Point/Counterpoint

Point: From Animal Models to Prevention of Colon Cancer. Systematic Review of Chemoprevention in Min Mice and Choice of the Model System

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Abstract

The *Apc*$^{Min/+}$ mouse model and the azoxymethane (AOM) rat model are the main animal models used to study the effect of dietary agents on colorectal cancer. We reviewed recently the potency of chemopreventive agents in the AOM rat model (D. E. Corpet and S. Tache, Nutr. Cancer, *43:* 1–21, 2002). Here we add the results of a systematic review of the effect of dietary and chemopreventive agents on the tumor yield in Min mice. The review is based on the results of 179 studies from 71 articles and is displayed also on the internet http://corpet.net/min.[2] We compared the efficacy of agents in the Min mouse model and the AOM rat model, and found that they were correlated ($r = 0.66$; $P < 0.001$), although some agents that afford strong protection in the AOM rat and the Min mouse small bowel increase the tumor yield in the large bowel of mutant mice. The agents included piroxicam, sulindac, celecoxib, difluoromethylornithine, and polyethylene glycol. The reason for this discrepancy is not known. We also compare the results of rodent studies with those of clinical intervention studies of polyp recurrence. We found that the effect of most of the agents tested was consistent across the animal and clinical models. Our point is thus: rodent models can provide guidance in the selection of prevention approaches to human colon cancer, in particular they suggest that polyethylene glycol, hesperidin, protease inhibitor, sphingomyelin, physical exercise, epidermal growth factor receptor kinase inhibitor, (+)-catechin, resveratrol, fish oil, curcumin, caffeate, and thiosulfonate are likely important preventive agents.

Introduction

Puzzling results have been presented at recent meetings of the AACR. NSAIDs[3] piroxicam and sulindac were reported to strikingly increase tumor yield in mutant mice, mice susceptible to spontaneous colon tumors (1–3), although NSAIDs are widely accepted as chemopreventive agents for such cancers in humans (4). These results raise questions about either the animal model or the NSAID protection. Thus, we have reviewed the results of dietary chemoprevention studies for two animal models of colorectal cancer, compared the results obtained with the two models, and then compared these results with the results of clinical intervention studies. We looked for consistency between two widely used animal models, and between these animal models and the results of clinical intervention trials.

Two Animal Models for Preclinical Testing of Chemopreventive Agents

For >30 years investigators have searched for dietary or chemopreventive agents that could suppress colorectal tumors in rodents. Because rodents such as rats have almost no spontaneous colon cancer they are given a carcinogen to induce colon tumors. The most commonly used are dimethylhydrazine derivatives. Dimethylhydrazine is metabolized to AOM and methylazoxymethanol in rats, and they are referred to as AOM studies in this review. AOM-induced tumors share many histopathologic characteristics with human tumors. They, like human tumors, are often mutated on *K-ras* and β-catenin genes (5), and show microsatellite instability, but, unlike human tumors, are seldom mutated at the *Apc* gene (15%), are never mutated at the *p53* gene (6), and have a low tendency to metastasize. Other colon carcinogens are used less frequently (7) including specific nitrosamines and heterocyclic amines like PhIP. PhIP induces the *Apc* mutation frequently (40–60%; Ref. 8) and microsatellite instability (9), but no *K-ras* or *p53* mutations (10, 11). It is present in our daily diet, although AOM is not. However, it is almost never used, because AOM is less expensive, more potent, and more convenient to use. Chemopreventive treatment can be begun before exposure to the carcinogen, and during the initiation phase, during the promotion-progression phase, or through both phases. The major end point in most rats studies is the incidence of colon tumors.

A mutant mouse, Min, was found with multiple intestinal neoplasia in 1990 (12). It was shown to have a mutated *Apc* gene, similar to that in patients with familial adenomatous polyposis, and in many sporadic cancers. This promising animal model mimics the rapid development of adenomatous polyps that affect humans with germ-line inactivation of one *Apc* gene. However, adenocarcinomas are seldom observed in this model, and no typical ACF arise above the intestinal mucosa. Consequently, the ACF to carcinoma progression is not established in this model. Moreover, the *K-ras* mutations

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[2] Internet address: http://corpet.net/min.

[3] The abbreviations used are: NSAID, nonsteroidal anti-inflammatory drug; AOM, azoxymethane; ACF, aberrant crypt foci; Apc, adenomatous polyposis coli; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; PEG, polyethylene glycol; DFMO, difluoromethylornithine; PPAR, peroxisome proliferator-activated receptor.

Table 1 Effect of dietary agents on the tumor number in the small intestine and in the colon of Min mice, and other mutant mice

