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Animal Models for the Study of Dietary Factors and Cancer of the Large Bowel[1]

Bandaru S. Reddy, T. Narisawa, R. Maronpot, John H. Weisburger,[2] and E. L. Wynder

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595

Summary

Studies in metabolic epidemiology have shown a strong association between dietary fat intake, level of fecal anaerobic bacteria, fecal acid, and neutral sterols and the risk of colon cancer among different populations. Current concepts visualize that colonic bile acids and cholesterol metabolites play a modifying role in large bowel carcinogenesis, that these compounds are derived from dietary factors (directly or indirectly), and that they subsequently are modified by the intestinal bacteria. In the animal model, 2 bile acids (lithocholic and taurodeoxycholic) acted as colon tumor promoters.

Rats fed a high-fat diet were more susceptible to colon tumor induction by 1,2-dimethylhydrazine compared to animals fed a normal-fat diet. The intestinal microflora also played a modifying role in enhancing colon tumor production by 1,2-dimethylhydrazine.

Introduction

Epidemiological studies suggest that diet, particularly as it relates to fat and animal protein, may be among the most important causal factors associated with colon cancer in man. These dietary effects may be mediated through changes in the composition of endogenous compounds secreted into the gut as well as the composition of intestinal bacteria, which could convert endogenous secretions into tumorigenic compounds. Dietary fat, intestinal bacteria, and acid and neutral sterols as possible causes of colon cancer in man have been detailed elsewhere in this symposium (17).

No apparent relation has been observed between diet-mediated agents responsible for colon cancer in man and the carcinogens affecting the large intestine in animals. There is, however, some merit in assuming a link because experimental studies in animal models may explain the complex sequence of events leading to colon cancer in man. To determine whether data in any of the animal systems can mimic the human system as it relates to the etiology of the colon cancer, it is of major importance to consider the composition of bile metabolites, the bacterial flora, the constituents of feces, and the ability of the animals to adjust to different types of diet. Ideally, the type of colorectal neoplasm induced in animals should also bear close similarity to those in man. Within the past few years, animal models have been developed to induce colon cancer by the administration of cycasin, DMH,[3] AOM, methylazoxymethanol acetate, MNU, and MNNG (2, 3, 5, 15, 18). These animal models have been used to study the relationship of dietary fat to colon cancer.

Experimental Leads

Standardization of Bioassay System Involving i.r. Administration of Direct-Acting Carcinogens

A number of alkylnitrosamides administered i.r. at several dose levels were studied to delineate a dose-response curve (Tables 1 to 5). This is important for future studies on cocarcinogenicity of relevant environmental agents and endogenous chemicals to determine whether they might augment or accelerate the carcinogenic process thus standardized (4, 6).

Colon Cancer Induction in Mice, Rats, and Guinea Pigs by i.r. MNU. *Mice.* In female ICR/Ha mice, 30 individual doses of 0.3 mg of 0.2% solution of MNU, each given 3 times/week when the mice were 7 to 8 weeks old, induced large bowel neoplasms with high incidence after the 17th week, compared with 6 doses of 1.5 mg each given 3 times/week which also led to leukemia in all mice until the 18th week (6). Small adenomatous nodules of the lung appeared in all mice. Neoplasms of the large bowel were found in 64 and 47% of mice given injections of MNU at dose levels of 1.5 or 0.3 mg, respectively. In the large intestine, adenocarcinomas and adenomas were found in the distal colon and rectum, and squamous cell carcinomas were found at the anal canal.

Rats. Nine- and 18-week-old CD Fischer rats were given i.r. injections of MNU at a dose level of 1.0 and 2.5 mg, 3

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[2] Presenter.

[3] The abbreviations used are: DMH, 1,2-dimethylhydrazine; AOM, azoxymethane; MNU, methylnitrosourea; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; i.r., intrarectal; i.g., intragastrically.

