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EFFECT OF METAMUCIL ON TUMOUR FORMATION BY 1,2-DIMETHYLHYDRAZINE DIHYDROCHLORIDE IN MICE

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Abstract—The effect of the plant cellulose metamucil on the tumorigenicity of 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) was studied in random-bred Swiss mice. Three groups of mice, which were 5, 6 and 6 weeks old at the beginning of the experiment, were given the following treatments: (1) metamucil (20%, w/w) in powdered diet for their lifespan; (2) 1,2-DMH, ten weekly subcutaneous injections at 20 mg/kg body weight; (3) combination of treatments given to groups 1 and 2. The administration of metamucil enhanced the appearance of colon tumours induced by 1,2-DMH in males only. Metamucil had no statistically significant effect on the development of tumours elicited by 1,2-DMH at seven additional sites. It was expected that a high amount of dietary fibre would inhibit carcinogenesis in the large intestine. Instead, metamucil increased the incidence of colon tumours induced by 1,2-DMH, although only in males.

INTRODUCTION

Cancer of the bowel is the second most common tumour in the human population of the Western hemisphere (Doll, Muir & Waterhouse, 1970). The high incidences of large-bowel tumours and also of lung cancer are probably recent developments, since their incidences are substantially lower in developing countries (Burkitt, 1971; Higginson, 1966; Wynder & Shigematsu, 1967).

As reported earlier, one of the most notable differences between the Western diet and that of less developed countries is the lower consumption of dietary fibre in the former (Cleave, Campbell & Painter, 1969). Therefore, it was suggested that since the high fibre content accelerates intestinal transit time, the contents of stool, including perhaps carcinogens, would remain in contact with intestinal epithelium for a substantially shorter time (Walker, 1961). In addition, the excess amount of carbohydrate prevalent in the Western diet may alter the bacterial content of the faeces and increase the extent of bacterial decomposition (Hoffman, 1964). Bacterial metabolism could be another reason for suspecting the role of diet in cancer causation (Aries, Crowther, Drasar *et al.* 1969; Hill, Crowther, Drasar *et al.* 1971).

To substantiate the first part of the above hypothesis, the effect of a diet containing a high level of metamucil, a plant cellulose used for the treatment of constipation, on the tumorigenicity of 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) in mice was investigated.

EXPERIMENTAL

Animals. Swiss albino mice from the colony randomly bred since 1951 at the Eppley Institute for Research in Cancer and Allied Diseases were housed in plastic cages with granular cellulose bedding, separated according to sex in groups of five, and given Wayne Lab Blox powdered diet (Allied Mills, Inc., Chicago, IL) and tap-water *ad lib.* with the exception described below.

Chemicals and diets. 1,2-DMH (mol wt 133.02; m.p. 168°C) was obtained from K and K Laboratories, Inc. (Plainview, NY) and metamucil, a psylium hydrophilic mucilloid, was purchased from Searle Laboratories, Division of G.D. Searle and Co. (Chicago, IL). 1,2-DMH was dissolved in sterile physiological saline for sc injection into mice as previously described (Toth, Malick & Shimizu, 1976). Metamucil was mixed with the powdered diet in a Norton Jar Mill. These mixtures were prepared once a week and the total consumption of diet containing metamucil was measured three times weekly during the treatment period. Preliminary toxicity studies were performed with 1,2-DMH, as described previously (Toth *et al.* 1976), and with metamucil given at 50, 40, 30, 20 and 10% levels in the powdered diet for 35 days to Swiss mice. Taking account of four parameters—survival rates, body weight, chemical consumption

Table 1. Survival rates of mice treated with metamucil, 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) or with a combination of metamucil + 1,2-DMH, for their lifespan

Group	Sex	No. of survivors at age (wk)								
		10	20	30	40	50	60	70	80	90
1	M	50	39	34	29	21	11	5	1	–
	F	50	49	43	38	33	20	12	4	–
2	M	50	48	37	29	12	5	1	–	
	F	50	48	43	35	24	13	5	1	
3	M	47	38	16	3	–				
	F	50	40	34	20	8	–			

Male and female Swiss mice (50/group) were treated with metamucil (20%, w/w, in the diet; group 1), 1,2-DMH administered sc (20 mg/kg body weight) once/wk for 10 wk (group 2) or with a combination of both treatments (group 3).