Category	Treatment/mutation[a]	Dose[b]	Duration[c]	n^d	Small Intestine			Colon			Ref.
					Adenoma number[e]		Treatment effect[f] %	Adenoma number[g]		Treatment effect[f] %	
					Treated	Control		Treated	Control		
Bile acid	Chenodeoxycholic	0.50%	10 wk	1	25	33	**77**	0.6	0.4	150	16
Bile acid	Ursodeoxyc. + sulindac	1500–4500 +75 ppm		2	3	18	**15**				17
Bile acid	Ursodeoxyc. + sulindac	500 +75 ppm		1	5	18	**30**				17
Bile acid	Ursodeoxycholic acid	500–1500 ppm		2	13	18	**73**				17
Fat	Arachidonic acid	1%	7 wk	1	55	54	101				18
Fat	Corn Oil	10% *vs.* 3%	150–300 day	2	32	23	**137**	3.8	1.8	**212**	19
Fat	DHA/*Apc*716	3% female	7 wk	1	68	219	**31**				20
Fat	DHA/*Apc*716	3% male	7 wk	1	278	193	144				20
Fat	Fish oil K85	0.4% male	17 wk/w1	1	36	108	**33**	1.6	1	160	21
Fat	Fish oil K85	1.25–2.5% male	17 wk/w1	2	80	108	74	0.7	1	65	21
Fat	Fish oil K85	0.4–1.25–2.5% female	17 wk/w1	3	40	73	**55**	0.3	0.8	38	21
Fat	High-fat low-fiber diet	fat:cellul. 22:0 vs 7:5%	5–6 wk	1	34	35	98	1.4	1.8	78	22
Fat	Low soybean oil	5% *vs.* 20, no fiber diet	60 day	1	19	30	**62**	0.2	0.4	57	23
Fat	Western diet/*Apc*1638	Fat+, Ca−, vit. D−	14–34 wk	2				2.3	1	233	24
Fiber	Cellulose	5–10% vs 0, in 20% fat	60 day	2	14	30	**45**	0.4	0.4	107	23
Fiber	Fructo-oligosaccharide	5.8%	42 day	1	47	50	93	0.7	2.1	**33**	25
Fiber	Fructo-oligosaccharide	5.8% in T-cell depleted	42 day	1				0.8	0.4	**200**	26
Fiber	Guar gum	5–10% *vs.* 0, in 20% fat	60 day	2	18	30	**59**	0.4	0.4	93	23
Fiber	High fiber rodent chow	fiber:fat 18:6 *vs.* 0:20%	60 day	1	28	29	96	1.1	0.4	**271**	23
Fiber	Inulin	2.5%	5–6 wk	1	49	35	140	2.4	1.8	133	22
Fiber	Inulin	10%	9 wk	1	68	55	123	1.3	0.6	211	27
Fiber	Oat bran	10%	5–6 wk	1	47	35	133	1.8	1.8	100	22
Fiber	Resistant starch	18.8%	42 day	1	50	50	99	3	2.1	143	25
Fiber	Rye bran	10%	5–6 wk	1	26	35	**75**	1.4	1.8	78	22
Fiber	Rye bran	10%/inulin diet	5–6 wk	1	36	40	91				28
Fiber	Wheat bran	10%	5–6 wk	1	35	35	99	2.1	1.8	117	22
Fiber	Wheat bran	7.1%	42 day	1	47	50	94	1.5	2.1	71	25
Fiber	Wheat bran	5–10% *vs.* 0, in 20% fat	60 day	2	16	30	**53**	0.6	0.4	**143**	23
Fiber	Wheat bran, fat-/*Apc716*	bran:fat 20:5 *vs.* 3:20%	7 wk	1	135	211	**64**	0.7	2	**36**	29
HAA	IQ/*Apc*716	300 ppm	11 wk	1	213	254	84				30
HAA	MelQx/*Apc*716	400 ppm	11 wk	1	221	254	87				30
HAA	N-OH-PhiP/*Apc*716	50 mg/kg × 5 inj.	50 day/d26	1	57	17	**335**				30
HAA	PhiP	50 mg/kg × 8 inj.	pups 3 wk	1	237	30	**789**	3.1	0.6	**519**	31
HAA	PhiP	25 mg/kg × 8 inj.	pups 3 wk	1	157	30	**524**	0.9	0.6	145	31
HAA	PhiP	50 mg/kg × 1 inj.	7–11 wk	2	163	47	**346**	1.8	0.6	**297**	32
HAA	PhiP	10 mg/kg × 1 inj.	7–11 wk	2	88	47	**188**	0.9	0.6	157	32
HAA	PhiP	50 mg/kg × 4 inj.	4 wk	2	78	72	108	0.5	0.7	78	33
HAA	PhiP/*Apc*1638	0.03% male and fem.	182 day	1	7	3	**234**				34
HAA	PhiP/*Apc*716	400 ppm	8 wk	1	168	147	114				30
inhib. EGF	EKB-569	150 ppm	60 day	1	3	20	**13**				35
inhib. EGF	EKB-569 + sulindac	150 +37.5 ppm	60 day	1	1	20	**4**				35
inhib. EGF	EKI-785	300 ppm	60 day	1	10	17	**56**				35
inhib. EGF	EKI-785 + sulindac	300 +150 ppm	60 day	1	1	17	**5**				35
inhib. INOS	Aminoguanidine	1500 ppm in water	10 wk	1	50	73	69	0.5	0.8	63	36
inhib. INOS	Arginine deficient diet	No arginine diet	10 wk	1	68	102	**67**	0.6	0.2	300	36
inhib. ODC	DFMO	2%	69 day	1	21	45	**46**	3.2	3.5	92	37
inhib. ODC	DFMO	1%	61 day	1	24	42	**57**				38
inhib. ODC	DFMO + piroxicam	1% +50 ppm	61 day	1	11	42	**27**				38
inhib. desat.	SC26196 n-6 desaturase	100 mg/kg/day	7 wk	1	34	54	**63**				18
NSAID	4-ASA or 5-ASA	500 ppm	75 day	2	102	84	122				39
NSAID	5-ASA balzalazide	250 mg/kg	90 day	1	6	22	**28**			**21**	40
NSAID	5-ASA balzalazide	62–125 mg/kg	90 day	2	11	22	**49**			**19**	40
NSAID	Aspirine	250–500 ppm	7 wk	2	17	36	**48**	0.7	1	65	41
NSAID	Aspirine	400 ppm	370 day/d-21	1	25	34	74				42
NSAID	Aspirine	200 ppm	130 day/d21	1	39	34	115				42
NSAID	Piroxicam	0.5 mg/mouse/day	7 day	1	2	49	**5**				18
NSAID	Piroxicam	25–50–100 ppm	61 day	3	28	42	**66**				38
NSAID	Piroxicam	50–100–200 ppm	6 wk	3	4	17	**23**	0.9	0.6	150	43
NSAID	Piroxicam	50 ppm	25 day/d55	1	8	23	**35**	1.4	0.8	169	44
NSAID	Piroxicam	50 ppm	50 day/d30	1	5	22	**23**	0.6	1.5	40	44
NSAID	Piroxicam	200 ppm	90–180 day	3	2	53	**4**				45
NSAID	Piroxicam	200 ppm	6–14 day	3	4	53	**7**				45
NSAID	Piroxicam	200 or 220 ppm	75 day	2	12	68	**18**				39
NSAID	Piroxicam	200 ppm	2–4 day	2	34	53	64				45
NSAID	Piroxicam/*Apc*1309	0.05%	10 wk	1	16	31	**52**	1.5	2.6	**56**	46
NSAID	R-flurbiprofen	10 mg/kg/day or/2d gav.	21 day	2	8	23	**35**				47

