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Are Animal Models of Colon Cancer Relevant to Human Disease

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Since its original description by Druckrey (1), the rodent model of colon cancer induced with 1,2-dimethylhydrazine has become widely used in the study of colonic carcinogenesis. At present, colonic tumors induced by DMH, or its metabolites, azoxymethane (AOM) or methylazoxymethanol (MAM), are the most popular experimental models of colon cancer. It is, therefore, appropriate to question whether the DMH animal models are relevant to human colon cancer. In order to answer this question we must actually ask two more specific questions. First, do the tumors induced by DMH or its metabolites have similar characteristics to those known for human colon cancers? While similarities in known characteristics of both DMH-induced and human colon cancers do not necessarily mean that other characteristics of the DMH models will hold for human colon cancer, major discrepancies in the features of DMH-induced cancer and human colon cancer would certainly make one more skeptical about the relevance of the DMH model to human disease. The second question we need to answer is whether the study of the animal model of colon cancer is more productive and more useful than the study of human colon cancer itself. After all, an animal model is only useful if it can provide more information than the human disease itself.

IS THE DMH MODEL OF COLON CANCER SIMILAR TO HUMAN DISEASE?

There are many well-documented similarities between the disease induced by DMH and human colon cancer. The pathologic features of DMH-induced colonic tumors span the entire spectrum of tumors seen in the human colon from tubular adenomas through polypoid and sessile carcinomas to mucinous adenocarcinomas (2–4). Studies of the kinetics of the colonic epithelial population have resulted in analogous alterations in the proliferative compartment in the animal model and in human colon cancer (4, 5). There are also similarities in colonic mucin synthesis in DMH-induced tumors and human colonic neoplasia (6).

There are several other features which cannot be compared between human colon cancer and the DMH models because of lack of data. Whereas a great deal is known about the metabolism of DMH and its metabolic conversion to the proximate carcinogen, the proximate carcinogen in human colon cancer is unknown. The finding that DMH-induced cancers are less frequent in germ-free animals and animals treated with antibiotics suggests an important role for the colonic flora in DMH-induced carcinogenesis; similar data is not available for human colon cancer. Similarly, selenium, ascorbic acid, disulfiram, *cis*-retinoic acid, and butylated hydroxyanisole have all been shown to inhibit the induction of cancer in the DMH models, but there is inadequate information to determine whether similar effects are found in human colon cancer. Certainly none of these features can serve as a basis for determining if the DMH model effects a disease similar to human colon cancer.

There are three areas of apparent discrepancy between the DMH-induced model and human colon cancer that we should examine in more detail: (1) the effect of dietary fat and fiber on colon cancer risk, (2) the presence of an adenoma as the precancerous lesion, and (3) the metastatic potential of colon cancer.

The association of human colon cancer with a high-fat, low-fiber diet, has been widely stated

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(7–9). Dr. Rogers has detailed the conflicting data regarding the effect of fat and fiber on the induction of colon cancer in the DMH models. Is there a serious discrepancy between the animal model and human colon cancer with regard to the influence of diet? I don't believe so because I don't believe that the human data is strong enough to prove an independent association between a high-fat diet or a low-fiber diet and colon cancer risk. There is clearly an association between per capita consumption of fat and national incidence rates of colon cancer (10). Case control studies, however, have not consistently shown such a relationship. While studies from Canada and Puerto Rico showed a positive correlation between fat intake and colon cancer, similar studies from the United States and Israel show no such correlation (11, 12). Indirect measures of fat intake, such as fecal bile salts, and neutral and acid steroids, also show a correlation with colon cancer mortality rate between nations, but again such a correlation has not been consistently shown in case control studies within nations (11, 12).

A similar story holds for the relationship between fiber intake and colon cancer risk. Epidemiologic data generally shows a positive correlation between a low-fiber diet and colon cancer risk (12). Case control studies from Israel and American blacks also show a negative correlation between fiber intake and colon cancer, but similar studies in Canada and Puerto Rico show no such correlation (12). Thus, the support for a high-fat or a low-fiber diet as the cause of colon cancer in man consists largely of correlative epidemiologic data. Although such data can provide important clues to the etiology of colon cancer, it by no means offers proof. Such clues must be directly tested prior to acceptance as truth. Thus, there does not seem to be a significant discrepancy at present between the DMH model and human colon cancer with regard to dietary influences.