Abbreviation: 1,2-DMH = 1,2-dimethylhydrazine dihydrochloride.

Table 2. Location and incidence of tumours in mice treated with metamucil (group 1), 1,2-dimethylhydrazine dihydrochloride (1,2-DMH; group 2) or with a combination of both treatments (group 3)

Group no. ...	2						3					
	Males			Females			Males			Females		
Location and type of tumour	M	T	Age at‡ death (wk)	M	T	Age at‡ death (wk)	M	T	Age at‡ death (wk)	M	T	Age at‡ death (wk)
Lungs												
Total tumours	10	14	49 (25–68)	17	24	57 (18–75)	1	1	39	3	3	35 (31–43)
Adenomas	7	8		13	16		1	1		3	3	
Adenocarcinomas	1	2		2	2		–	–		–	–	
Adenomas + adenocarcinomas	2	2 + 2		2	3 + 3		–	–		–	–	
Blood vessels												
Total tumours	6		47 (31–66)	20		54 (30–83)	4		33 (28–41)	6		44 (36–55)
Angiomas	1			11			2			3		
Angiosarcomas	5			9			2			3		
Kidneys												
Total tumours	9	16	61 (43–68)	–	–	–	1	1	46	–	–	–
Adenomas	8	13		–	–		1	1		–	–	
Adenocarcinomas	–	–		–	–		–	–		–	–	
Adenomas + adenocarcinomas	1	2 + 1		–	–		–	–		–	–	
Duodenum												
Total tumours	–	–	–	1	1	75	–	–	–	–	–	–
Polypoid adenomas	–	–		1	1		–	–		–	–	
Caecum												
Total tumours	3	3	41 (34–46)	1	1	49	–	–	–	1	1	39

Polypoid adenomas	1	1		–	–		–	–		–	–	
Adenocarcinomas	2	2		–	–		–	–		1	1	
Adenomas + adenocarcinomas	–	–		–	–		–	–		–	–	
Carcinoma	–	–		1	1		–	–		–	–	
Colon												
Total tumours	19*	36	42 (22–68)	21	37	50 (30–83)	8*	10	34 (24–41)	5	14	41 (39–52)
Polypoid adenomas	2	2		5	5		3	3		1	6	
Adenocarcinomas	14	24		15	24		4	4		3	3	
Adenomas + adenocarcinomas	–	–		2	3 + 5		–	–		–	–	
Polypoid adenomas + adenocarcinomas	3	3 + 7		–	–		1	2 + 1		1	3 + 2	
Rectum												
Total tumours	17	22	46 (22–70)	18	29	49 (30–66)	1	1	33	–	–	–
Polypoid adenomas	1	1		2	2		–	–		–	–	
Adenocarcinomas	16	21		15	24		–	–		–	–	
Polypoid adenomas + adenocarcinomas	–	–		1	1 + 2		–	–		–	–	
Squamous-cell carcinomas	–	–		–	–		1	1		–	–	
Anus												
Total tumours	6	6	47 (28–68)	2	2	33 (20–46)	1	1	28	2	2	39 (30–49)
Carcinomas	–	–		2	2		1	1		2	2	
Squamous-cell papillomas	2	2		–	–		–	–		–	–	
Squamous-cell carcinomas	4	4		–	–		–	–		–	–	

M = Total no. of mice with total or specified tumours T = No. of total or specified tumours

†The only listed tumours found in group 1 mice were pulmonary adenomas in one male dying in wk 44 and in five females dying between wk 47 and 83 (mean 69) and an angiosarcoma in a female dying in wk 64.

‡Average, range in parentheses.

Male and female Swiss mice (50/group) were treated with metamucil (20%, w/w diet) for their lifespan (group 1), with 1,2-DMH administered sc (20 mg/kg body weight) once/wk for 10 wk (group 2) or with a combination of both treatments (group 3).