Table 1 Continued

Category	Treatment/mutation[a]	Dose[b]	Duration[c]	n^d	Small Intestine			Colon			Ref.
					Adenoma number[e]		Treatment effect[f] %	Adenoma number[g]		Treatment effect[f] %	
					Treated	Control		Treated	Control		
NSAID	Sulindac	160 ppm	11.5 wk	1	0	12	**1**	0.1	0.4	25	48
NSAID	Sulindac	320 ppm	80 day	1	3	41	**7**	0.3	0.8	33	49
NSAID	Sulindac	0.6 mg/mouse/day	7 day	1	22	46	**48**				18
NSAID	Sulindac	160 ppm	10 wk	1	9	17	**51**	3	4.5	67	50
NSAID	Sulindac	75–150 ppm		2	4	18	**22**				17
NSAID	Sulindac	160 ppm	75 day	1	13	84	**16**				39
NSAID	Sulindac	300 ppm	10 wk	1	49	72	68				51
NSAID	Sulindac	150 ppm	60 day	1	4	17	**26**				35
NSAID	Sulindac/*Apc*716	150 ppm	8 wk	1	125	201	**62**				52
NSAID	Sulindac/*Apc*716	12 mg/kg/day	8 wk	1	356	424	84	0.9	1.8	50	53
NSAID	Sulindac/Min*Msh2*−	13 mg/kg/day	4 wk	1	294	354	**83**	14	13	108	54
NSAID	Sulindac/Min*Msh2*±	13 mg/kg/day	22 wk	1	33	44	74	5	4.8	104	54
NSAID	Sulindac sulfone	50 mg/kg/day gav.	42 day	1	19	25	77				47
NSAID/2	Celecoxib	1500 ppm	25 day/d55	1	11	23	**48**	2	0.8	253	44
NSAID/2	Celecoxib	500–1500 ppm	50 day/d30	2	6	22	**29**	0.6	1.5	37	44
NSAID/2	Celecoxib	150–500 ppm	25–50 day	3	17	23	**73**	0.7	1.1	65	44
NSAID/2	JTE-523	100 ppm	8 wk	1	84	123	**68**				55
NSAID/2	MF-tricyclic/*Apc*716	3.5–14 mg/kg/day	8 wk	2	187	424	**44**	0.3	1.8	**19**	53
NSAID/2	MF-tricyclic/Min*Msh2*±	13 mg/kg/day	4–22 wk	2	92	200	**46**	5.7	9	**63**	54
NSAID/2	ONO-AE2-227	300 ppm	7 wk	1	42	61	**69**	0.2	0.5	40	56
NSAID/2	Rofecoxib/*Apc*716	25–75 ppm	8 wk	2	111	201	**55**				52
Other	Beef meat	24%	5–6 wk	1	53	35	150	3.2	1.8	178	22
Other	Bovine lactoferrin	0.2% or 2%	8 wk	1	44	54	82	1	1.2	83	57
Other	*Citrobacter rodentium*	10E8 cfu, one gav.	152 day	1	7	9	79	2.8	0.8	**350**	58
Other	Copper	6 vs 1 ppm	13 wk	1	22	47	**46**	0.3	1	27	59
Other	DSS	4% in water	4 or 8 day	2				24.2	1.7	**1421**	60
Other	Exercise	1.2 km/1 h/day	7 wk	1	33	37	89	2.4	3.2	75	61
Other	Food restriction	20% restriction	7 wk	1	51	55	93	1.1	2.3	48	62
Other	Germ-free status	germ-free	85 day	1	28	32	88	0.9	1.4	66	63
Other	Methionine	0.7%	4 wk	1	25	26	96	0.9	0.5	180	64
Other	PEG 3350	10%	10 wk	1	22	42	**53**	2.6	3.2	81	65
Other	PEG 8000/Min*Msh2*+ or −	5% in male and fem.	60 day	1	13	12	104	0.7	0	**3020**	66
Other	Selenium in broccoli	2.1 ppm Se	10 wk	1	48	67	**71**	0.4	1.9	**22**	67
Other	Selenium *p*-XSC	10–20 ppm	80 day	2	24	41	**58**	1.2	2.8	**43**	68
Other	Sphingomyelin-ceramides	0.1%	8 wk	3	31	56	**56**	0.7	1.4	**50**	69
Other	Uroguanylin	26 μg/mouse/day	11 wk	2	27	48	**57**	0.1	0.7	**14**	70
Other	Vegetables and fruits mix.	20% mix in 9% fat diet	110 day/d-20	2	14	17	82	2.3	1.5	152	71
Other	Vegetables and fruits mix.	22% mix in 20% fat diet	110 day/d-20	2	28	17	**162**	2.3	1.5	152	71
Phytochem	Acarbose/*Apc*1309	400 ppm	10 wk	1	29	31	93	2.2	2.6	84	46
Phytochem	BB protease inhibitor	0.1–0.5%	92 day/d-2	2	7	11	**63**	0.4	0.6	**67**	72
Phytochem	Caffeic CAPE	0.15%	75 day	1	12	33	**37**				73
Phytochem	Catechin (+)	0.1–1%	75 day	2	7	26	**27**	0.1	0.6	17	74
Phytochem	Curcumin	0.2%	10 wk	1	13	14	94				75
Phytochem	Curcumin	0.1%	75 day	1	12	33	**36**				73
Phytochem	Lignan HMR	200 ppm/inulin diet	5–6 wk	1	27	40	**67**	1.7	1.3	131	28
Phytochem	Resveratrol	100 ppm in water	7 wk	1	9	30	**30**	0	4	**0**	76
Phytochem	Rutin-quercetin	2%	75 day	2	28	33	85				73
Phytochem	Soy isoflavones	475 ppm *vs.* 16 ppm	11 wk	2	31	31	99				77
Phytochem	Tea extract + sulindac	0.1% water +300 ppm	10 wk	1	32	72	**44**				51
Phytochem	Tea extract (green)	0.1% in water	10 wk	1	56	72	78				51
PPAR activ.	BRL49,653	20 mg/kg/day	8 wk	1	31	27	113	3.2	0.6	**525**	78
PPAR activ.	Troglitazone	150 mg/kg/day	8 wk	1	23	22	104	1.7	0.6	**283**	78
PPAR activ.	Troglitazone	0.2%	5 wk	1	78	67	116	3	1	**300**	79
Vitamin B	Folate	8–20 ppm	13 wk/w3	2	19	24	80	3.7	4.6	80	80
Vitamin B	Folate	2–8–20 ppm	26 wk/w3	3	23	18	126	3.1	2.6	120	80
Vitamin B	Folate/Min*Msh2*−	8 ppm	8 wk/w3	1	111	299	**37**	0.6	1.7	35	81
Vitamin B	Folate/Min*Msh2*−	8 ppm	5 wk/w6	1	295	70	**422**	2.4	2.4	100	81
Vitamin B	Folate + choline	2 ppm + 3% *vs.* 0+ 1.4	70 day/d21	3	33	29	114				82
Vitamin D	1a,25 (OH)2-D3	3 × 0.01 μg/wk	10 wk	1	18	17	108	4	4.5	89	50
Vitamin D	Ro 26-9114	3 × 5 μg/wk	10 wk	1	17	17	102	4.7	4.5	104	50

