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How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men.

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Abstract

Tumours in rodent and human colon share many histological and genetic features. To know if rodent models of colon carcinogenesis are good predictors of chemopreventive efficacy in humans, we made a meta-analysis of aspirin, beta-carotene, calcium, and wheat bran studies. Controlled intervention studies of adenoma recurrence in human volunteers were compared with chemoprevention studies of carcinogen-induced tumours in rats, and of polyps in Min (*Apc*(+/-)) mice: 6714 volunteers, 3911 rats and 458 mice were included in the meta-analyses. Difference between models was small since most global relative risks were between 0.76 and 1.00. A closer look showed that carcinogen-induced rat studies matched human trials for aspirin, calcium, carotene, and were compatible for wheat bran. Min mice results were compatible with human results for aspirin, but discordant for calcium and wheat bran (no carotene study). These few results suggest that rodent models roughly predict effect in humans, but the prediction is not accurate for all agents. Based on three cases only, the carcinogen-induced rat model seems better than the Min mouse model. However, rodent studies are useful to screen potential chemopreventive agents, and to study mechanisms of carcinogenesis and chemoprevention.

Keywords: Animal model; Diet; Chemoprevention; Colon-carcinogenesis; Min mice; Chemically-induced; Aspirin; b-carotene; Calcium; Wheat bran; Meta-analysis; Systematic review

1. Introduction

Some 100,000 rodents have been sacrificed on the chemoprevention altar. This number was estimated from the colon cancer chemoprevention database : **http://www.inra.fr/reseau-nacre/sci-memb/corpet/indexan.html** The estimate also includes liver, mammary, oesophagus, pancreas prostate, and skin cancer studies. Were these sacrifices useful? Were the time, efforts, and money needed to raise rodents, and to try to prevent their tumours of any use? The answer may seem obvious, since rodents and humans share many biological functions, and rodents are valuable for toxicity tests. Rodent studies are needed in the chemoprevention area, because epidemiological studies do not lead to firm conclusions: confusing factors cannot be fully eliminated. Thus, the hypotheses generated by epidemiology must be tested in controlled experiments, ideally in humans (1). But this is very long and costly, and it could jeopardize volunteers' health. Thus, animal trials should precede human trials. For instance, animal studies should have been completed before beta-carotene administration to smokers (2, 3). It is not, however, so obvious that animal chemoprevention studies are useful (4). Major differences between rodents and humans in lifespan, body weight, intestinal morphology (e.g., caecum), gut microflora, way of eating (e.g., meals, chewing, coprophagia), and gene regulation may change the outcome of dietary interventions. Also, the profound differences in efficacy seen, even in different studies using one model, cast doubt on their relevance for clinical studies (5). The question thus needs to be scrutinized.

How good are rodent models of carcinogenesis in predicting chemopreventive efficacy in humans? From a theoretical viewpoint, how similar, or dissimilar, are rodent and human tumours? From an empirical viewpoint, are the chemopreventive effects of agents tested in rodents and humans consistent or discrepant? This review focuses on colorectal cancer prevention only, and goes through four steps: (i) Comparison of the mechanisms of colon carcinogenesis in humans and in animal models. (ii) Review of human intervention studies aimed at preventing colorectal tumours. (iii) Meta-analysis of animal intervention studies (4). The meta-analysis was restricted to aspirin, beta-carotene, calcium and wheat bran, the only agents tested in several human trials. (iv) The efficacy of chemopreventive agents in animals and in humans was then compared.

2. Comparison of the mechanisms of colon carcinogenesis in humans and in animal models

Let us look first at colon carcinogenesis in humans, then in rodent models. Vogelstein model relates the histological

