colonic neoplasms; Sulindac; Methyl-nitrosourea; Adenocarcinoma;

Disease models; Animal; Anti-inflammatory agents; Nonsteroidal

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### INTRODUCTION

Large bowel cancer is one of the leading causes of cancer deaths in humans. It was reported in recent years that sulindac, a nonsteroidal anti-inflammatory drug (NSAID), was capable of controlling tumor growth and reducing the numbers of adenoma in patients with familial polyposis (FAP), with the likelihood of reducing the risk of colon carcinogenesis<sup>[1,2]</sup>. In order to further understand these effects, the present study was designed to investigate the chemopreventive efficacy and modulating role of sulindac on colon carcinogenesis and on tumor growth in the colorectal cancer model induced by N-methyl-N-nitrosourea (MNU) in mice.

### MATERIALS AND METHODS

#### Colorectal cancer model induced by MNU

Randombred ICR female mice from the Shanghai SIPPR/BK Experimental Animal Ltd. Co. aged 5-6 wk and weighing 18-21 g were used in the experiments. They were fed with a standard pellet diet and allowed tap water ad libitum. The MNU (obtained from Sigma Chemical Co, St. Louis, MO., United States) was kept frozen in powdered form prior to use. The carcinogen needed to be reconstituted in distilled water and maintained at 4 °C until intrarectal instillation. MNU 0.1 mL of 0.4% (0.4 mg) was given using a 5.0 cm 18 gauge cannula needle which was inserted about halfway into the lumen of the colorectum through the anus, three times for the first six weeks and only once for the following six weeks. The method used for colorectal cancer induced in mice followed in detail the method described in our previous study<sup>[3]</sup>.

#### Treatment and grouping

Sulindac, cis-5-fluoro-2-methyl-1-[p-(methyl sulfinyl)benzylidene]indene-3-acetic acid, was dissolved in tap water in a concentration such that each animal received an average dose corresponding to 5 mg/kg a day. The study consisted of two experiments. In the first experiment, two groups of 48 animals were treated with MNU as described above, either with or without sulindac, for 24 wk. In the second experiment, 48 mice were treated with MNU for 12 wk at first. Then they were randomized, with 24 animals given sulindac solution for 18 wk while the other 24 animals only received water as

**Table 1** The experimental design and animal groups

Group	No.	MNU intraducal time (wk)	Sulindac time (wk)
Experiment one			
Sulindac group	24	12	18
Control group	24	12	No
Experiment two			
Sulindac group	24	12	18
Control group	24	12	No

**Table 2** Effect of sulindac on the morphological characteristics of colorectal tumors induced by N-methyl-N-nitrosourea

	Experiment one		Experiment two	
	Sulindac group	Control group	Control group	Sulindac group
Average number of adenoma	4.7	8.4	3.5	4.7
Average number of adenocarcinoma	9.3	3.9	8.7	9.4
Average ratio of adenoma to adenocarcinoma	0.51	2.15	0.40	0.50

**Table 3** Effect of sulindac on the number of colorectal tumors induced by N-methyl-N-nitrosourea

	Experiment one		Experiment two	
	Sulindac group	Control group	Control group	Sulindac group
No. of mice	24	24	24	24
No. of mice with tumors	19	9	21	20
No. of mice without tumors	5	15	3	4
Incidence of mice with colon tumors (%) <sup>1</sup>	79.2	37.5	87.5	83.3
No. of tumors <sup>2</sup>				
0	5	15	3	4
1-3	11	8	8	7
4-6	4	1	5	7
7-9	1	0	5	3
10-12	1	0	2	2
13-16	0	0	1	1

<sup>1</sup> $\chi^2$  test, **Experiment one:**  $\chi^2 = 7.563$ ,  $P < 0.01$ ; **Experiment two:**  $\chi^2 = 1.003$ ,  $P > 0.05$ . <sup>2</sup>Wilcoxon rank test, **Experiment one:**  $\mu = 5.078$ ,  $P < 0.01$ ; **Experiment two:**  $\mu = 1.611$ ,  $P > 0.05$ .

a control (Table 1).

Mice were killed by neck dislocation at the end of the experiments. The whole large intestine was removed and then fixed in 10% buffered formalin. For each colonic tumor the maximum longitudinal and transverse dimensions ( $d_1$  and  $d_2$ ) were obtained. Tumor average diameter ( $D$ ) and volume ( $V$ ) were estimated using the following formula:  $D = (d_1 + d_2)/2$ ,  $V = (\pi/6) (d_1 + d_2)^3$ . After 48 h fixation, the entire colon specimens were coiled up into "swiss rolls" and embedded in paraffin. The blocks were sectioned at 6  $\mu$ m and stained with hematoxylin and eosin (HE) for evaluation of histological changes.