[a] Mutation given when different from the *Apc*850 mutation (classical Min mouse).
[b] Dose: ppm, part per million; % of diet; mg/kg of body weight; inj., injection; gav., gavage.
[c] Treatment start: /w1: from 1 week after birth; /d-20: from 20 days before birth.
[d] Number of similar studies from a single article which were pooled before reporting mean value.
[e] Number of adenomas in the small intestine of treated and of control mice (some studies report total number of intestinal adenomas, and do not give colonic values).
[f] Treatment effect, calculated as: 100 × (number of adenomas in treated mice)/(number in control mice). A percentage smaller than 100 denotes a protective effect. Boldface: significant effect.
[g] Number of adenomas in the colon of treated and of control mice.

observed in many human tumors were not detected in Min mice polyps (13), and *p53* inactivation, frequent in human cancers, does not raise tumor number in Min mice (14). After the Min mouse discovery with truncated *Apc* in position 850, other mice have been genetically modified so that one or more oncogenes hold a germ-line mutation (*e.g.*, truncated *Apc* in positions 716, 1309, or 1638, and mutated *Msh2* or *Mlh1*). A mutation on *Msh2* or *Mlh1* genes leads to mismatch repair defect, which makes these mice a model for human hereditary nonpolyposis colorectal cancers (15). Like in humans, different mutations lead to different phenotypes; for instance, as shown in Table 1, more adenomas are found in the gut of *Apc*716 mutant mice (250 polyps ± 95) than in classical Min mice (40 ± 20), or in *Apc*1309 and 1638 (30 and 3 polyps, respectively). Compared with Min mice, the number of polyps is higher in mice bearing both *Apc*850 and *Msh*2 mutations (160 ± 140/small intestine, and 5 ± 5/colon). These mutant mouse models have increased our understanding of carcinogenesis. They have also provided a model to evaluate the effect of diets and chemopreventive agents. The model avoids the hazard of carcinogen handling and leads to shorter assay, but is more expensive than the AOM rat model. Dietary treatments are typically begun for the mice by the age of 4–5 weeks, when tumors may already be present. A few studies have exposed the animals *in utero*, when neoplastic foci are already present. The timing mimics that of clinical intervention trials. Dietary interventions are given to adults that likely bear Min polyps, remaining after the visible ones have been removed. In most mouse studies it is the number of tumors in the small intestine that is the primary end point, although some authors have split the small intestine between proximal and distal parts, and showed that some agents afford a protection limited to the distal part. The major drawback of these mutants as models of human colon cancer is that their tumors occur predominantly in the small intestine, not the colon.