progression from normal tissue to cancer with the sequential accumulation of mutations (6, 7). Most human adenocarcinoma would evolve from aberrant crypt foci (ACF) and adenoma. This model has been progressively enriched, and several interdependent pathways are now accepted, based on the analysis of sporadic tumours and of two inherited syndromes: the familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancers (HNPCC). Germline mutation of the *Apc* gene determines the FAP syndrome. Most colorectal cancers are sporadic (90%), but they share with FAP tumours the same early *Apc* mutation in 50 to 80% of cases. In most sporadic colon cancers, like in FAP, a consequence of *Apc* gene mutation is b-catenin accumulation. Indeed APC protein forms a complex with b-catenin, axin, and glycogen synthase-3β kinase (GSK3β). Axin promotes β-catenin phosphorylation that mediates its degradation in the proteasome (8). In normal cell, this is regulated by the Wingless/Wnt signaling pathway. But *Apc* mutation prevents the formation of the complex, and b-catenin level rises in the cytoplasm. The stabilized β-catenin associates with transcription factor Tcf4. b-catenin-Tcf4 translocates into the nucleus, and induces constitutive activation of *c-myc*, *cyclin D1* and *c-jun* (9). The disruption of the Wnt/b-catenin/Tcf pathway is thus a major event in most colon cancers. Chromosomal instability (CIN), a common feature of 8/10 colorectal cancers (10), goes with *Apc* mutation. Truncated APC protein may loose its ability to connect chromosomes to microtubules. Defective chromosome segregation, and CIN, would thus result from mutated *Apc*. Furthermore, in the tumours where *Apc* is intact, the b-*catenin* gene is mutated, and stabilized β-catenin translocates into the nucleus and triggers *c-myc*, *cyclin D1* and *c-jun*. In the multiple steps process from normal cell to carcinoma, other genes are mutated or deleted. The oncogene K-*ras* is mutated in the early stage of colon carcinogenesis, while tumour suppressor genes (*DCC* and *p53*) are involved in later stages (11). The process is also associated with over-expression of iNOS and COX-2, with resulting increase in nitric oxide and prostaglandin E2 levels. HNPCC syndrome is not due to *Apc* mutation but to a mutation in a mismatch repair (MMR) gene: several MMR genes are implicated as first event (*Mlh1*, *Msh2*, *Msh6*, *Pms1*, *Pms2*). Mutation rate is 100–1000-fold greater in MMR-deficient cells than in normal cells. This is evidenced by microsatellite instability (MSI), which participates to the hypermutable phenotype (12). Most microsatellites are found in noncoding DNA, but some mutations due to MSI modify genes involved in later stages of carcinogenesis, e.g., transforming growth factor-β receptor II and insulin like growth factor II receptor. Besides mutations, human tumours have a general DNA hypomethylation, but the aberrant hypermethylation of promoter CpG islands leads to transcriptional silencing of key growth-controlling genes and contributes to cancer progression (13).

Do tumours in animal models, i.e. carcinogen-initiated rats and mutated mice, share the genetic events and the histological features of human cancers? The use of carcinogens has been necessary because laboratory rodents have extremely low spontaneous rates of colon cancer. Most published studies were done in rats injected with dimethylhydrazine (DMH) or its metabolite, azoxymethane (AOM). AOM-induced tumours in rats share many histopathologic characteristics with human tumours, and similarly go through ACF, adenoma (often polyps) and carcinoma. They, like human tumours, often bear *K-ras* mutation (30-60%), but, unlike human tumours, they seldom have a mutated *Apc* (8%), and never a *p53* mutation. However, like *Apc* mutated human tumours, rat tumours accumulate b-catenin in the nucleus. This is due to *Ctnnb1* mutation, which produces a b-catenin resistant to degradation (14). Alternatively, a mutation in the GSK3b phosphorylation motif of the b-catenin gene can reduce b-catenin degradation (15). Heterocyclic amines, e.g., 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), are also used to induce tumours in rats or mice. PhIP induces *Apc* (15%) and □-catenin mutations (50%) in the colon of rats (16). The direct acting nitrosamine methylnitrosourea (MNU) has been used in few studies. In contrast with DMH-, AOM- and PhIP-induced tumours, no *Apc* or b-*catenin* mutations were detected in MNU-induced tumours. Thus, Wnt/□-catenin/Tcf pathway plays a major role in human tumours and in carcinogen-induced rat tumours. Like in humans, COX-2 and iNOS are over-expressed in these tumours. However, these rodent carcinogens are not found in human diet (except PhIP), and use of large doses of a carcinogen is not comparable to the human situation. Although the carcinogen-induced tumours look similar to human tumours, we do not really know if they develop like spontaneous tumours. Perhaps the protection (or the promotion) depends on the tumour initiator.

A mutant mouse, Min, was found with multiple intestinal neoplasia in 1990 (17). It was shown to have a germline inactivation of one *Apc* gene, similar to that in patients with FAP, and in many sporadic cancers. This promising animal model mimics the rapid development of adenomatous polyps that affect FAP patients. The Apc protein deficiency in Min mice results from a premature translational stop codon at amino acid 850. Other mice have also been genetically modified on *Apc* with truncations in positions 580, 716, 1309, or 1638. Like in humans, different mutations lead to different phenotypes and Wnt/b-catenin/Tcf pathway plays an important role in mutant mice carcinogenesis. For instance, Min mice have ten times more polyps than *Apc*1638, but six times fewer than *Apc*716 mutant mice (18). In addition, COX-2 and iNOS play an important role in Min mice carcinogenesis, like in humans: knockout Min mice with deleted COX-2 or iNOS gene(s) develop fewer adenomas than "wild-type" Min mice (19, 20). Like in humans, methylation plays a role in Min mice carcinogenesis, since a reduction in DNA methyltransferase activity suppresses polyp formation (21). *K-ras* and *p53* mutations are not detected in Min mice tumours, in contrast with human tumours. Besides *Apc* mutant mice, mice with *Msh2* or *Mlh1* gene mutations were obtained, but their phenotype does not make them a clear model for HNPCC patients (22). However, *Msh2*-deficient mice develop small intestinal tumours and sebaceous gland tumors analogous to *Msh2*-mutated patients (Muir–Torre syndrome). Like human HNPCC, *Msh2-/-* and *Mlh1-/-* mouse cells display high mutation frequencies and MSI (23).

Table 1: Experimental colon tumour prevention in Man. Randomized double-blinded placebo-controlled published intervention studies are ranked by potency to prevent polyp recurrence, and grouped by agent.