Table 1

Histological classification of large bowel neoplasms in ICR/Ha Swiss mice by i.r. instillation of MNU

Group[a]	MNU dosage (mg)	No. of doses	Total no. of mice with large bowel neoplasms	No. of colorectal neoplasms		Ratio of adenocarcinomas to adenomas	No. of anal neoplasms	
				Adenocarcinoma	Adenoma		Squamous cell carcinoma	Papilloma
I	1.5	15	10	6	3	2.0	5	0
II	1	6	14	8	10	0.8	5	0
III	0.3	30	19	13	9	1.4	15	1
IV	1.8	1	1	0	0	0	1	0
V	0.3	6	12	3	7	0.4	3	1
VI	0.06	30	10	8	2	4.0	2	0

[a] There were 30 mice in each group.

Table 2

Large bowel tumors with i.r. MNU in female CD Fischer rats

MNU treatment (mg)	Starting age (wk)	Large bowel tumor	Thymoma	Time (wk)
2.5[a]	9	22/22	9/22	14–21
	18	5/5	0	20–25
1.0[a]	9	21/24	0	25
	18	5/5	0	30
2.5[b]	9	11/16	0	20

[a] Each solution was given 3 times/week for 10 weeks.
[b] Three times/week for 2 weeks.

Table 3

Large bowel tumors with i.r. MNU or MNNG in female strain 2 guinea pigs at Week 70

Treatment[a]	Dose (mg)	Large bowel tumor	Histological classification		
			Adeno-carcinoma	Squamous carcinoma	Sar-coma
MNU	2.5	5/5	6	1	
	1.25	10/10	9	2	1
MNNG	1.25	2/4	3		
	0.625	8/15 (5)[b]	13	1	1
Vehicle control		0/15 (12)			

[a] Animals were given 0.5 ml of each solution 2 times/week. The treatment was stopped at Week 42 in MNU groups and at Week 53 in MNNG and vehicle control groups.
[b] The number of surviving animals at Week 70 is in parentheses.

times/week for 10 weeks, and developed colon tumors between 14 and 30 weeks.[4] MNU induced colon tumors faster than MNNG did as used previously in our studies.

Instillation i.r. of carcinogenic chemicals such as MNU into rodents is a reliable method for obtaining animal models of colon cancer for studies on the mechanisms involved.

Guinea Pigs. Administration of 1.25 mg MNU i.r. 2 times/week for 42 weeks to female inbred strain 2 guinea pigs induced large-bowel adenocarcinomas in all animals in 38 to 56 weeks (7). The lesions were infiltrative or constrictive, which distinguished them from chemically induced large bowel cancers of rats and mice. Usually, rats and mice develop polypoid or plaque-shaped carcinomas and adenomatous polyps. A transplantable colon cancer in strain 2 guinea pigs is being maintained.

MNNG Administered i.r. to Rats on 2 Different Diets. Administration of MNNG i.r. exhibited a steep dose-response curve at dose levels of 0.31 to 1.25 mg/week for 50 weeks. A high yield (88%) of colon tumors was present with dosage of 1.25 mg, a much lower yield (17%) was present with a 0.6-mg dosage, and no tumors were present with a 0.31-mg dosage.[4] At the higher dosage level, the multiplicity of colon tumors was higher when animals were fed a crude diet of Purina laboratory chow, compared to a semipurified diet.

Vitamin A Deficiency and Colon Tumor Incidence. Groups of weanling female CD Fischer rats were fed vitamin A-free diet, vitamin A-supplemented diet, or Purina laboratory chow; after 5 weeks they were given i.r. injections of MNNG at dose levels of 1.25, 0.63, or 0.31 mg, 3 times/week for 30 weeks.[4] The large bowel tumor incidence in vitamin A-deficient animals was one-half that of vitamin A-supplemented animals at 1.25- and 0.62-mg dose levels.

Rogers *et al.* (14) found little evidence that vitamin A affects DMH-induced colon tumors in rats. However, in 1 experiment in which the largest dose of DMH was given, rats fed high levels of vitamin A developed fewer tumors than did rats fed normal diets. In this study, DMH was given i.g. (14). In another study, Newberne and Rogers (8) found that vitamin A-deficient rats were more susceptible to colon tumor induction by aflatoxin B_1 than were normal rats.