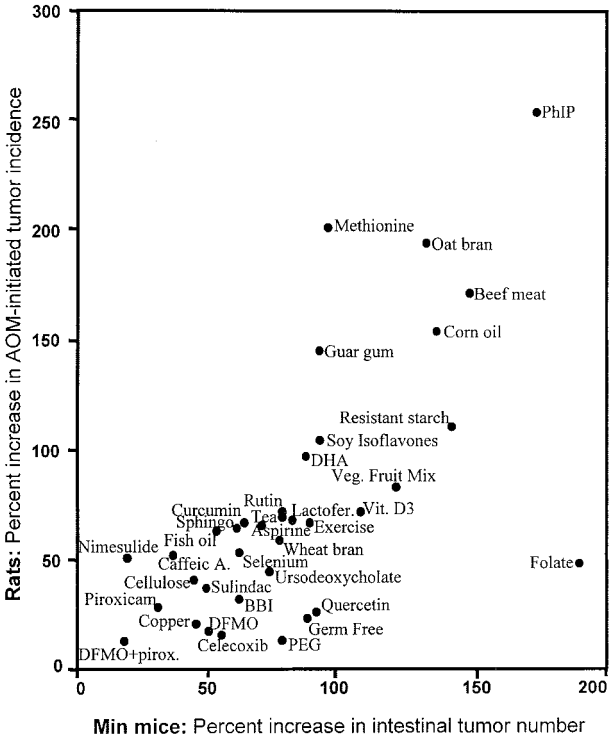

Fig. 1. Correlation between the effect of various agents on the number of adenomas in Min mice small intestine and on the incidence of colon tumors in AOM-initiated rats. The correlation coefficient was 0.66 with all of the points ($P < 0.001$) and 0.82 after exclusion of two outliers (methionine and folic acid).

Review on Dietary Chemoprevention in the AOM Rat and Min Mouse Models

Data from our previous systematic review on chemoprevention in the rat model (7) were gathered from 146 articles with the tumor end point, and 137 articles with the ACF end point, a putative preneoplastic lesion. Tables were built with potency of each agent or diet to reduce the tumor incidence or the number of ACF in the colon of rats. Both tables are available on a web site with sorting abilities.[2] Agents of outstanding potency that fully suppress colon adenocarcinoma and/or consistently inhibit adenoma and ACF in several independent AOM rat studies, were (ranked list): PEG, celecoxib, hesperidin, DFMO, and piroxicam (combined or not), sulindac (sulfone or sulfide), and ursodeoxycholic acid. In addition, treadmill exercise, and S-methyl-methane-thiosulfonate suppressed carcinoma (supported by a single study each). Last, Bowman-Birk protease inhibitor and sphingomyelin were consistently efficient in AOM-initiated mice (7).

All of the publications relating to the effect of dietary agents tested in Min mice and other mice with mutations resulting in intestinal tumors were identified from three databases: Institute for Scientific Information Current Contents, Medline, and the AACR web site, for the period from 1990 to May 2002. Data were gathered from 63 articles and 8 meeting abstracts, yielding 178 comparisons between a control and a treated group of mice (16–82). A primary table (data not shown) was built including the following data: mouse strain, mutation, treatment dose duration and vehicle, the number of intestinal adenomas in treated and control groups, the significance of the treatment effect, and, when reported, the size of intestinal adenomas and the specific number of colonic adenomas. Some papers did not report small and large bowel data separately. In those cases, we reported the total number of adenomas instead of small intestinal adenomas. Some meeting abstracts do not report detailed data and were not included in the tables. This primary table was abstracted to give the efficacy of each treatment to reduce the number of adenomas in the small intestine and in the colon of mutated mice (Table 1). The results are reported as a percentage of control values. The table is available on a web site with sorting abilities, allowing ranking of agents by potency.[2] Like the rat database, this mouse database will be kept updated as a resource for the research community.

Table 1 clearly shows that NSAIDs are, by far, the most potent agents to suppress tumor formation in the small intestine of Min mice. Notably, piroxicam and sulindac decreased the tumor yield by ≥90% in several independent studies. Piroxicam or sulindac were used in all but one of the top 25 studies, ranked by potency (see Table in web site). The other study involved the epidermal growth factor receptor kinase inhibitor, EKB-569. Specific anticyclooxygenase-2, like celecoxib or MF-tricyclic, were not more potent than nonselective NSAIDs. Other agents were less potent than NSAIDs, and the best ones decreased the tumor yield by 60–70%: (+)-catechin, resveratrol, fish oil (two studies), curcumin, folic acid, and caffeic acid phenetyl ester. The following agents were clearly less potent: cellulose, copper, DFMO, PEG, wheat bran, sphingomyelin, uroguanylin, and selenium compound, p-XSC.