Agent or Diet	Reference	Relative Risk (95% confidence interval)	No. of treated patients	Length, months	Daily dose	Colon endpoint	Primary endpoint
Selenium	Clark 96	0.42 (0.18-0.95)	653	54	200 µg	Cancer incid.	Skin cancer
vitC,vitE,Bcar,Se,Zn	Hercberg 04	0.71 (0.39-1.31)	2520	90	176 mg	Cancer incid.	All cancers
Celecoxib	Steinbach 00	0.72 polyp/patient	30FAP	6	800 mg	Polyp no.	
Sulindac	Giardiello 02	0.78 (0.4-1.5)	21FAP	48	300 mg	Polyp no.	
Calcium	Baron 99	0.85 (0.74-0.98)	464	18	1.2 g	Polyp recur.	
Calcium	Bonithon 00	0.66 (0.38-1.17)	176	36	2 g	Polyp recur.	
Calcium +vit. Mix	Hofstad 98	0.71 (0.5-1.0)	42	36	1.6 g	Polyp recur.	Polyp growth
Aspirin	Baron 03	0.81 (0.69-0.96)	377	33	81 mg	Polyp recur.	
Aspirin	Baron 03	0.96 (0.81-1.13)	372	33	325 mg	Polyp recur.	
Aspirin	Benamouzig 03	0.61 (0.37-0.99)	60	12	300 mg	Polyp recur.	
Aspirin	Benamouzig 03	0.85 (0.57-1.26)	66	12	160 mg	Polyp recur.	
Aspirin	Gann 93	0.86 (0.68-1.10)	11035	60	162 mg	Polyp incid.	Heart attack
Aspirin	Sandler 03	0.65 (0.46-0.91)	317	31	325 mg	Polyp recur.	
Ursodeoxycholic acid	Alberts 05	0.88 (0.73-1.05)	661	32	75 0 mg	Polyp recur.	
Wheat bran	Alberts 00	0.88 (0.7-1.1)	719	35	+11 g	Polyp recur.	
Wheat bran	MacLennan 95	1.2 (0.8-2.0)	150	48	+25 g	Polyp recur.	
Wheat bran	McKeown 94	1.2 (0.6-2.2)	99	24	+15 g	Polyp recur.	
Low fat	MacLennan 95	0.9 (0.6-1.5)	151	48	-7%	Polyp recur.	
Low fat	McKeown 94	1.2 (0.6-2.2)	99	24	-9%	Polyp recur.	
Low fat	Schatzkin 00	1.00 (0.90-1.12)	958	36	-10%	Polyp recur.	
Beta-carotene	Greenberg 94	1.01 (0.85-1.20)	359	48	25 mg	Polyp recur.	
Beta-carotene	MacLennan 95	1.5 (0.9-2.5)	156	48	20 mg	Polyp recur.	
Beta-carotene	Hennekens 96	1 NS	11035	144	25 mg	All cancers	Heart attack
Beta-carotene	Malila 99	0.98 (0.71-1.35)	7761	78	20 mg	Polyp incid.	Lung cancer
Fruits & vegetables	Schatzkin 00	1.00 (0.90-1.12)	958	36	+2serv	Polyp recur.	
Vit. C + vit. E	Greenberg 94	1.08 (0.91-1.29)	380	48	1+0.4 g	Polyp recur.	
Vit. C + vit. E	McKeown 88	0.86 (0.51-1.45)	70	24	0.4+0.4 g	Polyp recur.	
Vit. E	Malila 99	1.66 (1.19-2.32)	7768	78	50 mg	Polyp incid.	Lung cancer
Psyllium	Bonithon 00	1.67 (1.01-2.76)	198	36	3.5 g	Polyp recur.	

The (*Apc*(+/-)) mice are promising models of human colorectal cancer (24). However, a major drawback is that the tumours occur predominantly in the small intestine, not the colon. In addition, ACF and adenocarcinomas are not or seldom observed in this model. However, two new mutant mice may avoid these drawbacks. Germline targeted deletion of *Apc* exon 14 leads to severe colon polyposis: 5-15 polyps develop in these mice colo-rectum, vs. 0.4-4 in other *Apc* mutants (25). Other mice, with a N-terminal truncated □-catenin (*A33*$^{\square N \square cat}$), develop few spontaneous ACF in the colon, like human and rat models (26).

Taken together, rodent models grow tumours that share many histological and genetic features with humans. The major differences between rodents and humans are the small bowel location of tumours in Min mice (vs. human colon), and the mutation of beta-catenin gene in AOM-injected rats (vs. human *Apc* mutations). These conclusions render it pertinent to examine studies of intestinal tumour chemoprevention in humans, and to compare them with results obtained in rodent models.