Study of Widely Used Food Additives, Asbestos, and Bile Acids as Cocarcinogens in Colon Cancer. We are studying the cocarcinogenicity of carrageenans, tannins, asbestos, and bile acids in the colon. These compounds are being given i.r. to rats or mice along with MNNG or MNU.

Promoting Effect of Bile Acids in Colon Carcinogenesis

i.r. Administration. We reported the effect of 2 bile acids, taurodeoxycholic and lithocholic, in colon carcinogenesis (4) (Table 6). MNNG was administered i.r. as a single

[4] T. Narisawa, unpublished data.

Table 4

Dose-response experiment of MNNG on colon carcinogenesis by i.r. instillation in female Buffalo rats fed different kinds of diets

Treatment group[a]	MNNG dosage (mg)	Incidence of large bowel neoplasms (40–63 wk)	No. of neoplasms	
			Adenocarcinoma	Adenoma
Purina laboratory chow	1.25	15/17 (88%)	32	6
	0.625	3/18 (17%)	5	0
	0.313	0/12	0	0
Semipurified	1.25	9/14 (65%)	11	1
	0.625	3/16 (19%)	3	0
	0.313	0/15	0	0

[a] There were 24 rats in each group at the start. Animals received 0.5 ml of each solution 2 times/week for 50 weeks. A number of animals in each group died due to an outbreak of acute pneumonia at 15 experimental weeks.

Table 5

Incidence of colorectal neoplasms induced by i.r.-instilled MNNG in CD Fischer rats fed deficient and adequate vitamin A diets

Diet and MNNG dose	Effective no. of rats[a]	No. of rats with neoplasms	No. of neoplasms by histological classification		
			Adenocarcinoma	Adenoma	Other
Vitamin A deficient					
MNNG-H[b]	20	9 (45)[c]	5	12	Hemangioma
MNNG-M	24	3 (13)	1	2	Fibroma
MNNG-L	13	2 (15)	0	2	
Vitamin A supplement					
MNNG-H	24	23 (96)	17	49	Leiomyosarcoma
MNNG-M	22	6 (27)	0	10	
MNNG-L	22	3 (14)	0	3	
Purina laboratory chow					
MNNG-H	23	22 (96)	44	45	
MNNG-M	23	17 (74)	9	19	
MNNG-L	21	3 (14)	0	3	

[a] Number of rats that died or were killed between 40 and 45 weeks after beginning of experiment.
[b] MNNG-H, 1.25 mg MNNG; MNNG-M, 0.63 mg MNNG; MNNG-L, 0.31 mg MNNG.
[c] Number in parentheses, percentage.

Table 6

Colorectal tumor incidence in rats by bile acids after initiating with i.r. MNNG

Treatment[a]	% of rats with tumors	Total	Tumor classification	
			Adeno-carcinoma	Adenoma
LC	0	0	0	0
TDC	0	0	0	0
MNNG	25	10	5	5
MNNG + LC	52	30	8	22
MNNG + TDC	62	28	4	24

[a] MNNG group were given single dose of 4 mg i.r. MNNG; LC or TDC group received 1 mg bile acid i.r. 5 times/week for 13 months; MNNG + LC or MNNG + TDC group were given i.r. MNNG and bile acid as above. LC, lithocholic acid; TDC, taurodeoxycholic acid.

initiating dose followed by taurodeoxycholic acid or lithocholic acid applied repeatedly to the mucosa of the same segment of the colon for about 47 weeks. Taurodeoxycholic acid given i.r. is deconjugated into deoxycholic acid by bacterial enzymic hydrolysis. The development of adenomas significantly increased among those rats initiated with MNNG and receiving the bile acids as promoters compared to the group that was given only the carcinogen. The bile acids themselves did not produce any tumors. Hence, in this animal model, these 2 bile acids, which are present in high concentration in the colonic contents of man, do act as tumor promoters.

Experiments are in progress in our laboratory to test whether many other bacterially modified bile acids have colon tumor-promoting or accelerating activity. Germ-free rats are being used in this study because, under those conditions, the bile acids will be presented to the colon without further microbial modification. We thus found deoxycholic acid to be a promoter.

