Animal studies of colon carcinogenesis and altered epithelial cell differentiation

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ABSTRACT: Chronic inflammatory bowel disease (IBD) appears to predispose to subsequent colon cancer. Factors that influence the degree of this risk require definition since the reported incidence of malignant change varies widely. Differing environmental factors such as diet may be critical, and several approaches have been used to explore the role of specific variables in colon cancer pathogenesis; one has employed the use of animal models. Naturally occurring models of colon cancer exist including cotton-topped tamarins with colitis. Best studied, however, are animal models of colon cancer induced with specific chemical carcinogens. Cycasin and hydrazine derivatives, eg, 1,2-dimethylhydrazine, are most widely used. After parenteral administration of an active carcinogen, metabolic activation occurs, resulting in colonic adenocarcinomas. Sessile and polypoid neoplasms may be induced, particularly in the distal colon, similar to human colon cancer. Using this model, the effect of differing dietary and therapeutic variables has been explored. Studies with purified single dietary fibres, such as microcrystalline cellulose and hemicellulose, but not pectin, have demonstrated reduced numbers of colonic tumours; these in vivo observations correlate with in vitro effects of fibres on rat luminal and fecal mutagenic activities. Specific therapies used in IBD have also been evaluated – metronidazole, for example, a bacterial mutagen, enhances the development of chemically induced rodent colon cancer. In addition, a significant increase in colonic tumour development occurs after intestinal resection or bypass, two procedures used in the surgical management of IBD. In this setting, surgical sutures, particularly nonabsorbable materials including stainless steel, may play a critical role. Although the extent and duration of disease in patients with chronic IBD may be important in colon cancer pathogenesis, other variables, including diet and treatment, may be critical modulating factors. **Can J Gastroenterol 1990;4(7):372-377** *(pour résumé, voir page 373)*

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CHRONIC INFLAMMATORY BOWEL disease (IBD) appears to predispose to subsequent colon carcinoma (1). Although risk seems to be greatest for some individuals with longstanding pancolitis (2), the precise magnitude of this risk in specific populations or geographic areas is not clear. Indeed, some studies from referral centres may have overemphasized colon cancer ask in patients with ulcerative colitis. For example, high rates reported from the Mayo Clinic in Rochester (2) and Mount Sinai Hospital in New York (3) contrast with lower rates in published studies even from the same areas (4,5). Risk for cancer in colitis may also reflect geographic variables. Studies from the United States (2,3) and the United Kingdom (6) reporting high risks contrast with lower risks from other countries. In Copenhagen, 783 patients were observed for a median period of 6.7 years and colonic cancer was seen in only seven with a calculated cumulative risk after 18 years with ulcerative colitis of only 1.4% (7). Similar data were recorded by Gilat et al (8) from Tel Aviv, who followed 504 patients with ulcerative colitis for a mean period of 7.54 years. A very low incidence of colonic carcinoma of 0.6% was noted, comparable to a similarly low incidence of 0.5% from Prague, Czechoslovakia (9). Thus, the incidence of malignant

Etudes chez l'animal de la carcinogenèse colique et de la différenciation altérée des cellules épithéliales: Application possible aux MII

