Trihalomethanes and Other Environmental Factors That Contribute to Colorectal Cancer

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Colorectal cancer is a major cause of morbidity and mortality in the United States; it is the third most common site of cancer in men after prostate and lung cancer, and the second most common site of cancer in women after breast cancer (1). Furthermore, the incidence of colorectal cancer is increasing in industrial countries (2). Because familial forms of colon cancer are thought to account for only about 15% of all cases (2), there is a strong likelihood that both environmental factors and inheritance play important roles in the pathogenesis of this disease.

The National Toxicology Program reported that trihalomethanes, which are by-products of water chlorination, induce colorectal cancers in rats (3). Epidemiological studies also suggest an association between consumption of chlorination by-products in drinking water and an increased risk for colorectal cancer in humans (4). Although disinfection of drinking water by chlorination has been a major disease prevention treatment, these findings raise an important public health concern because of the large number of people who consume chlorinated water. In recent years there has been extensive study of the molecular events involved in the development of human colorectal cancer. Consequently, the molecular genetics of colorectal carcinoma are among the best understood of any common human cancer.

To foster an exchange of information and ideas on potential interactions of environmental factors and molecular genetic events that may occur in the development of colorectal cancer, a workshop, "Trihalomethanes and Other Environmental Factors That Contribute to Colorectal Cancer," was held at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, 14 September 1993.

The objectives of the workshop were 1) to review the current status of research on the biology, epidemiology, and genetics of colorectal cancer, and 2) to identify areas in which further research would advance our understanding of the influence of genetic susceptibility and environmental factors (e.g., trihalomethanes) on the occurrence and development of this disease in humans.

Colorectal Carcinogenesis

An overview on the etiology of colon cancer, focusing on mechanistic relationships between factors identified through studies of the epidemiology, physiology, and molecular biology of this disease was presented. Increased colon cancer risk has consistently been associated with increased consumption of fat, meat, or protein, and the risk rises markedly with consumption of heavily browned meats. Decreased risk has been consistently associated with high consumption of vegetables (and perhaps fruit) and with physical activity. Cause-and-effect relationships are difficult to establish because of the multifaceted and incremental etiology of this disease. Arylamines produced in cooked meats may provide a link between diet and genetic susceptibility because the metabolism of these compounds to DNA-reactive intermediates is genetically variable. Colon cancer risk is increased in individuals with high N-oxidation (hepatic P450IA2) and high N-acetylation (colon NAT2) activities. A K-ras mutation at codon 12 is consistent with the formation of an arylamine adduct as an early event in the multistep carcinogenic process. Other than family history, where two genes have been identified (APC and hMSH2), there is much uncertainty on how to relate population risk factors and physiology to molecular genetic events in colon cancer.

The molecular biology of colorectal neoplasia has been studied intensely because this is a relatively common tumor with well-recognized environmental and familial aspects and because precursor lesions are easily identifiable and accessible. The model generally accepted for this disease involves changes in the normal epithelium to an abnormal state of proliferation and differentiation; this is followed by the development of benign neoplasms (adenomas), of which a small subset increases in size and degree of dysplasia. Eventually, some of these lesions progress to infiltrating adenocarcinomas. Genetic changes along this sequence involve a combination of alterations in oncogenes which drive cellular proliferation and in tumor-suppressor genes which lead to dysregulation of normal growth control. Germline mutations in the APC (adenomatous polyposis coli) gene are responsible for familial adenomatous polyposis, a syndrome characterized phenotypically by the development of hundreds to thousands of colorectal adenomas, with 100% occurrence of colorectal neoplasia if colectomy is not performed. Somatic mutations in the APC gene are frequent in sporadic colorectal tumors. The sporadic disease refers to those cases (about 85%) in which a well-recognized inherited basis has not been established. Currently there is no information on how environmental factors influence sporadic mutations in the APC gene. APC mutations are probably an early event in the development of sporadic neoplasia because they have been detected in aberrant crypt foci and in a high percentage of adenomas. Most mutations in the APC gene lead to truncation of the gene product. Mutations in K-ras and N-ras genes also occur at high rates in colorectal neoplasms. Because ras mutations do not increase in frequency as the adenoma to carcinoma sequence occurs, it is believed that they are typically early events.

High rates of deletion of the DCC (deleted in colorectal cancer) gene and high rates of mutations and deletions in the p53 gene have also been observed in human colorectal neoplasms. These are late events because they are uncommon in early adenomas and are increased in frequency in later phase adenomas and in carcinomas. Some carcinomas do not show any identifiable abnormalities in the DCC gene or in p53. One of the functions of the normal p53 gene product is to arrest cells with damaged DNA in the G1 phase of the cell cycle and thereby allow DNA repair before the cells progress to S-phase. Comparisons of genetic abnormalities in hereditary and sporadic colorectal cancer cases reveal nearly equal frequencies of APC and p53 mutations, whereas ras mutations may be slightly higher in cancers of patients with hereditary nonpolyposis colorectal cancer syndrome (HNPCC).