Table 2 Agents producing a very different effect on the tumor number in the small intestine and in the colon of Min mice (protection ratio below 0.4 or above 2.5 data from Table 1)

Category	Treatment	n^a	Treatment effect: polyps in treated mice percent polyps in control mice		Protection ratio: effect in small intestine *versus* effect in colon	Ref.
			Small intestine	Colon		
NSAID	Sulindac	1	1	25	0.04	48
NSAID	Sulindac	1	7	33	0.22	49
NSAID	Piroxicam	3	23	150	0.15	43
NSAID	Piroxicam	1	35	169	0.20	44
NSAID/2	Celecoxib	1	48	253	0.19	44
PPAR activ.	BRL49,653	1	113	525	0.22	78
PPAR activ.	Troglitazone	1	104	283	0.37	78
PPAR activ.	Troglitazone	1	116	300	0.39	79
inhib. INOS	Arginine deficient diet	1	67	300	0.22	36
Fiber	High fiber chow	1	96	271	0.35	23
Fiber	Wheat bran	2	53	143	0.37	23
Fat	Fish oil K85	1	33	160	0.20	21
Other	*Citrobacter rodentium*	1	79	350	0.23	58
Other	PEG 8000	1	104	3020	0.03	66
Fiber	Fructo-oligosaccharide	1	93	33	2.8	25
Other	Selenium in broccoli	1	71	22	3.2	67
Other	Uroguanylin	2	57	14	4.0	70
Vitamin	Folate/Min*Msh*2−	1	422	100	4.2	81
Phytochem	Resveratrol	1	30	0	100	76

[a] See abbreviations and notes to Table 1.

Comparison of Results Obtained with the Two Prevention Models

Fig. 1 shows that many agents that suppress tumors in the Min mouse intestine (Table 1) also decrease the incidence of colorectal cancer in AOM-initiated rats (7). A significant correlation was found between the efficacy of agents tested in both models ($r = 0.66$; $n = 36$; $P < 0.001$). It is clear that the most potent chemopreventive agents in the Min mouse small intestine are also potent in the colon of AOM-initiated rats, and, thus, the animal models seem consistent.

Min mice, as noted above, have many tumors in the small intestine but few tumors in the colon (median number 34 and 1, respectively; Table 1). By contrast, human tumors are rarely found in the small intestine but frequently in the colon. This discrepancy between the mouse model and the human situation led us to examine the effect of diets on the tumor yield in the colons of mutated mice. To do so we calculated the ratio between treatment effect in the small intestine and in the colon from Table 1. The table, sorted by this ratio, showed that the median ratio was 0.95. That is on average, the agents have similar efficacy on large and small intestinal tumors. The top and bottom of this ranked table are shown on Table 2, which shows agents with a ratio below 0.4 or above 2.5. Several studies with NSAIDs fell in the first group and show a much weaker protection to the colon than to the small intestine. PPAR agonists, high fiber diets, PEG and *Citrobacter rodentium* were also in this group, and increase tumor yield in the colon but not in the small intestine. In contrast. resveratrol, folic acid, uroguanylin, selenium in broccoli, and fructo-oligosaccharides fall in the latter group, and afforded a specific protection to the colon (Table 2).

Some discrepancies between the small and large bowel are easy to explain. This is the case when the effect is a consequence of changes in the gut flora. For instance, fructo-oligosaccharides are not digested in the small intestine, but fermented by the microflora in the colon, where they yield butyrate, a possible apoptosis inducer. Formation of butyrate may explain why fructo-oligosaccharides decrease the tumor yield in the colon of Min mice but not in the small intestine (25). The promotion of tumors by *C. rodentium* is limited to the colon, where the bacterial density is much higher than in the small intestine (58). In this case the presence of the colonic microflora is associated with an increased risk. When the effect of an agent is a consequence of changes in the gut flora it is understandable that the colon of Min mice is an adequate model of the human colon. Tumor promotion by PPAR γ agonists also shows an understandable discrepancy much stronger in the colon than in the small intestine. This pattern may reflect PPAR γ expression, high in the colon, low in the rest of the gut, in both mice and humans (83).

Other discrepancies are less easily explained. The most potent chemopreventive agents in the Min mouse small intestine, also potent in the colon of AOM-initiated rats, sometimes increase the tumor yield in the colon or in the ileum of mutated mice. The NSAIDs piroxicam, sulindac, and celecoxib strongly decrease the number of tumors in the small intestine of mutant mice (Table 1), and markedly decrease the tumor incidence in AOM-initiated rats (84, 85, 86). However, piroxicam increased the number of tumors in the colons of Min mice in four of six studies (Table 1). In addition, piroxicam caused a 10-fold dose-dependent increase in tumor multiplicity in the distal intestine of *Msh2*−/− mice (1). In two of three studies, the sulindac protection in the colon of Min mice is much weaker than in the small intestine (Table 2). In three of four studies, no protection is seen in the colon of mice with *Msh2* or *Apc*716 mutations (Table 1). In addition, sulindac treatment significantly increased colonic tumors in four mutated mouse models: *Apc* Min, *Apc*1638, *Apc*1638/*Mlh1*, and *Mlh1* mice (2, 3). In *Mlh1*+/− mice, for instance, sulindac treatment increased the colon tumor incidence from 20% to 91% (3). Also, a late treatment with a high dose of celecoxib increased the tumor yield in the colon of Min mice (44). Thus, NSAIDs that are very potent chemopreventive agents promote tumors in the colons of mutated mice.