3. Experimental chemoprevention of intestinal tumours in humans

Randomized, placebo-controlled trials directed at preventing the recurrence of colonic adenomatous polyps in human volunteers are considered the gold standard for chemoprevention studies though they do have limitations. The major one is that the study end-point is not cancer incidence but adenoma recurrence. Other limitations are the short length of the intervention 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 (3). Two agents, calcium (27-29) and aspirin (30-32), consistently reduced polyp recurrence in several intervention studies (Table 1). The estimated "weighted mean RRs" for calcium and aspirin were 0.79 and 0.85 respectively (weighted by study size). A recently published meta-analysis finds an RR= 0.80 (CI: 0.68, 0.93) for calcium supplement (33), which is close to the value estimated here, 0.79. Interventions with high wheat bran and/or low fat diet, beta-carotene or vitamin C and E had no effect at all on polyp recurrence (34-39). The "weighted

Table 2: Meta-analysis of chemoprevention studies in carcinogen-initiated rats, dealing with aspirin, beta-carotene, calcium and wheat bran protection. Relative risks (RRs) calculated with Random Model, except underlined values, calculated by Chi-square test on 2x2 tables. Data subsets shown in italics (full data and figures on http://corpet.net/min)

Treatment	2x2 Table : No. of Rats		RR	95% C.I.	p Value
	With tumour	Total			
Aspirin treated rats	313	559	0.84	0.75-0.95	0.006
No aspirin controls	167	252	0.86	0.77-0.96	0.007
Aspirin during initiation only			*0.68*	*0.42-1.16*	*0.13*
Aspirin "both" periods			*0.80*	*0.67-0.95*	*0.012*
Aspirin post-initiation only			*0.92*	*0.79-1.08*	*0.32*
Beta-carotene treated rats	54	95	0.76	0.61-0.93	0.005
No beta-carotene controls	82	109	0.72	0.47-1.08	0.11
High calcium treated rats	548	984	0.91	0.84-0.99	0.03
Low calcium controls	456	748	0.92	0.85-1.00	0.06
Calcium in High Fat diets			*0.93*	*0.86-1.02*	*0.11*
Calcium in Low Fat diets			*0.92*	*0.77-1.11*	*0.38*
Calcium lactate			*0.72*	*0.55-0.94*	*0.02*
Ca phosph., carbon., gluconate			*0.99*	*0.95-1.04*	*0.74*
Wheat bran treated rats	307	595	0.83	0.75-0.91	0.0002
No wheat bran controls	355	569	0.87	0.77-0.97	0.015
Wheat bran in High Fat diets			*0.79*	*0.66-0.93*	*0.006*
Wheat bran in Low Fat diets			*0.91*	*0.78-1.07*	*0.26*

mean RRs" were estimated to be 0.96, 1.00, 1.00 and 1.04 respectively. Table 1 shows the effect of other interventions: mixtures, complex dietary changes, or once only tested agents. We chose to focus this meta-analysis on agents fulfilling two criteria: (i) well-defined agent, (ii) several concordant human trials. Accordingly, aspirin, beta-carotene, calcium, and wheat bran effect in rodents were further examined.

4. Chemoprevention in animal models of intestinal carcinogenesis.

According to the provocative article by Pound et al. (4), systematic reviews should become routine to ensure the best use of existing animal data, and improve the estimates of effect from animal experiments. We thus made a systematic review of aspirin, beta-carotene, calcium, and wheat bran dietary chemoprevention studies in two animal models of colorectal cancer: carcinogen-initiated rats (and mice), and mice mutated on the Apc gene (Min mice mainly).

4.1. Methods.

The meta-analysis of carcinogen-injected rats was done as follows: we searched articles on Medline/PubMed database and in "references" sections (cut-off date, January 2005). Some papers were not included: not in English, poor protocol, missing or aberrant data (list given on http://corpet.net/min). Studies were far from homogeneity (all Q Cochran's $p<0.01$), which disqualified "Fixed Effects" model (40). "Random Effects" model was used to calculate common RR, 95% confidence intervals (95%CI) and p values (40), which are shown in Table 2. Funnel plots were drawn to detect publication bias, which were tested by rank test (40). However, the Random model calculation needed to duplicate some control data, because many studies use a single control group for several treated groups. Each control rat was thus included several times in the table, which should not be. We thus added a second approach, by pooling data. It is not recommended as a rule because it gives too little weight to studies with low baseline levels of adenomas. Raw number of tumour-bearing rats, and of tumour-free rats, in control and treated groups, were included in a table, and summed up as if all rats had been treated in a single study (each control rat was included only once). The 2x2 contingency table with all rats (shown on Table 2) was then analysed with Chi-square statistics without Yates correction, and 95%CI were calculated and shown in Table 2. Pooling of data from all studies was chosen, including rats and mice, initiated by various carcinogens, and treated with various doses. We reasoned that when a human population is treated with a chemopreventive agent, people are exposed to various carcinogens, and have different genetic backgrounds and different diets. We thus had no *a priori* reason to exclude any rodent protocol.

The meta-analysis of Min mice intestinal polyp studies was done as follows: Global Effect Size and p value were first calculated with "Random Effects" model (40), and given in the "Results" section. However a second approach was also used, because "Effect Size" cannot be compared with RR. We thus chose to use ratios instead of differences. Number of adenomas per mouse in treated group was divided by corresponding value in control group and multiplied by 100, for each study. The mean of these percentages was compared with the hypothetical 100% value (H0 hypothesis)

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Table 3 Summary of dietary prevention of colorectal tumours in rats, mice and humans: Efficacy of agents to reduce polyp recurrence in humans, tumour incidence in rats, and polyp number in mice.