The replications error phenotype (RERT), characterized by instability in microsatellite repeat sequences throughout the genome, is present in the tumors of about 75% of patients with HNPCC and in only 10% of seemingly sporadic tumors. This instability has been attributed to a...
mutation in a gene (hMSH2) on chromosome 2 that results in a defect in nucleotide mismatch repair. Little or no information is available on how environmental factors interact at the level of the genome to affect the molecular genetic events in colorectal neoplasia.

The multiple intestinal neoplasia (Min) mouse characterized by Amy Moser of the University of Wisconsin in C57Bl6 mice possesses a nonsense mutation in the APC gene and is a potential model to study factors that influence hereditary colorectal cancer. Mice with a homozygous genotype die in early embryogenesis; however, heterozygous Min mice have a high incidence of colon cancer. Min reflects the human phenotype in that the average intestinal tumor load is greater than 50, all regions of the intestine are affected, and most of the intestinal lesions are adenomas with moderate dysplasia. Min mice have detectable intestinal tumors by 35 days of age and the average life span is 120 days. In backcrosses with other strains of mice, the F1 hybrid mice live longer and have fewer tumors than the Min+ C57Bl6 strain. This observation suggests that modifier genes of the Min phenotype exist. Treatment of Min mice with exogenous chemicals (e.g., ethyl nitrosourea) can further increase tumor development. Therefore, both environmental and genetic factors can affect the Min phenotype. Future studies should determine if environmental factors affect the progression of colorectal carcinogenesis or influence the expression of modifier genes of the Min phenotype.

Transforming growth factor α (TGFα) and TGFβ can act in an autocrine manner to regulate the proliferative status of intestinal epithelial cells, such that the net balance determines the growth state of the cell. TGFα is an epithelial cell mitogen, while activated TGFβ can override the growth stimulatory effects of TGFα, resulting in cessation of growth without inducing differentiation. As an integral membrane glycoprotein, TGFα has the potential to influence adjacent epidermal growth factor (EGF) receptor-containing cells. During the transformation process in colon epithelial cells, overproduction of TGFα or enhanced signaling through the EGF receptor may result in growth stimulation and/or there may be loss or a defect in the growth inhibitory pathways. If environmental chemicals bind to receptors of these growth factors, they may alter the growth of intestinal epithelial cells by perturbing the balance between the stimulatory and inhibitory signal transduction pathways. Though cancer cells may have an upregulation of TGFβ, the immunosuppressive properties of this factor coupled to the refractoriness of these cells to growth inhibition may contribute to the transformed phenotype. At least six ligands have the potential to bind to the EGF receptor and signal through that pathway. Because TGFβ expression is not uniformly increased in expression in colon cancers compared to normal epithelial tissue, perhaps other members of the family are also involved. Further, the selective induction of specific genes, such as the cyclooxygenase genes, by TGFα may also influence colon neoplasia. Upregulation of TGFα has been observed commonly after treatment with various tumor promoters.

Animal Studies on Trihalomethanes

Of the 435 chemicals studied for their carcinogenic potential in long-term rodent studies by the NCI/NTP, 14 showed evidence of colorectal carcinogenicity in rats. Two of these chemicals that caused marked increases in tumor incidence were the brominated trihalomethanes, bromof orm, and bromodichloromethane. Three other brominated chemicals caused a colon cancer response that was greater than 10%. Oral exposure to bromodichloromethane caused adenomas or adenocarcinomas in greater than 90% of the high dose male rats. This is a dramatic chemical effect because colorectal cancers are uncommon in untreated rats, occurring at a rate of approximately 0.1%.

The morphological characteristics of the colorectal epithelial tumors induced by the trihalomethanes are similar to those observed in human colorectal cancers. Nodules observed grossly on the mucosal surface were frequently multiple. Macroscopically there was a spectrum of lesions present in the distal colon and rectum; the earliest precursor lesion was focal atypical hyperplasia (corresponding to dysplasia) with a high mitotic index. These lesions progressed to adenomas and carcinomas exhibiting invasion into the muscularis mucosa. No metastases were observed in this study, and the tumor response was greater in males than in females.