The asterisks indicate values that differ significantly ($P < 0.01$) by Peto's method (Peto, 1974). See Table 3 for details of the statistical analysis.

levels and histological changes—the 20% dose of metamucil was found to be suitable for lifelong treatment. This technique was developed in this laboratory (Toth, 1972).

Treatments

Group 1. The diet containing 20% (w/w) metamucil was given for life to 50 male and 50 female mice, which were 37 days old at the beginning of the experiment.

Group 2. 1,2-DMH was administered as ten weekly sc injections of 20 mg/kg body weight in 0.01 ml physiological saline to 50 male and 50 female mice which were 43 days old at the beginning of the experiment.

Group 3. The metamucil-containing diet was given for life to 50 male and 50 female mice. Commencement of feeding was followed 2 days later by 1,2-DMH treatment as described for group 2. The mice were 44 days old at the beginning of the experiment.

All experimental animals were carefully checked and weighed at weekly intervals, and the gross pathological changes were recorded. The animals were either allowed to die or were killed with ether when moribund. Complete necropsies were performed on all animals. All organs were examined macroscopically and fixed in 10% buffered formalin. Sections from the liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinals, duodenum, jejunum, ileum, caecum, colon, rectum and at least four lobes of the lungs of each mouse and from organs with gross pathological changes were stained with haematoxylin and eosin and were examined by light microscopy.

Statistical analyses. Tumour incidences were statistically analysed by Peto's method (Peto, 1974), a modification of the Kaplan–Meier formula. This excluded the number of mice that died early in the experiment apparently because of the combined toxicity of metamucil and 1,2-DMH.

RESULTS

The average daily intake of metamucil was 984 mg (males) and 980 mg (females) in group 1 animals and 976 mg (males) and 912 mg (females) in group 3 animals.

The survival rates of the treated animals are shown in Table 1. The data demonstrate that the metamucil, 1,2-DMH, and metamucil + 1,2-DMH treatments markedly shortened survival compared with the untreated controls (Toth, Patil, Erickson & Kupper, 1979). In the metamucil and metamucil + 1,2-DMH groups a substantial number of mice developed intussusception of the intestine, mainly of the colon.

The number of animals with tumours, their age at death (latent periods) and the most important neoplasms found in the treated animals are summarized in Table 2. In male animals treated with 1,2-DMH the observed number of animals with colon tumours was 19, while eight tumours were observed in the 1,2-DMH + metamucil-treated males. The expected number of colonic neoplasms, taking into account the differences in survival of the two groups, are 23 for the former group and four for the latter. This difference is significant at $P < 0.014$ (Peto, 1974; Table 3). The metamucil treatment however had no enhancing or inhibiting effects on tumours induced by 1,2-DMH in seven additional organs.

Table 3. Statistical analysis

Week of experiment	Observed no. of male mice with colon tumours/no. of male mice		Expected no. of male mice with colon tumours	
	Group 2	Group 3	Group 2	Group 3
22	1/46	0/31	0.60	0.40
24	0/45	1/29	0.61	0.39
26	1/41	0/25	0.63	0.37
27	0/40	1/22	0.65	0.35
28	1/40	0/18	0.70	0.30
31	1/39	1/13	1.46	0.54
32	0/35	1/11	0.76	0.24
33	1/35	1/8	1.63	0.37
34	0/34	2/7	1.66	0.34
36	3/34	0/4	2.68	0.32
41	1/27	1/3	1.80	0.20
45	2/21	0/1	1.91	0.09
46	2/20	0/1	1.91	0.09
47	1/17	0/0	1.00	
55	1/9		1.00	
57	2/8		2.00	
64	1/5		1.00	
68	1/3		1.00	
		Total		
	19	8	23.02	3.98

$$\left[\frac{(19-23.02)^2}{23.02}+\frac{(8-3.98)^2}{3.98}\right]^{1/2}=\sqrt{(4.0604+0.702)}=\sqrt{4.7624}=2.1823,\quad P<0.014 \text{ (Peto, 1974).}$$

Group 2 mice were administered 1,2-dimethylhydrazine (sc; 20 mg/kg body weight) once/wk for 10 wk. Group 3 mice were similarly treated but were maintained on a diet containing metamucil (20%, w/w).