RESUME: Les maladies inflammatoires de l'intestin (MII) semblent prédisposer au cancer colique. La survenue des transformations malignes étant rapportée de façon très diverse, il est nécessaire de définir les facteurs qui influent sur le degré de risque. Divers facteurs d'environnement tels que l'alimentation peuvent être d'importance essentielle et plusieurs approches, parmi lesquelles l'expérimentation sur l'animal, ont servi à explorer le rôle de certaines variables spécifiques dans la pathogenèse du cancer colique. Il existe des modèles de cancer colique survenant naturellement chez l'animal – les tamarins pinches atteints de colite notamment. Ceux qui se prêtent le mieux à l'étude sont toutefois les modèles animaux de cancer colique provoqué par des carcinogènes chimiques spécifiques. La cycasine et les dérivés de l'hydrazine tels que le diméthylhydrazine sont les plus utilisés. Ces carcinogènes sont administrés par voie parentérale et leur activation métabolique chez l'hôte donne lieu à des adénomes coliques. Des néoplasmes sessiles ou polypoïdes similaires au cancer colique chez l'homme peuvent ainsi être provoqués, surtout à hauteur du côlon distal. L'effet de diverses variables d'ordre alimentaire et thérapeutique a ainsi été étudié. Des études utilisant des fibres alimentaires uniques purifiées telles que la cellulose microcristalline et l'hémicellulose, mais non la pectine, ont démontré un nombre réduit de tumeurs coliques; ces observations in vivo révèlent qu'il y a corrélation entre les effets in vitro des fibres et les activités mutagènes luminales et fécales étudiées chez le rat. Les traitements spécifiques utilisés dans les MII ont été également évalués. Le métronidazole, par exemple, un mutagène bactérien, favorise le développement du cancer colique produit par les agents chimiques chez les rongeurs. De plus, on note une augmentation significative du développement tumoral au niveau du côlon après résection ou dérivation intestinale, deux procédures utilisées dans le traitement chirurgical des MII. En l'occurence, il se pourrait que les sutures jouent un rôle essentiel, surtout lorsqu'elles se composent de matériaux non absorbables comme l'acier inoxydable. Bien que l'étendue et la durée des entéropathies inflammatoires chroniques soient importantes dans la pathogenèse du cancer colique, d'autres facteurs telles que l'alimentation et les variables thérapeutiques pourraient intervenir de façon déterminante.

change in ulcerative colitis may vary widely in different population groups; the reasons for the different incidence rates are unknown. Geographic variables may be important, possibly reflecting the influence and interaction of differing genetic and environmental factors thought to be critical in the development of de novo colon cancer unrelated to chronic IBD. A number of approaches have been used to explore the role of specific variables in the pathogenesis of colon cancer; both naturally occurring or experimentally induced animal models of carcinogenesis may be employed.

COTTON-TOPPED TAMARIN MODEL

Colon cancer was initially observed in a colony of cotton-topped tamarins (*Saguinus oedipus*) housed in Oak Ridge, Tennessee (10). These new world primates were native to South America, largely northern Colombia, and were subsequently imported to the United States and Europe for research purposes as well as zoological displays. Since the initial identification of colon cancer in the cotton-topped tamarin over 100 additional animals have been described with colonic adenocarcinoma from several animal colonies in different countries (11). These findings have been considered strongly suggestive of a largely genetically determined neoplasm in this animal species. After the initial recognition of colon cancer, the observation of underlying colitis was also recorded (12).

Emaciation, palpable tumours and lymphadenopathy may be present and barium contrast studies may reveal suspect neoplastic lesions. The tumours may vary in size from microscopic foci to large 5 to 6 cm tumours, and these can apparently arise de novo from mucosal epithelium without prior adenomatous polyp formation; often an acute, chronic or quiescent form of colitis is present. A very high percentage of tamarin colon cancers appear to metastasize to regional lymph nodes as well as other tissues, including lung. Metastases to the liver are apparently rare, possibly due to some anatomical differences in the lymphatic drainage of the large bowel and the apparent lack of portal vein invasion. The colon cancers have been observed in both imported and colony-born cotton-topped tamarins from both sexes, and their intracolonic distribution approximates 60% from cecum to splenic flexure and 40% from splenic flexure to the rectum (13). The histology of colon cancer occurring in the cotton-topped tamarin has recently been reviewed (14). The carcinoma is most typically poorly differentiated, although periodic acid Schiff stains reveal mucin-producing malignant cells. Pre-existing colitis is usually present, cancers are often multicentric and, interestingly, multiple ileal sites may also show carcinoma.

RODENT MODELS

Despite the lengthening list of natural and experimentally induced animal models of IBD detailed elsewhere (15), superimposed development of colonic cancer in rodent models is exceedingly rare. Carrageenan, a sulphated polysaccharide fibre (in contrast to other forms of dietary fibre) derived from the red seaweed, has been associated with the development of an inflammatory process in the intestinal tract of mice, guinea pigs, rabbits and primates (16-18). Following oral administration, a clinical-pathological syndrome similar to human ulcerative colitis results, except that histological changes are reported to be localized to the cecum. Interestingly, chronic carrageenan administration in the presence, or even absence, of a chemical carcinogen may induce colorectal cancer in these animals (19,20).