Agent or Diet	**Humans, mean polyp recurrence**		**Carcinogen-initiated rats, colon tumour incidence**			**Min mice, Polyp number, (small bowel)**		
	RR [c]	**N [e]**	**RR (95%CI)[c]**	**Rats /men**	**N [e]**	**PR (95%CI)[i]**	**Mice /men**	**N [e]**
Aspirin [a]	0.85 S [d]	4	0.86 (0.77-0.96)	OK [h]	8	0.94 (0.73-1.15) [j]	±OK	7
Beta-carotene	1.00 NS [d]	4	0.72 (0.47-1.08)[g]	OK	4	No study		0
Calcium	0.79 S	3	0.92 (0.85-1.00)	OK	13	1.09-1.21	NO	1
Wheat bran	0.96 NS	3	0.87 (0.77-0.97)	±OK	12	0.64 (0.54-0.84)	NO	5
Selenium [b]	*0.42 S*	*1*	*0.50 S*	*OK*	*7*	*0.60 S*	*OK*	*3*
Celecoxib	*0.72 S*	*(1)* [f]	*0.20 S*	*±OK*	*2*	*0.60 S*	*OK*	*4*
Sulindac	*0.78 NS*	*(1)* [f]	*0.60 S*	*±OK*	*8*	*0.50 S*	±OK	*15*
Low fat	*1.00 NS*	*3*	*0.80 NS*	*OK*	*10*	*0.70 S*	*NO*	*1*
Fruits & veg.	*1.00 NS*	*1*	*1.00 NS*	*OK*	*8*	*1.20 NS*	*OK*	*4*
Vit. C + vit. E	*1.04 NS*	*2*	*1.00 NS*	*OK*	*11*			*0*
Psyllium	*1.67 S*	*1*	*0.36 S*	*NO*	*1*			*0*

Notes to table 3.

a- Top-panel data come from this meta-analysis (Table 2), full data and figures on http://corpet.net/min
b- Bottom-panel data (*in italics*) from ref. (18): no true meta-analysis approach.
c- RR: relative risk of polyp recurrence (humans) or of colon tumour incidence (rats).
d- S, significant. NS, not significant.
e- Number of articles included in the meta-analysis.
f- Small scale study of polyp number reduction in FAP patients.
g- Not significant by Random model analysis, but significant by chi-square analysis (see Table 2).
h- OK: rodent data match human data; ±OK: no direct match but human RR within 95%CI; NO: rodent data differ from human data.
i- PR: polyp ratio, number of intestinal polyps in treated mice divided by number in control mice.
Not significant by Student's t test, but significant by Random model analysis: Effect Size= -0.29, 95%CI= -0.55; -0.03

sample Student t test. Also, a weighted mean was calculated, taking in account the number of mice per study. Full rats and mice data and figures are shown on website http://corpet.net/min, and data are summarized here in Table 2 (rats) and Fig. 1 (Min mice).

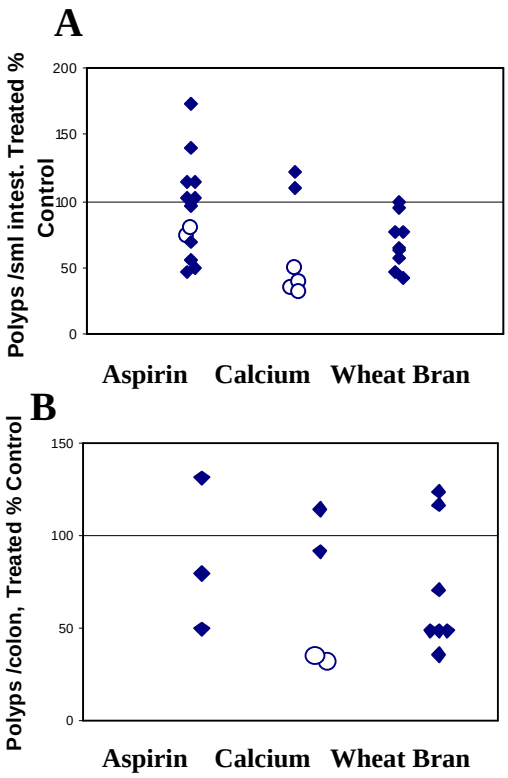
4.2. Results

4.2.1. Aspirin effect in carcinogen-injected rats.

The meta-analysis of eight publications (41-48) including 811 rats showed that aspirin reduces colon tumour incidence in rats: RR= 0.84 (p=0.006), with similar RR with Random model analysis (0.86, p=0.007). Analysis of subsets where aspirin was given only before or after the initiation is compatible with the hypothesis that the protection is higher when aspirin treatment is given during initiation (Table 2).

