SUPPRESSING EFFECT OF CROTON OIL ON INTESTINAL CARCINOGENESIS INDUCED BY METHYLZOXYMETHANOL ACETATE IN RATS

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Abstract.....The effect of croton oil on intestinal carcinogenesis by methylazoxymethanol acetate (MAM) was examined in ACI/N rats. Twenty seven male and 28 female ACI/N rats were given a single intragastric intubation of MAM at a dose of 25 mg/kg body weight, followed by croton oil at 0.25 ml/kg body weight, 3 times a week, by gastric intubation until the termination of this experiment (365 days). The animals had diarrhea with administration of the croton oil, but the diarrhea had no effect on their gain in weight.

Rats from all groups surviving more than 216 days were counted as effective animals. Seventeen out of 54 effective rats which were treated with MAM and croton oil developed intestinal tumors and the incidence of the intestinal tumors was significantly less than that of the group treated with MAM alone (30 out of 50 rats, $P < 0.01$). The average number of tumors per rat in the experimental group which was treated with MAM and croton oil (0.6 ± 1.1) was also smaller than that in the group which was treated with MAM alone (1.0 ± 1.8), although the difference was not significant.

These results suggest that croton oil may suppress some tumor growth at the proper dose in intestinal carcinogenesis which is initiated by MAM.

Key words : Methylazoxymethanol acetate, croton oil, carcinogenesis, rat intestine,

INTRODUCTION

In 1941, croton oil was found to possess cocarcinogenic activity (Berenblum 1941). The active cocarcinogenic principle of croton oil has been isolated and chemically identified as phorbol ester (Hecker 1967). 12-O-Tetradecanoylphorbol-13-acetate (TPA) which is one of these phorbol esters, is a potent promoter in mouse skin carcinogenesis (Boutwell 1974). It also stimulates the proliferation of epidermal cells *in vitro*. The mechanisms of promotion by TPA have been extensively studied utilizing cultured cells (Castagna and Rochette-Egly 1979, Cohen et al. 1977, Kasukabe et al. 1979, Kayano et al. 1982, Mondal et al. 1976, Yamasaki et al. 1977). Therefore, it is important to examine how far the findings obtained from these investigations can be extended to an *in vivo* system or carcinogenesis with epithelial cells other than epidermal cells.

Increased ornithine decarboxylase (ODC) activity correlates with TPA tumor promotion in several animal models (O'Brien et al. 1975). Induction of ODC activity and DNA synthesis after a single intrarectal administration of TPA in 2% DMSO solution was observed in the colon (Rozhin et al. 1984, Takano et al. 1984). The results of this investigation suggest that croton oil promotes intestinal carcinogenesis as well as skin carcinogenesis.

We have tried to examine the effect of croton oil on methylazoxymethanol acetate(MAM)-initiated intestinal carcinogenesis in rats.

MATERIALS AND METHODS

An inbred strain of conventional ACI/N rats which were 5 weeks old and which were maintained in our laboratory, was used in this experiment. For the experimental group, 27 males and 28 females were given a single stomach intubation with MAM in saline solution at a dose of 25 mg/kg body weight (Group 1). Beginning one week after the MAM intubation, the animals were given croton oil intragastrically 3 times a week at 0.25 ml/kg body weight, diluted in triple that amount of olive oil, and the treatment continued until the termination of this experiment (365 days). A standard diet (Diet CE-2, CLEA Japan Co. Ltd., Tokyo) and water were given *ad libitum*. MAM was purchased from Ash Stevens Inc., Detroit, Mich. Croton oil was purchased from Nakarai Chemicals Ltd., Kyoto, and olive oil from Nikko Seiyaku Co. Ltd., Gifu, Japan. Group 2, consisting of 25 males and 25 females, was given MAM and olive oil according to the same schedule as Group 1. Group 3, 15 males and 14 females, was given saline intubation instead of MAM at the same volume as Group 1 and croton oil. The control group, consisting of 10 males and 9 females, was intubated with saline and given olive oil. At weekly intervals the weight of the rats in each group was recorded.

Moribund animals were killed with chloroform and necropsy was undertaken immediately. All surviving rats were sacrificed on the 365th day after the initiation of

the experiment and complete necropsies were carried out. All areas of nodularity in the gastrointestinal tract were measured and the organs were fixed in 10% formalin. All sections taken from paraffin blocks were stained with hematoxylin and eosin.

Rats from all groups surviving more than 216 days were counted as effective animals.

RESULTS

At every stage the growth of the rats treated with MAM + croton oil (Group 1), MAM (Group 2), or croton oil (Group 3) was basically similar to that of the control rats. In Groups 1 and 3, the rats had diarrhea due to the irritating effect of the croton oil, but this had no effect on their body weight gain (Figure 1). However, 18 rats in Group 1 which were given MAM and croton oil and 8 rats in Group 3 which were treated with croton oil alone died or were sacrificed when moribund before the termination of the experiment. The incidence of intestinal tumors in such group is shown in Table 1. The earliest tumor was observed on the 216th day in an animal treated with MAM and croton oil. Tumor-induced intussusception was the cause of death. The total number of rats bearing intestinal tumors was 17 out of 54 effective rats in Group 1 and 30 out of 50 in Group 2 ($P < 0.01$, χ^2 test). At the termination of the experiment 11 out of 37 surviving rats had intestinal tumors in Group 1. Twenty nine of 49 surviving rats in Group 2 also had intestinal tumors ($P < 0.01$, χ^2 test).

Group 1 also showed a lower multiplicity of intestinal tumors compared to that of Group 2, but not significantly lower. Also, the average number of tumors in intestine was 0.6 ± 1.1 in Group 1 and 1.0 ± 1.8 in Group 2.

