Environmental Management & Pollution

Environmental and Chemical Carcinogenesis

8.1 Abstract

People are continuously exposed exogenously to varying amounts of chemicals that have been shown to have carcinogenic or mutagenic properties in experimental systems. Exposure can occur exogenously when these agents are present in food, air or water, and also endogenously when they are products of metabolism or pathophysiologic states such as inflammation. It has been estimated that exposure to environmental chemical carcinogens may contribute significantly to the causation of a sizable fraction, perhaps a majority, of human cancers, when exposures are related to "life-style" factors such as diet, tobacco use, etc.

This lesson summarizes several aspects of environmental chemical carcinogenesis that have been extensively studied and illustrates the power of mechanistic investigation combined with molecular epidemiologic approaches in establishing causative linkages between environmental exposures and increased cancer risks.

A causative relationship between exposure to aflatoxin, a strongly carcinogenic mold-produced contaminant of dietary staples in Asia and Africa, and elevated risk for primary liver cancer has been demonstrated through the application of well-validated biomarkers in molecular epidemiology. These studies have also identified a striking synergistic interaction between aflatoxin and hepatitis B virus infection in elevating liver cancer risk. Use of tobacco products provides a clear example of cancer causation by a life-style factor involving carcinogen exposure. Tobacco carcinogens and their DNA adducts are central to cancer induction by tobacco products, and the contribution of specific tobacco carcinogens (e.g. PAH and NNK) to tobacco-induced lung cancer, can be evaluated by a weight of evidence approach.

Factors considered include presence in tobacco products, carcinogenicity in laboratory animals, human uptake, metabolism and adduct formation, possible role in causing molecular changes in oncogenes or suppressor genes, and other relevant data. This approach can be applied to evaluation of other environmental

carcinogens, and the evaluations would be markedly facilitated by prospective epidemiologic studies incorporating phenotypic carcinogen-specific biomarkers.

Heterocyclic amines represent an important class of carcinogens in foods. They are mutagens and carcinogens at numerous organ sites in experimental animals, are produced when meats are heated above 180 ◦C for long periods. Four of these compounds can consistently be identified in well-done meat products from the North American diet, and although a causal linkage has not been established, a majority of epidemiology studies have linked consumption of well-done meat products to cancer of the colon, breast and stomach.

Studies employing molecular biomarkers suggest that individuals may differ in their susceptibility to these carcinogens, and genetic polymorphisms may contribute to this variability. Heterocyclic amines, likemost other chemical carcinogens, are not carcinogenic per se butmust be metabolized by a family of cytochrome P450 enzymes to chemically reactive electrophiles prior to reacting with DNA to initiate a carcinogenic response.

These same cytochrome P450 enzymes—as well as enzymes that act on the metabolic products of the cytochromes P450 (e.g. glucuronyl transferase, glutathione *S*-transferase and others)—also metabolize chemicals by inactivation pathways, and the relative amounts of activation and detoxification will determine whether a chemical is carcinogenic. Because both genetic and environmental factors influence the levels of enzymes that metabolically activate and detoxify chemicals, they can also influence carcinogenic risk.

Many of the phenotypes of cancer cells can be the result of mutations, i.e., changes in the nucleotide sequence of DNA that accumulate as tumors progress. These can arise as a result of DNA damage or by the incorporation of non-complementary nucleotides during DNA synthetic processes. Based upon the disparity between the infrequency of spontaneous mutations and the large numbers of mutations reported in human tumors, it has been postulated that cancers must exhibit a mutator phenotype, which would represent an early event in cancer progression.

A mutator phenotype could be generated by mutations in genes that normally function to guarantee genetic stability. These mutations presumably arise via DNA damage by environmental or endogenous agents, but it remains to be determined whether the acquisition of a mutator phenotype is a necessary event during tumor progression.

8.2 Aflatoxin

Keywords: Aflatoxin; Liver cancer; Hepatitis viruses; Tobacco carcinogens; Lung Cancer; Heterocyclic amines; Colon cancer; Carcinogen metabolism; Genetic polymorphism; Mutator phenotype

Validation of a causal relationship between aflatoxin exposure and hepatocellular carcinoma risk in humans: a molecular epidemiology paradigm demonstrating the power of biomarkers. Monographs Program on the Evaluation of Carcinogenic Risks to Humans of the International Agency for Research on Cancer (IARC) publishes authoritative carcinogenic risk assessments based on examination by experts of all relevant information to assess the strength of available evidence that exposures to the chemicals could alter the incidence of cancer in humans.