4.2.2. Aspirin effect in mutated mice.

Seven articles including 232 mice with an *Apc* mutation provide data on aspirin (49-55). Number of intestinal adenomas in treated mice was 94% of number in controls (Fig.1, p= 0.59). Effect Size analysed by Random Model was -0.29 (p=0.03). This small reduction of small intestinal polyps was thus significant or not, according to model. Furthermore, aspirin treatment did not reduce the number of colonic polyps (Fig.1-B). According to Perkins et al. (55) aspirin prevents the early phase of carcinogenesis, and would be active only before birth and until weaning. Data subsets were analysed to test this hypothesis. Mean number of polyps in the two early-treated groups of mice were 74 and 80% of controls (Fig. 1, open circles), vs. 102% in mice only treated after weaning. This is compatible with the hypothesis or early protection.



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Figure 1: Effect of interventions on number of tumours in *Apc* mutated mice, expressed as percent of control (full data on http://corpet.net/min). A: Small intestine. B: Large intestine. Open circles: pre-birth administration (aspirin), or "Western diet" (data not included into calcium meta-analysis)

4.2.3. Beta-carotene effect in carcinogen-injected rodents. The meta-analysis of four studies (56-59) including 204 rats and mice showed that beta-carotene reduces colon tumour incidence in rodents: RR= 0.76 (p=0.005). However, this RR was not significant using Random model analysis (0.72, p=0.11, Table 2). No study of beta-carotene in Min mice was found.

4.2.4. Calcium effect in carcinogen-injected rats.
The meta-analysis of 17 publications (44, 47, 60-75) including 1732 rats showed that calcium reduces colon tumour incidence in rats: RR= 0.91 (p=0.03), with similar RR with Random model (0.92, p=0.06). The hypothesis that calcium specifically reduces high-fat diet promotion was tested by analysing separately studies with high fat (>20% fat, w/w) and low fat diets (< 6%), but both subsets yielded similar RRs and p values (Table 2). Also, we tested the hypothesis that some calcium salts were more protective than others. This was indeed the case: calcium lactate was protective in rats (RR=0.7, p=0.02, Table 2), but phosphate, carbonate and gluconate afforded no protection (RR=1).

4.2.5. Calcium effect in mutated mice.
Small intestinal polyp yield increases by +9 and +21% when dietary calcium doubles (ref. (76), 79 mice). Calcium did not reduce the number of colonic polyps either (Fig. 1-B). In contrast, mice fed the high-calcium AIN76 diet had fewer polyps than mice fed the low-calcium Western diet designed by Newmark (77-79). This polyp reduction to 37% of control value (weighted mean, p<0.001) cannot however be attributed to calcium alone, since diets also differed for phosphate, fat, and vitamin D content (Fig 1, open circles).

4.2.6. Wheat bran effect in carcinogen-injected rats.
A significant protection by wheat bran is shown in two out of twelve publications (80-91). Meta-analysis, including 1164 rats, showed that wheat bran reduces colon tumour incidence in rats (RR= 0.83, p=0.0002), with similar RR in Random model analysis (0.87, p=0.015). The hypothesis that wheat bran specifically prevents fat promotion was tested by analysing separately studies with high fat and low fat diets. Wheat bran indeed protected rats given a high-fat diet (RR= 0.79, p=0.006), but not rats given a low-fat diet (Table 2).

4.2.7. Wheat bran effect in mutated mice.
The eight studies (92-96) gathering 147 Min mice showed a protective effect of wheat bran (Fig 1-A). Number of small intestinal polyps in wheat bran-fed mice was 69% of control number (weighted mean, 66%, p=0.001), and effect size was -0.74 by Random model analysis (p<0.001). Bran also marginally decreased colonic tumours (p=0.07, Fig.1-B).

5. Comparison of intestinal chemoprevention in humans and in animal models.

Table 3 shows that aspirin, beta-carotene, calcium, and wheat bran effect in men, rats and mice led to RRs comprised between 0.72 and 1.00 (and PRs between 0.64 and 1.15): no promotion and no strong protection were observed (Fig. 2). The effects of four agents in three models were thus similar. However, Table 3 significances and 95%CIs suggest that: (i) Aspirin protected men and rats, but not Min mice (but human RR was within mice PR 95%CI), (ii) Beta-carotene did not protect rats or men (no published Min mice study), (iii) Calcium protected men and rats, although effect in men was stronger than in rats. In a single study, Min mice were not protected (76), and (iv) Wheat bran protected mice and rats, but not men (but human RR was within rat 95%CI). Carcinogen-induced rat studies matched human trials for aspirin, calcium, carotene, and were compatible for wheat bran. Min mice results were compatible with human results for aspirin, but discordant for calcium and wheat bran (no carotene study). However, the size of these discrepancies was small and may not be meaningful. Bottom of Table 3 reports rodent data from a previous review (18). These results should be considered with caution, because the true meta-analysis approach was not undertaken in rodents, and because the effect in humans relied on single studies (except low fat). The effect of most of the diets or agents was consistent across the various models except one striking discrepancy: psyllium afforded strong protection in one rat study, and significant promotion

