

Point/Counterpoint

Counterpoint: From Animal Models to Prevention of Colon Cancer. Criteria for Proceeding from Preclinical Studies and Choice of Models for Prevention Studies¹

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Abstract

Corpet and Pierre (D. E. Corpet and F. Pierre, *Cancer Epidemiol. Biomark. Prev.*, 12: xx-xx, 2003) have reviewed the prevention studies made with the azoxymethane rat and Min mouse colon cancer models, and have shown that many agents reduce the numbers of these experimental tumors. They suggest that agents with preventive activity with little or no toxicity should be evaluated in clinical intervention studies without delay. I think that the decision to proceed to a clinical trial is more complex, and involves an understanding of the safety of the agent and of the strength and consistency of the preclinical data. However, I am also impressed by the wide range of agents that have been found to affect the development of colon cancer in animals. This suggests that human colon cancer may be the consequence of many different dietary and lifestyle deficiencies, a view supported by the observation that normal mice develop colon cancer when fed diets deficient in several food components known to prevent tumors with the azoxymethane rat model [Newmark, H. L. *et al.*, *Carcinogenesis* (Lond.), 22: 1871–1875, 2001]. There is a clear need to evaluate the preventive effects of additional combinations of these agents, identified perhaps from the Corpet and Pierre review (D. E. Corpet and F. Pierre, *Cancer Epidemiol. Biomark. Prev.*, 12: xx-xx, 2003), or from actual human high-risk diets. With diets that increase the risk of “spontaneous cancer” in hand, the stage would be set for assessing the most effective ways to reduce colon cancer risk, again first with animal studies, then clinical trials, and then perhaps population studies.

Introduction

Corpet and Pierre (1) have reviewed the many animal studies that have tested dietary and pharmaceutical agents for their ability to prevent colon cancer. They focused on two animal

study models, one based on colon carcinogenesis in rats treated with the colon carcinogen, AOM³ (2), and the other based on carcinogenesis in mice with a defective *Apc* gene, such as Min mice (1). The results of experiments with these two models are clearly summarized in their tables and web pages. It is evident that many agents inhibit colon carcinogenesis in one or both models. Some, notably calcium (3) and possibly nonsteroidal anti-inflammatory drugs (4), reduce the recurrence of colonic polyps in clinical trials, suggesting that they may be important in colon cancer prevention. Others, including exercise (*e.g.*, Ref. 5), fish oil (6), curcumin (7), catechin (8), and PEG 8000 (9), inhibit colon carcinogenesis in animals, have no apparent toxicity in rodents or humans, but have not yet been tested for clinical activity. The point of Corpet and Pierre (1) is that these agents should be tested quickly for their activity in clinical intervention studies.

The review by Corpet and Pierre (1) is thought-provoking, and their impatience with the apparent delay in the application of possibly active preventive agents is understandable. However, my conclusion on viewing this array of results is different from theirs. First, I think that there may be good reasons for the delay in proceeding from preclinical investigations to clinical trials; the criteria for proceeding from the results of animal studies to the conduct of clinical trials requires additional consideration. Second, I think that the perspective provided by the review points to possible important future studies; it suggests that active agents should be combined and optimized, first in animal studies and then in clinical trials.

Criteria for Proceeding from Preclinical Investigations to Clinical Trials

Corpet and Pierre (1) suggest that agents that show clear evidence for preventive activity in an experimental model and that have no or limited toxicity should be considered for clinical intervention studies without delay (1). I think this underestimates the complexity of the decision making required before initiating a clinical trial. The clinical investigator in prevention studies hopes to expend effort in an optimal way, that is, to identify possible important interventions for cancer prevention without compromising the health of any subject. This involves two extrapolations. The first concerns the safety of the intervention agent administered over a period of years. Frequently, information on human safety is incomplete, and it may be necessary to generate it with Phase I and II studies before proceeding to a more definitive clinical trial. The second concerns the extrapolation from the results of animal intervention studies. It is well known that there are significant differences in

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³ The abbreviations used are: AOM, azoxymethane; PEG, polyethylene glycol; ACF, aberrant crypt foci; FAP, familial adenomatous polyposis; NWD, New Western Diet.

the response of different species of animals and of humans to chemical carcinogens (10), and that there are clear differences in the genetic pathways to cancers in mice and humans (11). This is, of course, the reason for the trial.

The agents Corpet and Pierre (1) identify as active and suggest as ready for clinical trials differ substantially in the degree to which they are known to be safe for study subjects. Some, like exercise and fish oil, are “really safe” with long use by humans and with epidemiological evidence indicating that they are protective (12–15). Others have been used widely in humans and are “probably safe,” although there may be surprises as there was with retinol and β -carotene (16). Still others are not routinely used in humans, so they are “not known to be safe.” Ideally only really safe agents would be used in trials, but agents that are probably safe, with properties that are well understood, have also been evaluated.