In the groups which had not been intubated with MAM, no tumors were found (Group 3 and the control group).

Histologically, the intestinal tumors were classified as (1) adenomas, (2) tubular adenocarcinomas including well and moderately differentiated adenocarcinomas, and (3) mucinous carcinomas and signet-ring cell carcinomas, according to Pozharisski (1973).

The data regarding sites and histological types of tumors are shown in Table 2. A smaller incidence of colonic adenomas was observed in the group given MAM + croton oil than in the group given MAM alone ($P < 0.01$, Student's *t*-test).

No epithelial injuries other than tumors were detected histologically in the mucosa of surviving rats treated with MAM + croton oil or croton oil alone at the termination of the experiment.

Fig. 1 : Mean body weight of rats in each group plotted every two weeks for 30 weeks and at the 50th week.

Table 1. Time at which rats in each group died or were sacrificed and the corresponding incidence of tumors.

Time after initiation of Exp. (months)	Group 1 (MAM + croton oil)					Group 2 (MAM)				Group 3	Control
	No. of deaths	No. of rats with tumor of:				No. of deaths	No. of rats with tumor of :			No. of deaths	No. of deaths
		small intestine	cecum	colon	others		small intestine	cecum	colon		
— 6	1(0) ^a									5(0)	
6— 7											
7— 8	4(2)			2(2)							
8— 9	3(0)									3(0)	
9—10	5(4)	1(1)	1(1)	2(2)		1(1)	1(1)				
10—11	5(2)			1(1)	1(1) ^b						
11—12	37(24)	6(10)	5(8)	5(5)	1(1) ^c	49(49)	12(17)	4(4)	20(28)	21(0)	19(0)
Total	55(32)	7(11)	6(9)	10(10)	2(2)	50(50)	13(18)	4(4)	20(28)	29(0)	19(0)

a: number of tumors in parenthesis

b: nephroblastoma in the kidney

c: adenoma in the liver

Table 2. Sites and histological types of tumors in the intestine of rats treated with MAM + croton oil or with MAM

Tumor sites :	Number of tumors					
	Group 1			Group 2		
Histological types	Male	Female	Total	Male	Female	Total
Duodenum :						
Mucinous carcinoma	1	1	2			
Jejunum-ileum :						
Adenoma	4	1	5	7	3	10
Tubular adenocarcinoma		1	1	3	1	4
Mucinous carcinoma	3		3	2	2	4
Cecum :						
Adenoma	1	1	2	1		1
Tubular adenocarcinoma		3	3	2		2
Mucinous carcinoma	2	2	4		1	1
Colon :						
Adenoma	3*	2	5**	18*	4	22**
Tubular adenocarcinoma	3		3	1		1
Mucinous carcinoma	2		2	5		5

* ; significant difference between mean tumor numbers (0.1 ± 0.3 in Group 1, 0.7 ± 0.9 in Group 2, $P < 0.001$, Student's t-test)

** ; significant difference between mean tumor numbers (0.1 ± 0.3 in Group 1, 0.4 ± 0.7 in Group 2, $P < 0.01$, Student's t-test)

DISCUSSION

In this experiment, the incidence and number of intestinal tumors induced by MAM was reduced by administration of croton oil. In our preliminary experiment, suppression of body weight gain of animals and earlier deaths after severe diarrhea were observed by administration of croton oil at 0.5 ml/kg body weight ; a dose which was twice that of the dose in this experiment. In the present study, croton oil also induced diarrhea in the rats. This result means that this dose of croton oil was enough to irritate the intestinal mucosa. But no histological changes other than tumors were detected in the intestinal mucosa of surviving animals treated with MAM+croton oil or croton oil alone at the termination of the experiment. The suppression of body weight gain caused by diarrhea seems to be rather insignificant at the present dose of croton oil as shown in Fig. 1. It has been suggested that diarrhea may indirectly affect intestinal carcinogenesis because extreme alterations in the fecal stream seem to be a contributing factor in colon carcinogenesis (Tomita et al. 1985), but the relationship between diarrhea and intestinal carcinogenesis is unclear and should be investigated in the near future.

Few papers have reported about the local toxic effect of croton oil or TPA on tumor cells. In contrast to the hypotheses given in these papers, regression occurred in about 50% of the tumors formed in response to croton oil applied to the skin as a promoter (Frei and Ritchie 1962 ; Saffiotti and Shubik 1963). Croton oil causes regression in transplants of murine fibrosarcoma at the injection site in mice and guinea pigs, as demonstrated by Yarkoni and Rapp (1979). They speculated that the destruction of tumor tissue within 24 hours after the intralesional injection of the agent is probably a result of indiscriminate killing of the injected tissue. Other investigators have reported a nonspecific destruction of tumor in mice and man after intralesional injection with various irritating agents (Felix et al. 1976 ; Cohen et al. 1976). TPA and croton oil may have regressive activity on initiated cells as well as promoting activity in mouse skin.

It has been speculated from stronger suppression of adenomas than carcinomas that there is a difference in the sensitivity to the toxicity of the croton oil between adenomas and adenocarcinomas in the intestine and that adenomas may have more sensitivity to croton oil than adenocarcinomas. It may be coincident with the results that premalignant epithelial cells secrete more protease in response to TPA than do malignant cells *in vitro* (Friedman, 1981).

Croton oil at the present dose had a suppressing effect rather than a promoting effect in the MAM-induced intestinal carcinogenesis. This suppressing effect may be due to the toxicity of croton oil if we hypothesize that the toxicity effects the carcinogenic mechanism antagonistically.

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