Table 3 Summary of dietary prevention of colorectal tumors in rats, mice and humans: efficacy of agents or diets to reduce tumor incidence in rats, polyp number in mice, and polyp recurrence in humans

Agent or diet[a]	AOM-rats, colon tumor incidence[b]		Min mice, polyp number, (small bowel)		Humans, polyp recurrence	References and notes
	%[c]	N[d]	%[c]	N[d]	%[c]	
Selenium	**50**	7	**60**	3	***50***	95[e]
Celecoxib	**20**	2	**60**	4	***70***	96[f]
Aspirin	90	9±	**70**	4	**80**	97[g]
Sulindac	**60**	8	**50**	15	80	98[h]
Calcium	70	6±			**80**	99[i]
Wheat bran	**60**	9	**80**	4	90	100
Low fat	***80***	10±	***70***	1	100	101–104[j]
Caloric reduction	**50**	3	*90*	1	*100*	102–104[k]
Fruits and vegetables	100	8	120	4±	100	102, 105–107
β-carotene	**80**	3			110	104, 108
Vit. C + vit. E	100	11			110	108–110
Psyllium	***40***	1			***160***	111

[a] Agents or diets tested in clinical trials, ranked by efficacy in humans.
[b] Data come from a systematic review of positive rat studies (7), pooled with null and negative published rat studies.
[c] Data are treatment effects, averaged from all published studies, and rounded to the nearest 10. Calculation: 100 × (colorectal tumor incidence in treated group)/(incidence in control group). A percentage <100 denotes a protective effect. Boldface, significant effect. Italics, value not firmly established (because from a single trial or a small size one or because the primary endpoint was not colon tumor). In mice column, polyp number was used in place of tumor incidence.
[d] Number of pooled studies. ±, some studies within the pool were clearly discrepant.
[e] Colon cancer was a secondary endpoint in the selenium trial, primarily designed to reduce prostate cancer.
[f] Polyp reduction shown in FAP patients. No data yet published on sporadic polyps.
[g] Significant effect of low dose of aspirin (80 mg/day), no effect of high dose (325 mg/day).
[h] Sulindac shows significant protection in FAP patients (three small-size trials), not on sporadic polyps (two trials) (4).
[i] In *Apc*1638 mice (24), the low-calcium "Western Diet" increased by +175% the tumor yield. This result was not included in the table because the diet was also low in vitamin D and high in fat.
[j] The effect of fat was significant in F344 rats (101) but not in SD rats (data not shown). In human volunteers, the interventions were in part, or led to, a dietary fat intake reduction (102–104).
[k] The above-cited low-fat interventions also led to a reduction in caloric intake, estimated as −18%, −10%, and −5% in studies 102–104, respectively.

Other agents in addition to NSAIDs also yield discrepant results in the colon of rats and mice, including DFMO, PEG, and inulin. DFMO, which blocks ornithine decarboxylase, strongly decreases colon tumor incidence in rats (87), and suppresses polyps in the small intestine of Min mice, but increased the number of large polyps in the colon of Min mice (37). PEG, a mild laxative, is a strikingly potent chemopreventive agent in rats (88, 89), but it strikingly increased the tumor yield in the colon of Min mice in one of two studies (Refs. 65, 66; Table 1). Last, inulin, a natural nondigestible oligosaccharide, decreases carcinogenesis in rats but increases the tumor yield in the colons of Min mice (22, 27).

The reason for these puzzling discrepancies is unclear. Some differences may be just chance findings linked to the very low number of tumors in the colon of Min mice. But most may be a result of differences in key enzymes between the small and large bowel of Min mice. Phospholipases A-2 and cyclooxygenase-2 are up-regulated in colonic tumors of humans and rats, and in small intestinal tumors of Min mice (90–92). The resulting increase in prostaglandin E2 level would promote cancer growth. This up-regulation is not seen in the colon of Min mice (93, 94), which may explain why NSAIDs do not always reduce carcinogenesis in the colon of Min mice. Polyamines levels are lower in the colon than in the small intestine of Min mice. Despite a high ornithine decarboxylase expression, a colonic antizyme decreases the polyamine pool (37). This low level of polyamines in the colon may explain why Min mice have few polyps in the colon. Moreover, DFMO treatment reduces polyamine levels in human colon but not in the colon of Min mice. This may explain why DFMO does not suppress colonic polyps in Min mice. These considerations would suggest that the colon of rats and the small intestine of Min mice are better models of the human colon than the Min mouse colon.

Comparison of Human Data with Animal Models Data

Finally, we would like to know how the results with the two animal models of colon cancer prevention compare with those obtained to date in clinical trials. Randomized, placebo-controlled trials directed at preventing the recurrence of colonic adenomatous polyps in human volunteers are considered to be the gold standard for chemoprevention studies, although they do have limitations. The major one is that the study end point is not cancer incidence but adenoma recurrence. Other limitations are the relatively short length of the intervention treatment compared with the duration of the disease, the possible lack of compliance with the protocol, and the inclusion of subjects that differ from the general population. How well do the animal models predict what happens in these clinical trials? To answer this question we built a table showing the effect of dietary interventions on tumors in rats and mice, and on the recurrence of colonic polyps in humans (Table 3). The mean effect in rats was extracted from a published database of positive studies (7), to which were added null and negative studies. The mean effect in Min mice was calculated from Table 1. Table 3 is obviously a first approach to such a comparison, because no account was taken of the dose used, and the data presented are not homogeneous across different models. Table 3, nonetheless, shows that the effect of most of the diets or agents is consistent across the various models (95–111), although discrepancies are seen between the effect of agents in humans and in animals as follows.

NSAIDs strongly decrease the tumor yield in the colon of

AOM-injected rats and in the small intestine of mutant mice. This is consistent with epidemiological studies suggesting that, taken collectively, NSAIDs might decrease the colorectal cancer incidence by 45% in humans (4, 112). It is also consistent with the effect of celecoxib and sulindac, which decrease the polyp number in familial adenomatous polyposis patient trials. However, as detailed above, several independent studies (but not all) show that some NSAIDs can increase the tumor yield in the colon of mutant mice.