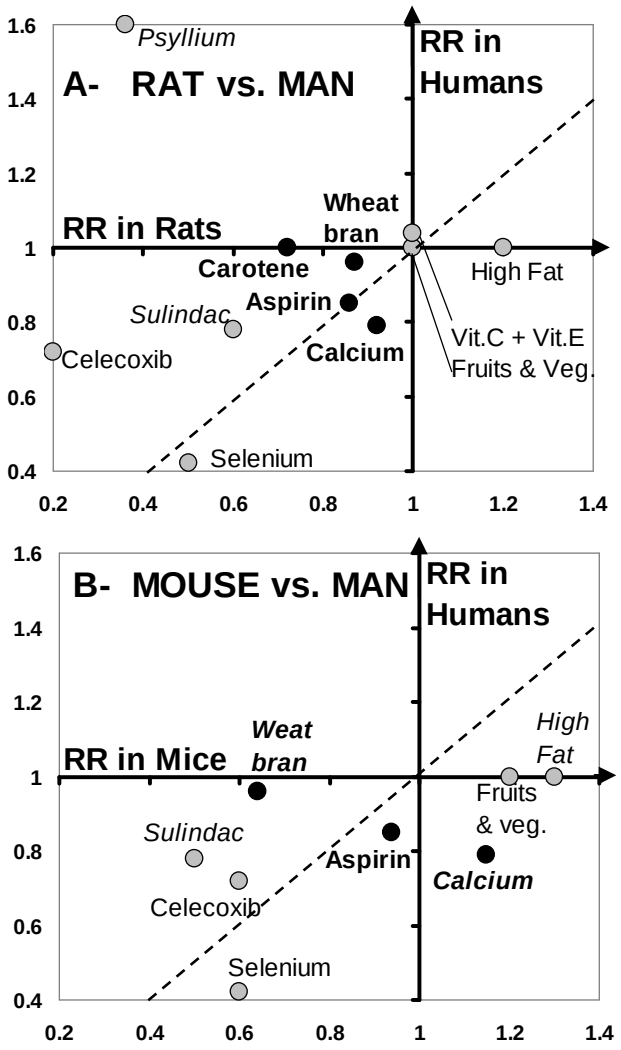


Figure 2: Chemoprevention in humans and rodents (data from Table 3). Colon polyp recurrence RR in humans vs. tumour RR in chemically-induced rats (panel RAT vs. MAN), or vs. Polyp Ratio in *Apc* mutated mice (panel MOUSE vs. MAN). Black points: meta-analysis data. Grey points: tentative values from ref (18). *Italics*: RR significance discordant in humans and rodents.

in one human study. However, the first published study of psyllium (not reported here) showed a strong promotion in DMH-initiated rats (97). The previous review concluded there was a reasonable agreement between the results of the animal studies and the more limited clinical studies (18). The present meta-analysis somewhat challenges this conclusion, because the prediction is not accurate for all agents, and carcinogen-induced rats model seems better than Min mice model.

6. Discussion

This meta-analysis of experimental studies suggests that the effects of aspirin, beta-carotene, calcium, and wheat bran were not strikingly different in humans, rats and mice (Fig. 2). However, the hypothesis that chemopreventive agents produce the same effect in animals and in humans has hitherto not been tested. Robust analysis would require solid data on more than four agents, and with more contrasted RRs (e.g., below 0.5 and above 1.0). Table 3 already suggests that selenium, celecoxib, and sulindac effect in rodents could match the effect in volunteers. Rodent models thus roughly predict effect in humans. A closer look at table 3 shows that carcinogen-induced rat studies matched human trials for aspirin, calcium, carotene, and were compatible for wheat bran. Min mice results were compatible with human results for aspirin, but discordant for wheat bran and calcium (single calcium article, and no carotene study). Table 3 also suggests discordances for psyllium in rats, and low fat diet in mice. Thus the rodent models do not predict accurately the outcome of intervention studies in humans for all agents, and Min mice do not appear to be superior to carcinogen-induced rats. The following four considerations may explain the apparent discrepancies between rodents and humans:

(i) Some agents may not afford the same protection in rodents and in humans (e.g., wheat bran). This means that rodent models would not be reliable predictors to detect chemopreventive agents.

(ii) Differences in study design could preclude any precise quantitative comparison between rodents and humans. Notably, genetic, diet, environment and treatment are fully controlled in rodent studies, not in human trials.

(iii) Publication bias could distort rodent results. Bias is probably much higher for rodent than for human studies. In contrast with human trials, null or negative rodent studies are less likely to be published than positive ones. This bends the mean of rodent results toward protection. For instance, several scientists have indicated to the authors that in their opinion their manuscripts were declined because the results contradicted a currently accepted dogma (e.g., calcium is protective). To illustrate this point, the funnel plot of aspirin data in rats showed a significant publication bias (plot shown on htt://corpet.net/min, p=0.0007). Calcium and wheat bran data show no clear evidence for bias. However, to reduce publication bias, there should be an ethical obligation to post all unpublished results on an internet archive.