The agents Corpet and Pierre (1) suggest also differ substantially in terms of their strength and consistency as preventive agents. Some show a consistent preventive effect with both assay models; others have given inconsistent, conflicting results: different for lesions in the rat and mouse colons, and/or different for the colon and small intestine of the mouse. Clearly, the consistency of the effect of an agent and the amount known about its action can affect how it can be tested. I think that active agents that are well understood and give consistent protection across the animal models could be tested without delay providing there is also evidence that they are really or probably safe. Agents with conflicting protection results, if they are known to be really safe, do not pose a problem. But agents with conflicting protective effects and with less safety information worry me. That unexplained difference in protection for the two assays may be a result of a mechanism that could lead to problems of increased tumor growth in the treated arm of a clinical trial.

Consider the following example taken from the review (1). PEG, molecular weight 8000 (PEG 8000) is probably safe and markedly reduces tumor development in AOM-initiated rats (1, 2), but it has been reported to increase tumor development in the colons of Min mice. It has been argued that PEG should not be used in clinical trials because of the known importance of *Apc* mutations in the development of human colon cancer and the lack of understanding of the colon tumor promotion effect in Min mice. PEG could in some way promote the loss of heterozygosity of the *Apc*(+/-) cells or the growth of clones of cells with such mutations. Corpet and Pierre (1) are now arguing that the results with the Min mice should be disregarded on the basis of the other conflicting data with the Min model. I think that it is illogical to use some of the results obtained with the assay and then to ignore the Min data here, without first gaining an understanding of the mechanism responsible for the protective effect of PEG in the rat and the promotional effect in the Min mouse colon. An alternative approach might be to bypass this problem by obtaining additional epidemiological evidence on the safety of PEG. Perhaps this could be done with a case-control study of polyp or cancer case subjects, by asking for the use or not of PEG consistently as a laxative. This might be practical in Europe where PEG appears to be more widely used than in North America. I think that, with either an explanation of the discordant Min data or with evidence of the long-term safety of PEG, there would be less worry of a promoting effect of PEG in clinical trials, and it would be reasonable to test the efficacy of PEG 8000 in a clinical trial in subjects with spontaneous polyps and with a polyp recurrence reduction end point or an ACF assay.

There are, of course, many agents that appear to be con-

sistently active with the models that Corpet and Pierre (1) have reviewed, that many of us would consider “really safe.” Most of us, I think, would agree that supplements, at appropriate dosage levels, of calcium, vitamin D, cellulose, fish oils, and other oils containing n-3 fatty acids, B vitamins, and organically bound selenium fit the category, and could be additionally promoted in public health interventions if clinical intervention show that they are protective. Should we not be sure that such safe interventions are tested for their possible protective effects in humans first before proceeding with pharmaceutical agents with less certain safety? In raising this priority I do not imply that we should not be interested in mechanisms of chemoprevention or of prevention strategies for very high-risk subjects.

Choice of Experimental Models for Colon Cancer Prevention

Corpet and Pierre (1) reviewed the results of the two animal models that are the most widely used for identifying colon cancer-preventive agents. Are they appropriate for simulating the development of colon cancers in humans?

The AOM rat model, which has been used so extensively in cancer prevention studies, is based on earlier epidemiological studies with the cycad nut and carcinogenesis studies of cycasin (17). In the classic form of the model described by Reddy (18), AOM is given to rats as two i.p. injections 1 week apart, the animals are randomized to control and experimental diets 1 week later, and the animals are scored for number of colonic tumors 40 weeks later. An earlier end point, the putative cancer precursor ACF, can be scored for size earlier, at 14 weeks (19), and appears to give similar results (2). These protocols assess the promotional or protective effects of the experimental diets and when followed closely provide data that is quite reproducible. AOM is a methylating agent that results in a great loss of colonic cells by apoptosis, an increase in proliferation, and an apparent increase in mutations of colonic epithelial cells (20). How do the conditions of these experiments correspond to those in human colon carcinogenesis? The most obvious difference is the deliberate exposure of the rat colon to a carcinogen, AOM, although the human colon may well be chronically exposed to carcinogens of different types. In humans, there may be a generalized increase in colonic proliferation, although this has not always been clearly observed (21), and the mechanism responsible for increased proliferation is not necessarily a carcinogen exposure. The AOM model, as usually used, only examines the later steps in colon carcinogenesis. It does not assess the effect of the diet on the early steps of colon carcinogenesis, mutation, increased proliferation, and initiation, steps that can proceed to the changes in molecular circuitry characteristic of cancer.

The Min mouse model as first described by Moser *et al.* (22) would appear to provide a better model of human colon carcinogenesis, at least for patients with FAP with a defective *Apc* gene (23). But there are again problems. The disease in the Min mouse develops in quite a different way from that in the human. Patients with FAP have a long period of years in which the colon appears normal after which ACF and adenomatous polyps appear before there is any evidence of carcinoma (24). In the Min mouse ACF are not readily observed, tumors can arise very soon after birth and, as noted, are seen primarily in the upper gastrointestinal tract (1). There are, of course, now many models of FAP-like disease with different mutations in the *Apc* gene (25). Unfortunately none simulates the pattern of disease in humans accurately. Perhaps more importantly, there is a genetic limitation to the Min model. In the Min mouse, it

is a loss of heterozygosity event that leads to the loss of *Apc* function and increased risk of intestinal polyps (26). In human colorectal neoplasia, somatic *Apc* mutations are frequent as the “second hit” in FAP (27), and as either “hit” in sporadic polyps and cancers (28).