Wheat bran consistently reduces carcinogenesis in animals but has apparently no significant effect in humans, a discrepancy for insoluble fibers, already pointed out by Giovannucci (113). A soluble fiber, psyllium, decreases carcinogenesis in rats but increases the tumor recurrence in human volunteers. However, both results are only supported by a single study each. In addition, other soluble fibers similar to psyllium often show promoting properties in AOM-induced rats, an effect that fits the human trial result.

Rats and mice fed a high-fat diet have usually more tumors than controls fed a low-fat diet. In rodents, the relationship between the colon cancer incidence and the intake of fat remains true when controlled for calorie consumption. Fatty diets with high linoleic acid content and n-6-polyunsaturated fatty acids seem to be particularly consistent promoters in rodents (101). In contrast, neither human trials nor observation studies support fat or linoleic acid as tumor promoters in humans (114), a discrepancy already pointed out by Giovannucci (113).

Caloric reduction is a strategy that seems very efficient in animals (Table 3). Overnutrition could be seen as the most potent "carcinogen" in rodents (115). According to Willett (116), a positive energy balance (caloric intake *versus* physical activity) is the most powerful and consistent dietary influence on human carcinogenesis. No published human trial specifically tested the effect of caloric reduction. However, a side effect of interventions with low-fat diet, and with fruits and vegetables, was a modest reduction in caloric intake (102–104). The lack of reduction in polyp recurrence seen in these trials (Table 3) suggests that the caloric reduction was too small to reduce insulin resistance, a supposed link between overnutrition and carcinogenesis (113, 117).

That fruit and vegetable consumption protects against colorectal cancer is a dogma supported by many epidemiological studies (118–119), an association that may have been overstated (120). This dogma is challenged by all of the experimental studies in rats, mice, and humans (Table 3). Indeed, a mixture of fruits and vegetables, reproducing what people typical consume, marginally increased the tumor yield in most animal studies (71, 105–107), although large amounts of black raspberries or of orange juice can inhibit carcinogenesis in rats (121).

Conclusions

There is a close agreement between the many results obtained in the colons of AOM-initiated rats and in the small intestine of Min mice (Fig. 1). There is a less, but perhaps reasonable, agreement between the results of these animal studies and the more limited clinical studies (Table 3). The discrepancies are interesting. Some results obtained in the colon of Min mice are discrepant from those of the Min mouse small intestine and the AOM rats. These exceptions could be keys to dissecting what is really important for tumor formation at the cellular and molecular levels. Understanding the mechanisms of tumorigenesis and its modulation will help to resolve discrepant opinions, and to make formal recommendations to the public. Many promising agents strongly and consistently suppress tumor formation or growth in the small intestine of Min mice, and/or in the colon of AOM-injected rats. Some of them have already been tested in completed clinical trials: selenium, celecoxib, aspirin, sulindac, calcium, wheat bran, low-fat diet, fruit and vegetable diet, β-carotene, and vitamins C and E (Table 3). Others are presently under study in humans: ursodeoxycholate, piroxicam, DFMO, and folic acid. Most published trials show no reduction in polyp recurrence, although celecoxib, aspirin, or calcium afforded significant, although modest, protection (Table 3). Thus, we need to identify new agents or strategies with animal models to reduce human colon cancer load.

A conservative approach to deciding which agents or diets to include in clinical intervention trials would be to chose only those that show preventive properties in all of the available models. We think that this approach might be too conservative. It would have disqualified the use of celecoxib, piroxicam, sulindac, DFMO, calcium, and folic acid in clinical trials, because they have shown promoting properties in some preclinical studies. In our opinion, well-conducted chemopreventive preclinical studies have established the efficacy and the safety of agents in rodents that do not meet this criteria.

Our point is that it is appropriate to proceed now with the agents that are particularly potent against carcinogenesis in either rats or mice. The data we have summarized and compared suggest that clinical trials could now be made with: PEG, hesperidin, Bowman-Birk protease inhibitor, sphingomyelin, physical exercise, and S-methyl-methane-thiosulfonate (from the AOM rat model), and EKB-569, (+)-catechin, resveratrol, fish oil, curcumin, and caffeic acid phenetyl ester (from the Min mouse model). These agents showed no toxicity in rodents, and some of them are already used daily by humans on a large scale (PEG, exercise, catechin, fish oil, and curcumin). The safety of others, notably EKB-569, still need to be evaluated (122).

Because human studies are extremely long and costly, they require stringent preliminary studies to determine optimal dosage and evaluate side effects. The long-term administration of untested agents to otherwise healthy people poses ethical problems, as β-carotene trials in smokers sadly showed. The use of surrogate end point biomarkers in step-wise clinical trials might help to decrease both cost and risk, although no single surrogate end point biomarker has been conclusively validated and established as the gold standard biomarker for colon cancer. Nevertheless we think that prospective prevention agents could be tested sequentially, first in short-term trials to determine whether they suppress ACF in the colon of volunteers (123), and then, if successful, with more standard trials to determine whether they reduce adenoma recurrence. This approach might be particularly appropriate for agents like PEG that clear ACF quickly from the mucosa before they reduce polyp numbers (89). Such a sequential strategy could eventually provide evidence for safe dietary interventions for the prevention of colorectal cancer.

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