It is generally, although not universally, accepted that most human colon cancers arise in benign adenomatous polyps. Several authors (5, 13, 14) explicitly state that such an adenoma–carcinoma sequence does not occur in the rat model of colon cancer induced with DMH. Such a discrepancy would cast serious doubt as to the usefulness of the DMH model in studying the premalignant mucosa in colon cancer. Again, I do not believe that a significant disparity between the animal model and human colon cancer exists with regard to the adenoma–carcinoma sequence. In this case, the data for the sequence in human colon cancer seems adequate, but the data suggesting that such a sequence does not occur in the DMH model is clearly inadequate. The arguments for an adenomatous polyp to cancer transition in the human are numerous and have been recently reviewed by Morson (15). The key elements in this argument are: (1) foci of intramucosal cancer are commonly seen in polyps but are extremely rare in normal mucosa; (2) there is clearly an incidence of invasive cancer in adenomatous polyps that increases with the size of the polyp; (3) although large colon cancers rarely contain evidence of residual adenomatous tissue, such tissue is commonly found in small colon cancers; (4) some apparently benign adenomatous polyps have developed into cancer; and (5) one study by Gilbertson and Helms (16) suggested that removal of polyps significantly decreased the subsequent colon cancer risk.

Thus, there is strong, if indirect, evidence to suggest that the adenoma–carcinoma sequence occurs in humans. Both Maskens (5) and Ward (13) strongly contend that DMH-induced rat colon adenocarcinomas arise *de novo* from flat mucosa and not from benign polyps. In both of these studies, high doses of DMH (20 or 26.6 mg/kg for 20 weeks) were used. Although polypoid adenomas were not observed by Maskens (5), focal areas of atypia that resemble flat adenomas were seen. In Ward's studies adenomas were observed; however, it was suggested that early neoplastic lesions (?flat adenomas) evolved into either polypoid neoplasms or adenocarcinomas rather than sequentially. The early mucosal lesions described appear indistinguishable from flat adenomatous change in the mucosa. On the other hand, Sunter et al (3) and Wiebecke et al (4) presented evidence that adenomas clearly develop in response to DMH in the rat. Their data were consistent with progression from benign adenomas to polypoid adenocarcinomas. Furthermore, studies by Haase et al (17) and Chang (18) are consistent with an adenoma-carcinoma sequence in the mouse model of DMH-induced cancers. Thus, the studies in the animal model appear to be inadequate to exclude the adenoma–carcinoma sequence, and there may not be a serious discrepancy between the animal models and human colon cancer in this regard. We have noted however, that early invasive experimental carcinoma can arise from polypoid lesions which histologically appear to be dysplastic and not polypoid carcinomas (unpublished observation).