Other sulphated products of high

molecular weight, including sulphated amylopectin (21) and sodium lignosulphonate (22) cause lesions similar to those observed in human ulcerative colitis in the cecum and proximal colon. Recently, colon cancer was noted to develop in rats following induction of chronic colitis with 2% dextran sulphate (23). At three months, two of eight rats had carcinoma in the cecum and/or ascending colon. At six months, 11 of 13 rats had carcinoma in the cecum and/or ascending colon and one of 13 had carcinoma of the rectum.

EXPERIMENTALLY INDUCED COLON CANCER

Different chemical and physical agents have been used to induce de novo colonic neoplasia in experimental animals (15). No agent has produced effects exclusively in the colonic epithelium or been completely reproducible in different animal species. The colonic mucosa of rats, hamsters and mice is highly sensitive to tumour induction with certain chemicals, particularly 1,2-methylhydrazine; in contrast, other agents produce tumours in the colon only incidentally (15). Normally, small laboratory rodents have a very low rate of spontaneous colon tumour development (24). Tumour induction depends on the specific agent used, dosage, route of administration, and time of autopsy following administration of the carcinogen. In addition, the species, strain, degree of inbreeding, and sex of the animals are important variables. Besides chemical agents, colonic neoplasia may be induced in experimental animals with ionizing radiation administered in different forms (25,26).

Chemical induction of colon cancer in experimental animals was initially reported in 1941 using a cholanthrene derivative (27). Similar results were later reported using several other structurally different compounds classified into various categories, including: cholanthrenes, eg, 3-methylcholanthrene; aromatic amines, eg, 2,3-dimethyl-4-aminobiphenyl compounds; alkylnitrosamides and nitrosourea compounds, eg, *n*-methyl-*n*-nitro-*n*-nitrosoguanidine; aflatoxins, eg, aflatoxin B_1; and cycasin and hydrazine derivatives, eg, 1,2-dimethylhydrazine and azoxymethane (28). The best studied, most potent and most selective agents are the hydrazine derivatives.

In 1963, colorectal adenocarcinoma was first observed in experimental animals administered cycad meals derived from a tropical plant, *Cycas circinales* (29). Subsequently, cycasin, the glucoside derivative of methylazoxymethanol, was observed to be the critical carcinogen in cycad meals (30). In 1967, intestinal tumours were induced in rats with the symmetrical hydrazine derivative, 1,2-dimethylhydrazine (31). Dimethylhydrazine is believed to be metabolically activated within the host to an active carcinogen, ie, it is a procarcinogen (32). It is thought to undergo at least two hepatic oxidation reactions, although nonhepatic tissues may also be involved (28). The first oxidation step is thought to result in azomethane, an agent expired by the host animal in its respiratory gases. The second oxidation step converts azomethane to azoxymethane, another agent commonly used in experimental colon carcinogenesis. Azoxymethane is then *n*-hydroxylated to methylazoxymethanol, which may decompose spontaneously or be altered by certain tissues enzymatically. In vivo studies indicate that an alkylating intermediate, methyldiazonium, is formed during this decomposition. Methyldiazonium or a subsequent derivative appears to be capable of methylating cellular nucleic acids, suggesting a possible explanation for the mutagenicity of these agents. Intraluminal factors may play some role, possibly after binary excretion of one or more carcinogen derivatives (28). Luminal bacteria seem to be involved, since tumour production is decreased in germ-free rats treated with dimethylhydrazine, but increased in those administered azoxymethane. In addition, carcinogen may be delivered to colonic cells by the blood stream.

Dimethylhydrazine produces tumours in the small and/or large intestine. In the small intestine, most tumours are found in the proximal few centimetres, often near the region of the bile ducts, providing additional evidence that either bile itself or some component excreted into bile or pancreatic juice is important. Tumours in the small intestine are polypoid or sessile lesions and locally invasive. In the large intestine, rat epithelial tumours are very similar to human neoplasms in both histologic type and distribution. These are pedunculated or sessile, and exhibit varying degrees of cellular atypia and mucus content. Most tumours are found in the distal colon, although they may also occur in proximal colon; frequently, tumours are multiple. Most have tubular or tubulovillous elements; serial sections may reveal local invasion through the muscularis mucosa. Small microulcerations occur on the surface of colonic tumours and, occasionally, large ulcerations may be observed with malignant epithelial cells, connective tissue elements, inflammatory exudate and variable amounts of necrotic debris. Occult luminal blood loss may be detected along with frank rectal bleeding. Most invasive tumours are well differentiated adenocarcinomas, particularly in the more distal colon. These neoplasms form tubular, acinar or papillary structures, and often the tumours are nonuniform in structure with occasional foci of poorly differentiated cells. Some tumours produce serosal umbilication. Pedunculated masses may lead to intussusception and obstruction; protrusion through the anal verge may occur. More advanced lesions may involve local lymph nodes, pericolonic fat and mesentery, although metastases to more distant sites, including the liver or lungs, is less common. While most carcinomas occur in the distal colon, administration of lower dosages of carcinogen reportedly produces an apparent shift in distribution to the more proximal colon. Even with large dosage schedules, however, tumours can be found in the proximal colon (33).