Thus, although the AOM rat and Min mouse models have proved to be useful, AOM is not the carcinogen to which humans are exposed, and the model may only reflect the later steps of carcinogenesis. The Min mouse develops colon tumors quite differently than humans with a consistent loss of heterozygosity at the *Apc* gene rather than the somatic mutation at this site in sporadic human colorectal neoplasia, and neither effectively assesses the effects of initiating agents. Ideally we would like a rat or mouse model with colon tumors resulting “spontaneously” from a “bad lifestyle.” It would be nice if the diet was not extreme in terms of human exposure, and the diet led to cancer in a process involving the development of ACF, polyps, and cancers like that seen in humans. Of course the problem with such a model is defining a bad lifestyle. It represents, in part at least, the absence of the preventive factors for which we are looking, risk factors that we are still attempting to fully define.

However, Newmark *et al.* (29) have made an important step in defining a bad lifestyle mouse diet. They developed what they called a NWD that was based on the standard sucrose-based rodent diet, AIN-76 (30), modified to model certain aspects of the western diet. It has reduced calcium (with the usual levels of phosphate), vitamin D, cellulose, and folic acid (with other methyl donors and B₁₂ also reduced), and increased fat with n-6, not n-3 fatty acids. Newmark *et al.* (29) found that with this diet, C57 Bl/6 mice develop hyperproliferative and hyperplastic colonic epithelium at 4 months and colon tumors in 18–24 months. This was seen without any typical exogenous carcinogen and in animals that seldom develop colon cancer on the standard AIN-76 rodent diet.

It is interesting that nearly all of the components of the NWD diet had been assessed previously in colon carcinogenesis studies as tabulated by Corpet and Pierre (1) and Corpet and Tache (2). It is also interesting to note that high levels of sucrose that may be associated with colon cancer risk (31) could have been partially responsible for tumors, as dietary sucrose increases the mutation frequency of colonic epithelial cells (32). Epidemiological studies suggest further additions to these components of a bad lifestyle. The western lifestyle tends to lead to excess accumulated energy and evidence of insulin resistance that is associated with risk of colon cancer (33). The lifestyle may also be associated with an excess of proinflammatory, n-6, fatty acids and a deficiency of natural anti-inflammatory agents, antioxidants, other vitamins, and trace minerals. Studies with these additional factors could build on the experience with the NWD. It is possible that, with the addition of the further risk factors, a simulated western diet could result in colon cancer in mice and rats in <18 months, and of colon cancer precursors in an even more interesting length of time, perhaps as short as 3–6 months. Of course, an alternative approach to picking many individual risk factors is to feed the rodents the actual bad lifestyle human diets, a strategy that Liberman *et al.* (34) have piloted.

Prevention studies with such carcinogenic diets would have an emphasis different from those with the AOM rat or the Min mouse models. The objective would be shifted as the method to prevent these colon cancers is already known: return the animals to their initial low-risk rodent diets. The questions asked of the animal studies would instead be how do we assess the relative importance of the multiple risk factors associated

with the western diet? And how do we determine the minimum changes necessary to our high-risk diet to reduce colon cancer risk? The answers to these questions could be of great help, first in the design of future clinical trials interventions and then in the choice of interventions for future public health studies to reduce colon cancer incidence.

Animal studies with bad lifestyle diets might sound expensive, but there are savings to be made. A bad lifestyle with relationship to colon cancer may well overlap with a bad lifestyle for other cancers. It could explain the similarity of the risk factors for colon cancer with those of breast and prostate cancers (35). It may explain the remarkable associations that have been observed between markers of colon cancer and atherosclerotic cardiovascular disease risk (36). It could be responsible for the association of risk with that of insulin resistance and type 2 diabetes (37, 38). Indeed, it appears that the NWD, that first approximation to a bad lifestyle diet, affects breast histology in a manner similar to that seen in early breast carcinogenesis (39). We clearly want to develop interventions for colon cancer that are consistent with preventing breast cancer and other chronic diseases seen too frequently in those adopting a western-style lifestyle.

Finally we should note that the database developed by Corpet and Pierre (1) raises many interesting questions. They have already noted the questions raised by the unexplained discrepancies between the results of different animals carcinogenesis assays and how the answers could be keys to the identification of mechanisms of tumorigenesis. But also unexplained are why many of the agents are protective at all, and why so many apparently different protective agents are each able to prevent a large fraction of experimental colon cancers. Such questions attract the theoretician in us all. Certainly I found that model building was irresistible with even a first look at the animal prevention data (37). Clearly defined mechanisms can be tested and refined with experimental and clinical studies that combine effective agents and lifestyle changes to provide a mechanistic understanding of human colon carcinogenesis. Such an understanding would provide a solid basis for population interventions.

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Note added in proof: The author notes that a recent abstract for the 2003 AACR conference (Abstract number 879: Dorval ED *et al.*) provides evidence of the type suggested to justify a trial with polyethylene glycol.

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