Pulmonary tumours were macroscopically and histologically similar to those previously described from this laboratory (Toth *et al.* 1979; Toth & Shimizu, 1974). Blood-vessel tumours were grossly and histologically similar to those described by Toth & Wilson (1971). Kidney tumours were macroscopically and histologically similar to those obtained under other experimental conditions (Terracini, Palestro, Gigliardi & Montesano, 1966). The duodenal tumour was grossly and histologically similar to those found in man (Evans, 1968). Tumours of the caecum, rectum, colon and anus were macroscopically and histologically similar to those described previously (Druckrey, 1970; Druckrey, Preussman, Matzkies & Ivankovic, 1967; Toth *et al.* 1976).

In a number of cases other types of tumours were observed. Since they occurred in low incidences, their appearance could not be attributed to the experimental treatments. They were limited to malignant lymphoma in the metamucil-treated group 1 animals (in one male and four females). In group 2, two malignant lymphomas, two preputial-gland carcinomas, one preputial-gland papilloma, one ileal adenocarcinoma, one jejunal polypoid adenoma and one forestomach papilloma occurred in males and two malignant lymphomas, two clitoral-gland carcinomas, one adrenocortical adenoma, one jejunal adenocarcinoma, one skin papilloma, one uterine fibromyosarcoma and one uterine myofibroma in females. One papilloma of the throat occurred in a group 3 female.

DISCUSSION

The current findings show that the administration of 20% metamucil in powdered diet to Swiss mice enhanced the tumorigenic action in the colon of 1,2-DMH (20 mg/kg body weight) given as ten weekly subcutaneous injections.

The hypothesis that dietary fibre plays a role in the development of colo-rectal cancer has previously found both support and contradiction in epidemiological and experimental studies. A number of epidemiological studies have shown that a high-fibre diet protects against colo-rectal cancer in man (Bjelke, 1978; Dales, Friedman, Ury *et al.* 1978; Maclennan, Jensen, Mosbech & Vuori, 1978; Malhotra, 1977; Modan, Barell, Lubin *et al.* 1975), while an equal number of investigators have found no evidence for such claims (Bingham, Williams, Cole & James, 1979; Drasar & Irving, 1973; Jain, Cook, Davis *et al.* 1980; Liu, Stamler, Moss *et al.* 1979; Lyon & Sorenson, 1978). Experimental carcinogenicity studies have also shown that certain types of dietary fibres (pectin, bran and cellulose) inhibit the carcinogenic action of certain chemicals (Fleiszer, Murray, Richards & Brown, 1980; Freeman, Spiller & Kim, 1980; Watanabe, Reddy, Weisburger & Kritchevsky, 1979; Wilson, Hutcheson & Wideman, 1977). However, a number of studies in which the diet contained a high amount of bran, pectin, cellulose or alfalfa showed no evidence for a protective effect (Freeman, Spiller & Kim, 1978; Ward, Yamamoto & Weisburger, 1973; Watanabe, Reddy, Wong & Weisburger, 1978). In one study the administration of a high amount of dietary agar was found to enhance the tumorigenicity of 1,2-DMH (Glauert, Bennink & Sander, 1981).

In man, cancer of the large bowel mainly occurs in the colo-rectal area, where retention of the faeces is the longest and where bacterial fermentation is the most active. This was a reason for postulating that any carcinogen ingested or formed in the gut would be present in more concentrated stools and would be held in contact with the colon and rectal mucosa for a prolonged time. The fibre-containing diet is thought to accelerate the transit time of the faeces. Therefore, to test experimentally the validity of this hypothesis, the effect of the high cellulose-containing diet on the tumorigenicity of 1,2-DMH, a classical large bowel cancer-inducing agent, was investigated in mice. Contrary to our expectation, the findings of this investigation surprisingly gave limited evidence that a high fibre-containing diet enhances the carcinogenicity of 1,2-DMH in the colon, adding new fuel to this controversial subject.

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