Nigro *et al.* (9) observed that feeding of cholestyramine, a nonabsorbable resin increasing bile salt excretion, enhanced AOM-induced colonic tumors in rats. Although this effect was mediated through an increased fecal bile salt excretion, one could not exclude the possibility of some direct effect of cholestyramine itself (1). In another study, Chomchai *et al.*

(1) observed that the carcinogenic effect of AOM in rats was enhanced by the increase of bile salts in the colon induced by surgical diversion of bile to the middle of the small intestine. Fecal excretion of bile salts was increased in the animals with bile duct implantation compared with normal rats. In an experiment pertinent to this area, we incubated cholestyramine-bound taurocholic acid or taurochenodeoxycholic acid with mixed cultures of anaerobic bacteria isolated from human stools. We found that 65 to 75% of cholestyramine-bound bile acids were deconjugated and further modified by intestinal microflora.

These results evidently add support to the concept that some bile acids can act as promoters or accelerators in colon carcinogenesis.

Mouse Skin Bioassay System: Bile Acids and Neutral Sterols as Cocarcinogens. In view of our findings that certain bile acids may act as cocarcinogens, it is important to ask whether they would accelerate carcinogenesis in the presence of a classic carcinogen. Mixed solutions of cholesterol and its metabolites or of bile acids and benzo(*a*)-pyrene are being applied to mouse skin. The question is whether these bile metabolites will result in a higher yield of skin tumors at an earlier time than will benzo(*a*)pyrene alone. These experiments are still in progress, but indications are that no enhancement is obtained. The mechanism requires clarification, but it may involve a diluting effect by the chemicals studied of the benzo(*a*)pyrene applied.

Modifying Effect of Intestinal Bacteria in Colon Carcinogenesis

The effect of diet in colon carcinogenesis may also be mediated both by altering the supply of bile metabolites and by changing the numbers of type of intestinal microflora available to act on bile metabolites. Thus, we have extended our studies to elucidate the effect of intestinal microflora on the sensitivity of colon to the carcinogenic effect of DMH using germ-free rats as a tool (11, 12). The DMH injected s.c. (20 mg/kg of body weight per week for 20 weeks) induced colonic tumors in 20% of germ-free rats and in 93% of conventional rats (Table 7). This suggests that the intestinal microflora directly or indirectly played a modifying role in promoting or accelerating colon tumor production by DMH.

We have extended our studies to elucidate the effect of the intestinal microflora on the carcinogenicity of AOM on the colon (11). Germ-free, gnotobiotic rats contaminated with *Clostridium perfringens*, and conventional rats were given 20 weekly i.r. instillations of AOM at a dose level of 10 mg/kg of body weight per week and autopsied 15 weeks later. AOM appreciably increased the multiplicity of colonic tumors in germ-free and gnotobiotic rats as compared to conventional controls (Table 8). Similarly, i.r. instillation of MNNG increased the multiplicity of colonic tumors in germ-free rats compared to conventional animals (12).

Table 7

Tumor incidence in germ-free and conventional rats with 1,2-DMH

Status	Body wt (g)	Animals with tumors: Ear canal No.	Ear canal %	Kidney No.	Kidney %	Small intestine No.	Small intestine %	Colon No.	Colon %	Classification and multiplicity of colon tumors (per rat): Total tumors	Adenocarcinoma	Adenoma
Germ-free (24)[a]	198	0	0	0	0	0	0	5	21	0.2 + 0.1[b]	0.1 ± 0.1	0.1 ± 0.1
Conventional (15)	179	13	87	3	20	12	80	14	93	2.1 ± 0.4	1.2 ± 0.2	0.9 ± 0.3

[a] Numbers in parentheses, number of rats.
[b] Mean ± S.E.