In this model, mucinous adenocarcinomas are also observed. These tend to occur more commonly in the proximal colon, parallelling the distribution of mucinous or colloid-type adenocarcinomas in humans (34).

TABLE 1
Intestinal tumours in resected or bypassed rats

Group	Mean tumours per animal	
	Small bowel	Large bowel
Control	0.20±0.14	0.87±0.22
Transsection	0.33±0.13	0.87±0.34
Resection	0.53±0.21	1.76±0.34*†
Bypass	1.24±0.42*	2.94±0.66*†

*Based on reference 56. Animals received 1,2-dimethylhydrazine and the surgical procedures included transsection with end-to-end reanastomosis, 50% jejunoileal resection, or 50% jejunoileal bypass. Results are expressed as mean± SE. *P<0.05 compared to control group. †P<0.05 compared to transsection group*

Usually these tumours are sessile masses, often ulcerated, and may be associated with mucosal and/or submucosal lymphoid aggregates. In these tumours, clusters of epithelial cells tend to form glandular structures. Large ductular spaces and varying amounts of non-epithelial stromal elements and inflammatory cells may be present; the most prominent features of these tumours are considerable amounts of extracellular mucus that appear to distend glandular structures. All muscle coats and serosa may be involved with these lesions, as they tend to be less well differentiated and more invasive. Undifferentiated or signet ring cell types with intracellular mucus also occur but are rare.

Scanning and transmission electron microscopy as well as freeze fracture techniques have provided more refined ultrastructural descriptions of these colonic tumours induced with hydrazine compounds (35,36). Tumour cells, although highly variable in size and shape, tend to be smaller and more rounded than normal colonic epithelial cells, and their microvilli appear to be fewer, blunted and smaller. In addition, fewer apical surface membrane particles are found than particles associated with the microvilli of surface membranes of normal colonic epithelial cells. Fewer tumour cell surface mucopolysaccharides seem to be present as assessed by ruthenium red staining, compared to normal colonic epithelial cells. Other histochemical studies have revealed reductions in total mucus content of the tumours and altered staining reactions, similar to findings in humans (37). Chemical and biochemical observations in dimethylhydrazine-treated animals indicate that the carbohydrate content of colonic tumours is reduced compared with non-cancerous colonic mucosa from both normal and carcinogen-treated rats (38). In addition, reduced activities of glycosyltransferases are seen, and the activities of glycosidases are reduced or remain unaltered in colonic tumours (39).

The relevance of synthetic and natural hydrazine compounds to human cancer is not known, although these are present in the environment as industrial and food contaminants (40,41). 1,1-Dimethylhydrazine is present in tobacco plants, although a correlation between smoking and colon cancer is not evident (40,42). Hydrazines are found in wild and cultivated mushrooms including *Gyromitra esculenta* and possibly some *Helvella* species (40,43). *n*-Methyl-*n*-formylhydrazine may be converted to methylhydrazine under conditions analogous to those present in the human stomach (44). Hydrazine compounds may be found in rocket propellants while hydroxyethylhydrazine has been used as a ripener for plants (45,46). Hydrazine and its many derivatives are also commercially used in pesticides, herbicides, blowing agents for plastics and water treatment. Several of these hydrazine analogues can produce tumours in experimental animals (40).