Studies on the biotransformation of the trihalomethanes are in progress and pharmacokinetic models are being developed to characterize both the metabolic pathways that produce carcinogenic intermediates and the delivery of these materials to target tissues. Bromodichloromethane (BDCM) can be oxidized by cytochrome P4502E1 or cytochrome P4502B1 to phosgene, a highly reactive intermediate that acylates a variety of macromolecules or undergoes hydrolysis to carbon dioxide. The same enzyme system can act reductively to form bromide ion and the dehalogenated free radical intermediate that can interact with membrane lipids. Alternatively, BDCM may form a dichloromethyl glutathione conjugate catalyzed by glutathione-S-transferase. All pathways of trihalomethane metabolism lead to reactive intermediates that potentially interact with DNA. Interactions with microflora and other contents of the gastrointestinal tract may produce additional reactive intermediates. In vitro studies show that brominated trihalomethanes are more active than chloroform in forming protein adducts under aerobic conditions or lipid adducts under anaerobic conditions. Maximal rates of cytochrome P4502E1-mediated oxidation are similar for BDCM and chloroform; however, qualitative differences in the uptake and metabolism of these chemicals in rats indicate that a second metabolic pathway may be active for BDCM. Among dihalomethanes, brominated chemicals have a more favorable reaction with glutathione-producing mutagenic intermediates.

Human lymphoblast cell cultures that had lost their ability to express cytochrome P450 activity were transfected with various cytochrome P450 isozymes and exposed to BDCM. A dose-dependent increase in
cytotoxicity, which was attributed to metabolic activation of the parent compound, was observed in cells expressing cytochrome P4502E1, but not in those specifically expressing cytochromes 1A2, 2A6, 2B6, or 3A4.

**Epidemiological Studies of Chlorinated Drinking Water**

A meta-analysis of data from nine published case–control and cohort studies that examined associations between chlorinated drinking water and colon cancer, colorectal cancer, or rectal cancer was conducted. By definition, the exposed groups in this analysis had higher exposure to chlorination by-products than the unexposed groups based on their use of either surface water (versus groundwater) or chlorinated water (versus unchlorinated or chloraminated water). Relative risk estimates for all sites included in these studies were compiled by pooling the study results. Although all of the sites had relative risks greater than one, significant increases were obtained only for bladder cancer and for rectal cancer. For those studies that gave some estimate of exposure, dose responses for colon and rectal cancer were evident when the exposure subgroups were categorized as low, moderate, and high. Other drinking water contaminants, besides the trihalomethanes, may have contributed also to the increased risks of rectal cancer.

An epidemiological study of drinking water in Iowa residents demonstrated an association between consumption of chlorination by-products from chlorinated surface water sources and increased risk for rectal cancer, but not for bladder cancer or colon cancer. Rectal cancer risk associated with chlorination by-products appeared to be influenced by lifestyle factors (e.g., physical activity).

**Conclusions and Future Needs**

A general discussion following the presentations arrived at the following conclusions:

- Both epidemiological data and animal data indicate that trihalomethanes pose a potential carcinogenic risk to humans. The animal studies are considered to be particularly predictive of potential human risk because of the site correspondence in tumor response, the fact that colon and rectal cancers are uncommon in rodents, and because morphological features of the tumors induced in animals are analogous to those observed in human colorectal tumors. The Min mouse, which possesses an APC mutation, is a useful model to study the influence of environmental factors on familial adenomatous polyposis. Because the brominated chemicals are more active than chlorinated chemicals in the animal models and because the brominated trihalomethanes are formed as a result of bromide ions in the source water, epidemiological studies of colorectal cancer in regions with high bromide content in the source water (e.g., coastal regions) would yield greater insight on risks associated with exposure to trihalomethanes.

- The distinction between rectal cancer versus colon cancer in many cases is a difficult one. There are no studies available comparing the molecular genetics of tumors at these two sites. Differences in p53 or ras gene mutation rates in the proximal versus distal colon suggest that a gradient of response rather than absolute distinctions may exist. Currently it is reasonable to consider colon and rectal cancers in animals as arising by a similar process.

- Epidemiological studies of by-products of water chlorination need better characterization of exposure and greater consideration of other risk factors. Studies of occupational cohorts exposed to trihalomethanes or other brominated chemicals may also shed light on the relationship between these chemicals in drinking water and cancer risk.

- To better understand the mechanisms of cancer induction by trihalomethanes, we need to know what enzymes are involved in the activation of these chemicals and in particular the relative roles of the cytochrome P450 isozymes and glutathione-S-transferase. The types and frequencies of mutations induced by these chemicals in critical target genes in colorectal tumors in animal models need to be characterized. There is no evidence at present for a non-mutagenic mechanism in trihalomethane-induced colorectal cancer.

- Polymorphisms that are involved in either the activation of the chemical (e.g., glutathione-S-transferase and cytochrome P4502E1), the deactivation of mutagenic intermediates, or the control of the disease process (e.g., oncogenes and tumor-suppressor genes) can influence the cancer risk associated with exposure to environmental carcinogens. In addition, the degree of induction of inducible metabolic enzymes can show substantial interindividual variability and environmental factors may influence the expression of various genetic susceptibilities. Thus, genetically susceptible subpopulations should be identified and analyzed separately for their cancer risk.

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