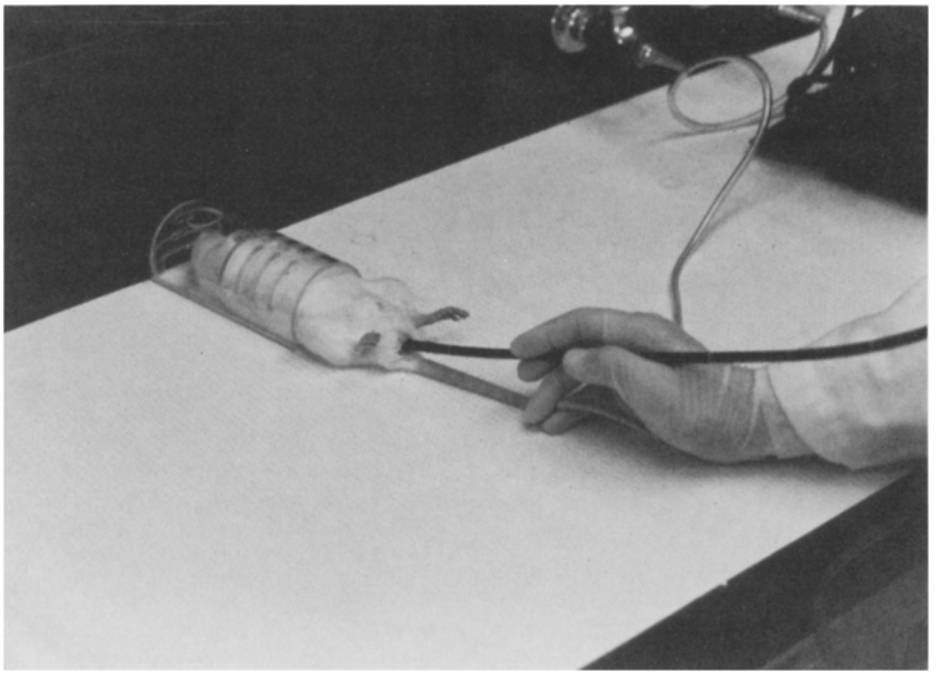

Fig 1. Distal colonoscopy in the rat is performed after pentobarbital anesthesia using a pediatric bronchoscope.

There does appear to be a significant difference between the animal models and human colon cancer with regard to metastatic potential of the colonic adenocarcinomas. In human colonic cancer, approximately 1/2 of patients have evidence of lymphatic metastases and 1/3 have evidence of hematogenous metastases at the time of presentation. With respect to lymphatic metastases, the usual pattern is an orderly progression of tumor through the lymphatic network from the pericolonic lymph nodes and progressively up the perivascular lymphatics (19–21). The usual route of hematogenous metastases is initially to the liver and subsequently to the lung. Initial hematogenous spread to the lung is unusual but can occur, particularly with rectal primaries (22). The incidence of hematogenous metastases increases with the stage of the primary tumor as well as with the histologic grade of the tumor (23). Most studies of the DMH models of colon cancer suggest a very low incidence of metastases. The tumors which are capable of metastasis are almost exclusively the mucinous adenocarcinomas of the proximal colon. The adenocarcinomas of the distal colon have not been shown to be capable of metastases (3, 5, 14, 15, 17, 18). In addition, the metastases that do occur are generally found in regional lymphatics or on the peritoneal surface. Whereas liver metastases are commonly found in the human disease, they are rarely, if ever, found in the DMH models. Thus, it appears that there is a major difference between the metastatic potential of DMH-induced cancers and human colon cancer. At the minimum, this difference implies that the DMH model, as it is currently used, does not appear to be relevant to the study of colon cancer metastasis.

IS IT MORE USEFUL TO STUDY THE ANIMAL MODEL THAN HUMAN DISEASE?

The second major question that relates to the relevance of the animal models of colon cancer is whether study of the animal models can be more productive than study of the human disease itself. It appears that in may areas the animal model is clearly more useful. Studies designed to assess the effect of dietary manipulation or chemoprevention can be done more efficiently and rapidly in the animal models than in human colon cancer. Studies of carcinogen metabolism are possible in short-term cultures of human mucosa, but serial observations or more long-term studies require an appropriate animal model. Similarly, studies of colon cancer promoters cannot be performed in the human. The animal model, however, is of more limited value in

the study of preneoplastic mucosa. The limitation of the models in this area stems from the necessity of sacrificing animals in order to make any observation about the characteristics of the preneoplastic mucosa. The need for sacrifice precludes the possibility of directly demonstrating that the preneoplastic mucosa would have subsequently developed into a colon cancer. The mere finding of a particular characteristic in the DMH mucosa prior to the development of identifiable cancers is insufficient to conclude that the alteration is a marker of mucosa at risk.

Because of these limitations, we set out to test the feasibility of making serial observations in the DMH model of colon cancer in the rat. We have determined that serial colonoscopy and biopsy (every two weeks) of the distal rat colon (Figure 1) can be performed in the rat. We have used a pediatric bronchoscope with its standard biopsy forceps. Not only can DMH-treated mucosa and tumors be observed endoscopically, multiple biopsies of the normal-appearing mucosa, as well as the tumors, can be performed safely. We believe that the ability to make serial observations will greatly enhance the utility of the rat model of colon cancer. Serial observations are important for the study of precancerous mucosa but are not limited to this area. Certainly, the analysis of any interventional therapy would be facilitated by serial observations of the effect of the intervention.