### Statistical analysis

Statistical analysis was made using the  $\chi^2$  test and Wilcoxon rank test. The difference was regarded as significant if the  $P$  value was less than 0.05.

## RESULTS

The tumors induced in mice were all situated in the distal part of the large bowel, predominantly in the small polypoid adenocarcinoma. There were no significant differences in the location and the gross appearance of tumors in the MNU-induced group and control group in both experiments. In the first experiment, there were fewer adenocarcinomas and more adenomas in the sulindac-treated group

**Table 4** Effect of sulindac on the volume of the colorectal tumor induced by N-methyl-N-nitrosourea

	Experiment one		Experiment two	
	Sulindac group	Control group	Control group	Sulindac group
Median tumor diameter (mm) <sup>1</sup>				
< 1	5	5	8	6
1.1-1.5	4	1	4	5
1.6-2.0	4	2	5	4
2.1-2.5	3	1	2	3
2.6-2.9	2	0	1	1
> 3.0	1	0	1	1
Median tumor volume (mm <sup>3</sup> ) <sup>2</sup>				
1-3	5	4	7	7
4-6	4	3	3	3
7-9	3	1	4	4
10-13	2	1	2	3
14-16	1	0	2	1
17-20	2	0	1	0
21-24	1	0	1	1
> 25	1	0	1	1

<sup>1</sup>Wilcoxon rank test, **Experiment one:**  $\mu = 8.155$ ,  $P < 0.01$ ; **Experiment two:**  $\mu = 1.464$ ,  $P > 0.05$ .

<sup>2</sup>Wilcoxon rank test, **Experiment one:**  $\mu = 5.500$ ,  $P < 0.01$ ; **Experiment two:**  $\mu = 1.333$ ,  $P > 0.05$ .

than in the untreated group. In the second experiment, there were more adenocarcinomas and fewer adenomas in the sulindac-treated group than in the untreated group (Table 2).

In the first experiment, sulindac caused a significant reduction in both the number of mice with colonic tumors and the number of tumors per mouse. There were no significant differences in the number of mice with colonic tumors and the number of tumors per mouse between the sulindac-treated group and control group (Table 3). It was obvious that sulindac had a significant inhibitory effect on the growth of the MNU-induced tumors. However, in the second experiment the inhibitory effect of sulindac was less or disappeared after initiation of the colon carcinogenesis.

The median tumor diameter and median tumor volume were reduced in the sulindac-treated group in the first experiment. In the second experiment there were no differences in the median tumor diameter and volume between the sulindac-treated group and control group (Table 4). This finding reflects a rather more obvious reduction in tumor growth in the initiation stage, while in the second experiment the inhibitory effect on tumor growth is not significant in this promotion stage of colon carcinogenesis.

## DISCUSSION

The main purpose of this investigation is to study the potential chemopreventive properties of sulindac, a NSAID, in MNU-induced colon carcinogenesis. Several other NSAIDs have been studied for their chemopreventive efficacy in colon carcinogenesis. The studies by Spagnesi and Giardiello demonstrated that administration of sulindac causes regression of colon polyps in patients with FAP<sup>[1,2]</sup>. In the present study, we suggested that sulindac has an inhibitory effect on the development of MNU-induced colonic tumors in mice. Both the number of mice with tumors and the number of macroscopic tumors were reduced when MNU and sulindac were used together. It is more obvious that sulindac has a protective effect against the chemical induction of colonic tumors by MNU in mice. The chemopreventive effect was more significant in the initiation stage of the tumor, while in the promotion or progression stage this effect was less or disappeared. Sulindac could not cause the regression of established tumors induced by MNU.

The exact biochemical action of the sulindac in these experiments is not certain, but it is possible that it acts via inhibition of prostaglandin synthesis. Such an action may be used to explain the beneficial response obtained when indomethacin is administered to mice with transplantable NC carcinoma cell lines<sup>[4]</sup>. Tumor cells are thought to escape host immune surveillance through the production of prostaglandins in colonic tumors, which contain more

prostaglandins than the adjacent mucosa<sup>[5]</sup> and restore normal immunological mechanisms in the host. In addition, several studies also demonstrated that sulindac not only inhibits the prostanoid synthesis by acting on the cyclooxygenase (COX) activity but also modulates the activities of phospholipase C, lipoxygenase and arachidonic acid uptake, which are known to play a role in inflammation and cell proliferation<sup>[6,7]</sup>. Further experiments are now being conducted to investigate this phenomenon in terms of both the biochemical mechanisms involved and the changes in proliferative parameters in colonic tissues exposed to sulindac.

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