STUDIES USING THE DIMETHYLHYDRAZINE MODEL

The dimethylhydrazine rodent model has been used to examine factors that influence the pathogenesis of colon cancer, including dietary and other therapeutic variables, that may be relevant in patients with IBD. Studies with pure dietary fibres, for example, were previously reviewed (47). Reduced numbers of colon tumours were found in animals fed different amounts of cellulose or hemicellulose, but not pectin (48-51). These in vivo effects also correlated with the observed in vitro effects of these fibres on rat fecal mutagens (52). Pharmacologic agents used in the treatment of IBD have also been evaluated in this model. Metronidazole, for example, an agent first reported for use in 1975 for IBD (53), particularly in perianal Crohn's disease, is known to be a bacterial mutagen (54). In one study, long term use of metronidazole was reported to enhance the development of chemically induced rodent colonic cancers (55). Besides drug therapy, patients with ulcerative colitis or Crohn's disease may require some form of surgical intervention during the course of their disease. Two procedures commonly used in the past in this setting have included resection or bypass of diseased intestinal segments. As very significant morphological and functional change may be observed in the residual intestine or bypassed loops of small bowel following these procedures, detailed studies of the effects of both procedures in carcinogen-treated rats were performed.

The incidence, distribution, size and histopathology of rat small and large bowel tumours induced by sequential administration of 1,2-dimethylhydrazine followed by either small bowel transsection, 50% jejunoileal resection or 50% jejunoileal bypass were examined (56). Table 1 shows some of the results from these studies. Even after limited small bowel resection or bypass, intestinal neoplasia was enhanced in both the small and large intestine of the rat. Additional studies were done to define possible mechanisms for changes in tumour incidence observed. No differences in transit times were detectable, but increased luminal (ie, bacterial) beta-glucuronidase activities in both the cecum and distal colon of the resected, but not bypassed, rats were observed. In addition, an apparent subsite redistribution of small bowel tumours to ileum and large bowel tumours to more proximal colon in bypassed rats further suggested that the mechanisms involved for this tumour enhancement differed substantially from those in resected rats. Interestingly, in this study, at least a portion of the increased numbers of tumours in the small bowel seemed to be related directly to the presence of anastomotic suture lines.

As a result of this observation, the effects of sequential administration of 1,2-dimethylhydrazine followed by cecal placement of one of six different types of suture materials were systematically examined (Table 2) (57). Slowly absorbed and/or nonabsorbable suture materials in the absence of a surgical anastomosis promoted local tumour induction in the rat cecum, a site within the rat colon where carcinogen-induced tumour induction is distinctly rare. In addition, cecal suture material composed of multifilament stainless steel wire enhanced tumour development at a 'downstream' site in the distal colon. This was associated with increased fecal (ie, bacterial) beta-glucuronidase activities indicating a possible luminal-mediated mechanism for distal colon tumour development in this animal model.

TABLE 2
Effect of cecal suture materials on tumour development

Group	Mean tumours per animal		
	Small bowel	Large bowel	Cecum
Control	1.7±0.5	5.5±1.3	0.2±0.1
Surgical gut	1.7±0.4	3.9±0.8	0
Polyglycolic	1.3±0.3	5.3±1.4	1.0±0.5
Polyglactin	2.3±0.4	4.1±0.4	0.9±0.3*
Surgical silk	1.6±0.4	4.8±0.5	0.8±0.3*
Polypropylene	2.0±0.6	6.7±1.1	0.7±0.3
Steel	2.4±0.5	9.4±1.4*	0.8±0.2*

*Based on reference 57. Animals received 1,2-dimethylhydrazine and a single cecal suture. Results are expressed as mean ± SE. *P<0.05 compared to control groups*

CONCLUSIONS

Although patients with extensive and longstanding ulcerative colitis appear to be at increased risk for subsequent development of colon cancer, the precise magnitude of this risk and factors that further influence or modify this risk require elucidation. Different animal models of colon cancer with pre-existing colitis are available for examination of variables that might influence disease pathogenesis but, in the past, carcinogen-induced animal models have largely been employed. These studies have demonstrated that specific dietary factors, medications (eg, metronidazole) and surgical procedures including small intestinal resection or bypass are important pathogenetic factors in the development of experimental colon cancer in this experimental animal model. Suture foreign body materials may also be important but direct extrapolation to conditions present in human intestine would be premature, especially since the surgical procedures used in these studies do not directly resemble operative methods ordinarily used in gastrointestinal surgery.

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