(iv) Lastly, the meta-analysis itself might be inaccurate. We may have missed important studies, or the pooling of studies with different protocols was perhaps not a good choice. Because RRs were close to 1.00, changing the calculation method could change the significance (see notes g and j to Table 3). However, these choices were made *a priori*, and there was no intention to bias the conclusion, which indeed contradicts the authors starting opinion.

Could the artificial use of a potent carcinogen, or of a germline mutation, be the cause of the poor predictivity of rodent models? In Newmark's model, normal mice were fed a "Western diet", which contains high fat and phosphate, and low calcium, vitamin D, fibres, folic acid and vitamin B12. Eighteen months later, spontaneous colon tumours were observed in five mice out of twelve (98). Could this model be the ultimate one to predict tumour prevention in humans, as advocated by Bruce (99)? This notion is a distinct possibility, because, like in humans, the addition of calcium (and vitamin D) to the diet reduced tumour incidence in mice (98).

Animal studies may "predict" what happens in humans. Here are two examples from our laboratory. The first example is the serendipitous discovery that polyethylene glycol (PEG) is a potent chemopreventive agent in rats (100). Four years later, a population study showed that humans taking PEG-based laxatives have only half the risk of developing colorectal adenoma compared to non-users (101). Another example is beef meat promotion of carcinogenesis in rats. According to epidemiological studies (102) consumption of beef has been suggested to increase colon cancer risk in humans. Tumour promotion by beef may be mediated by myoglobin haem iron, and is fully inhibited by a high calcium diet (103). These data prompted the authors to ask epidemiologists to re-evaluate cohort results. Such evaluation showed that high calcium intake was associated with a stronger protection in those eating high levels of red meat than in those eating less than 25 g red meat/day (A. Flood, unpublished observation).

Well known agents such as aspirin might perhaps not have been the best ones to be subjected to this analysis, since they seem to afford only modest protection in rats and in volunteers. One may surmise that the most potent agents discovered in animal studies might afford consistent protection when tested in volunteers. Rodent models suggest that PEG, hesperidin, Bowman-Birk protease inhibitor, sphingomyelin, physical exercise, EGF-receptor-kinase inhibitor, (+)-catechin, resveratrol, fish oil, curcumin, caffeic acid phenetyl-ester and S-methyl-methane-thiosulfonate might well be efficacious preventive agents that have not yet been tested in humans (1, 18). However, the safety of giving a daily pill to thousand of healthy people for many years needs to be carefully evaluated prior to a trial (99), in order to avoid the negative results associated with beta-carotene and specific COX2 inhibitors (104).

In conclusion, how useful are the animal models? Do we have to agree with the letter sent by R. Greek and J. Greek to the Brit. Med. J. on 5 February, 2001? (Full text on http://bmj.bmjjournals.com/cgi/eletters/322/7281/248#12407) "Animals can only be proven to be “models” empirically. That is to say, we must know what happens in humans first, then study animals to see if a particular animal replicates the human condition… But this is a catch-22. We can only know which animal mimics humans after we know what happens in humans. But after we know

how humans respond there is no need to use animals. This gives us no new knowledge, is obviously not predictive, and thus obviates the need for animals."

Although one cannot disagree completely with the underlying sentiment expressed in this letter and has to admit that the empirical approach is necessary, rodent studies remain undoubtedly useful for the following reasons:

(i) To screen for potential chemopreventive agents, and to eliminate agents that have no effect or promote tumour growth. In Table 3, all the agents which decrease polyp recurrence in volunteers also decrease tumour incidence in rats. Agents with no effect in rats produced no effect in humans. However in this demonstration tumour promoters have been omitted: no agent which promotes tumours in rodents has ever been tested in humans. It may therefore be prudent to use rodent models as screening tools: agents which turn out to be inefficacious or tumour-promoting in rodents should not be tested in humans. An appropriate role for animals in cancer chemoprevention is thus the “initial screen”. Such screens may well discover as yet unknown potent chemopreventive agents like PEG (1, 100).

(ii) To allow the study of mechanisms. Invasive procedures and use of toxic compounds pose less ethical problems in rodents than in humans. Less time and money are required to test a hypothesis in rodents than in humans. Mice with modified or knocked out genes can be constructed to directly test some hypotheses. However one has to bear in mind that the relevance for humans of mechanisms found in rodents is doubtful if not validated in humans. For instance, attractive mechanisms explain how wheat bran prevents carcinogenesis in rats (105), but human trials show that wheat bran does not prevent colorectal adenoma.

(iii) To help identify new biomarkers and novel target genes. These can subsequently be detected in humans. For instance, ACF were first identified in the rat colon exposed to carcinogens (106), and they have subsequently been identified in the human colon. The numbers of ACFs increase with increasing risk of colon cancer, and they represent an attractive target for intervention (107). Also, novel gene targets were identified in human tumours on the basis of evidence collected from transcriptional profiles in Min mice (108).

Finally, this meta-analysis suggests that rodent models roughly agree with human data, but do not predict accurately the efficacy of all chemopreventive agents in humans. Human beings will however not be able to find new ways to prevent cancer without the help of animal models.

Conflict of interest statement

None declared.

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