In summary, the rodent models of colon cancer induced by dimethylhydrazine and its metabolites appear to be a good but not ideal model of the human disease. Although the animal model and the human disease share many characteristics, the metastatic potential of adenocarcinomas induced by DMH appears to be significantly less than that of human colon cancers. This discrepancy makes the DMH model less than ideal and certainly inadequate for the study of colon cancer metastases. In most areas of cancer research, the animal model is more efficiently studied than the human disease and the ability to make serial observations should further expand the utility of the animal model.

REFERENCES

1. Druckrey H: Production of colonic carcinomas by 1,2-dialkylhydrazines and azoxyalkanes. *In* Burdette WJ (ed.) Carcinomas of Colon and Antecedent Epithelium. Springfield, Illinois, Charles C. Thomas, 1970, pp 267–279
2. Newberne PM, Rogers AE: Adenocarcinoma of the colon. Animal model of human disease. Am J Pathol 72:541– 544, 1973
3. Sunter JP, Appleton DR, Wright NA, Watson AJ: Pathological features of the colonic tumors induced in rats by the administration of 1,2-dimethylhydrazine. Virchows Arch B 29:211–223, 1978
4. Wiebecke B, Krey U, Lohrs U, Eder M: Morphological and autoradiographical investigations on experimental carcinogenesis in polyp development in the intestinal tract of rats and mice. Virchows Arch A 360:179–193, 1973
5. Maskens AP: Histogenesis and growth pattern of 1,2-dimethylhydrazine induced rat colon adenocarcinoma. Cancer Res 36:1585–1593, 1976
6. Filipe MI: Mucinous secretion in rat colonic mucosa during carcinogenesis induced by dimethylhydrazine. Br J Cancer 32:60–77, 1975
7. Committee on Diet, Nutrition and Cancer, National Research Council: Diet, Nutrition, and Cancer. Washington, DC, National Academy Press, 1982
8. Weisberger JH, Wynder EL, Horne CL: Nutritional factors in etiologic mechanisms in the causation of gastrointestinal cancers. Cancer 50:2541–2549, 1982
9. Walker ARP: Cancer and diet, with special reference to intakes of fat and fiber. Am J Clin Nutr 29:1417–1426, 1976
10. Armstrong B, Doll R: Environmental factors and cancer incidence and mortality in different countries with special reference to dietary practices. Int J Cancer 15:617–631, 1975
11. Kinlen LJ: Fat and cancer. Br Med J 286:1081–1082, 1983
12. Willett WC, MacMahon B: Diet and cancer. N Engl J Med 310:633–638, 697–703, 1984
13. Ward JM: Morphogenesis of chemically induced neoplasms of the colon and small intestine in rats. Lab Invest 30:505–513, 1974
14. Pozhariski KM, Likhachev AJ, Klimashevski VF, Shaposhnikov JD: Experimental intestinal cancer research with special reference to human pathology. Adv Cancer Res 30:165–237, 1979
15. Morson BC: The pathogenesis of colorectal cancer. Philadelphia, WB Saunders, 1978
16. Gilbertson VA, Nelms JM: The prevention of invasive cancer of the rectum. Cancer 41:11–37, 1978
17. Haase P, Cowan DM, Knolls JC, Cooper EH: Evaluation of dimethylhydrazine induced tumors in mice as a model system for colorectal cancer. Br J Cancer 28:530–543, 1973
18. Chang YL: Histogenesis of symmetrical 1,2-dimethylhydrazine-induced neoplasms of the colon in the mouse. J Natl Cancer Inst 60:1405–1418, 1978
19. Buckwalter JA, Kent TH: Prognosis in surgical pathology of carcinoma of the colon. Surg Gynecol Obstet 66:611, 1938
20. Grinnell RS: Lymphatic metastases of carcinoma of colon and rectum. Ann Surg 131:494–506, 1950
21. Coller FA, Kay EB, MacIntyre RS: Regional lymphatic metastases of carcinoma of the rectum. Surgery 8:294–311, 1940
22. Brown CE, Warren S: Visceral metastases from rectal carcinoma. Surg Gynecol Obstet 66:611, 1938
23. Dionne L: The pattern of blood-borne metastases in carcinoma of the rectum. Cancer 18:775–781, 1965