Table 8

Tumor incidence in germ-free, monocontaminated, and conventional rats treated with AOM

Status	Body wt (g)	Animals with tumors: Ear canal No.	Ear canal %	Kidney No.	Kidney %	Small intestine No.	Small intestine %	Colon No.	Colon %	Classification and multiplicity of colon tumors (per rat): Total tumors	Adenocarcinoma	Adenoma
Germ-free (15)[a]	188	0	0	2	13	8	53	14	93	4.3 ± 0.3[b]	1.8 ± 0.3	2.5 ± 0.4
Monocontaminated (7)[c]	187	3	43	3	43	3	43	7	100	6.6 ± 1.1	3.3 ± 0.7	3.3 ± 0.9
Conventional (25)	191	12	48	12	48	12	48	15	60	2.4 ± 0.4	1.2 ± 0.2	1.2 ± 0.3

[a] Number in parentheses, number of rats.
[b] Mean ± S.E.
[c] Seven of 14 monocontaminated rats died 10 weeks before the last injection of carcinogen due to possible toxemia.

Table 9

Tumor incidence in rats treated with DMH and fed 2 levels of dietary corn oil or lard[a]

Diets	Animals with tumors (%)				Animals with multiple colonic tumor(%)	Total colon tumors (per rat)
	Ear canal	Kidney	Small intestine	Colon		
Corn oil, 5%	32	4	27	36	14	0.77
Corn oil, 20%	59	14	36	64	32	1.55
Lard, 5%	13	0	4	17	4	0.22
Lard, 20%	67	0	50	67	29	1.50
Purina laboratory chow	15	0	20	25	0	0.25

[a] Number of animals per group ranged from 20 to 24. Animals received weekly s.c. injections of 10 mg/kg body weight for 20 weeks and were autopsied 10 weeks after the last injection.

Modifying Effect of Dietary Fat in Colon Carcinogenesis

Manipulation of diet can change the composition of bile. It might be expected that the quality and quantity of dietary fat would change the composition of the intestinal bacteria, bile acids, and cholesterol metabolites, which in turn would be reflected in colon tumor promotion. We have studied further the effect of quality and quantity of dietary fat on colon tumor-promoting activity in animal models (10).

Inasmuch as men in various population groups usually eat comparable regimens over generations, we have designed our experiments in a manner so that animals are exposed to a given regimen for 2 generations prior to treatment with a carcinogen. This is important, especially since the intestinal microflora are thus conditioned by the diet of the host. Virgin female rats fed diets containing 20% lard, 5% lard, 20% corn oil, and 5% corn oil and Purina laboratory chow were bred, and the litters were weaned to the same diets consumed by the mothers. At 50 days of age, all 2nd-generation animals, except controls, received 20 weekly s.c. injections of DMH (10 mg/kg of body weight per week) and were autopsied 10 weeks after the last injection. Animals fed 20% lard or 20% corn oil were more susceptible to colon tumor induction by DMH compared to other groups (Table 9). The quality of fat (corn oil *versus* lard) had no major influence on the incidence of colon tumors. Fecal excretion of acid and neutral sterols were higher in animals fed diets containing 20% fat compared to those fed a 5% fat diet (13). These results add support to the concept that bile salts in the colon have some role in colon carcinogenesis. We are currently studying the tumor-enhancing potential of many bile acids and other sterol metabolites to elucidate the relative importance of these compounds in colon carcinogenesis.

Rogers *et al.* (15) found that a diet, marginally deficient in lipotropes but high in fat, enhanced DMH-induced colon carcinogenesis in rats.

Conclusions

Although a specific carcinogen for the colon has not yet been identified in the feces, an association has been established linking colon cancer to dietary fat and fecal acid and neutral sterols. In the animal model, 2 bile acids (lithocholic and deoxycholic) acted as colon tumor promoters. High dietary fat intake and microflora (directly or indirectly) have been shown to enhance DMH-induced colonic tumors in rats. Although we still need to understand the specifics of the mechanisms whereby dietary fat influences colon cancer, it is clear that control of passage, production and metabolism of carcinogens, cocarcinogens, or promoters, all of which may have a function in the pathogenesis of colon cancer, are involved. Metabolic epidemiological and laboratory investigations must be pursued to dissect the complex factors responsible for large bowel cancer in man. Such efforts are important, since we have not been able to reduce significantly the death rate from colon cancer, although the reported rate of deaths from rectal cancer has declined. It is hoped that the data thus generated in animal models and in man can significantly enhance our knowledge of the etiological factors that play a role in cancer of the large bowel and that they can provide the basis for rational prevention.

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