The mold-produced aflatoxins are among the few environmental chemicals in this list that were first identified as carcinogens in animals, and subsequently shown to pose carcinogenic risks to humans through epidemiologic studies. Extensive research has produced a comprehensive database addressing risks resulting from the high prevalence of their contamination of major food staples in many parts of the world, together with their carcinogenic potency in animals. Indeed, ***the aflatoxin-liver cancer risk relationship is among the most extensively documented examples demonstrating the significance of a widely disseminated environmental chemical carcinogen as a determinant of increased risk for a major form of cancer.*** Continuing research efforts stimulated by their discovery in the early 1960s produced an extensive body of evidence regarding human health risks resulting from aflatoxin exposure. Collectively, epidemiologic data together with evidence from many types of experimental models defines the role of aflatoxin exposure in HCC causation.

It is informative to review this information for perspective regarding the multifactorial etiology of the disease and, in particular, the critical role of well-validated molecular biomarkers in establishing the causal exposure-risk relationship. Chronic infections by the hepatitis B (HBV) or hepatitis C (HCV) viruses are major risk factors for the great majority of HCC cases worldwide. They also are largely responsible for the geographical pattern of HCC incidence, HBV being the dominant cause in developing countries of subSaharan Africa and Asia, while HCV is the major risk factor in developed countries with a high or intermediate incidence.

Evidence supporting these conclusions has recently been summarized. Carrier rates of HBV in African and Asian populations may be as high as 20%, and the infection is acquired early in life as a result either of perinatal infection by a carrier mother or by horizontal passage from infectious siblings. Males acquiring carrier status early in life are at very high risk of developing HCC, with a lifetime relative risk (RR) of 100 calculated for Taiwanese men. Thus, chronic infection with HBV has been stated to be the single most common cause of global HCC. The importance of HCV infection in the causation of HCC has been recognized more recently, and interactive effects between the carcinogenicity of HBV and HCV have been demonstrated in most, but not all, populations in which it has been studied.

Mechanisms through which these infections cause HCC are still unknown, although both direct and indirect actions are thought to be involved. The potency of HBV infection overshadowed recognition of the significance of aflatoxin exposure as a cause of HCC, evidence of which has mounted over a period of several decades. Aflatoxins belong to a large group of mycotoxins, toxic metabolites that contaminate food and feed commodities during growth of certain spoilage molds.

In addition to causing acute toxicity, aflatoxins are also liver carcinogens in experimental animals and extensive quality control measures are necessary to minimize levels in human foods. Aflatoxin-contaminated feed was discovered to be a liver carcinogen in rats even before the active agent was isolated and characterized. Subsequent experiments with chemically pure toxin showed that HCC was induced in sensitive species when aflatoxin B1 (AFB1), the major component of mixtures typically found in food raw materials, was fed at levels as low as 15 ppb (_g/kg) in the diet. Bioassays in various species offish, birds, rodents and sub-human primates eventually revealed that AFB1 is a liver carcinogen in all animals tested. Although there is wide variation in sensitivity, no completely refractory species has been identified.

These data clearly implicate aflatoxin as a potential liver carcinogen in humans, and the plausibility of this implication is supported by much additional experimental evidence. Aflatoxin is strongly mutagenic in test systems ranging from bacteria to human cells in culture, requiring metabolic activation by cytochrome; pathways of aflatoxin metabolism are similar in cells and tissues of susceptible animals and humans, including the epoxidation pathway resulting in covalent binding to DNA; the DNA adduct profile, with the aflatoxin-*N*7-guanine adduct (AFB1-*N*7-gua) representing the major adduct, is identical in animal and

human cells mutagenized by aflatoxin; adduct level in liver DNA is quantitatively related to aflatoxin dose and to tumor yield; and chemoprevention of DNA adduct formation inhibits tumorigenesis in experimental animals.

A paradigm for validating causal relationships utilizing molecular epidemiology is in the carcinogenic potency of aflatoxin in animals together with their frequent contamination of human foods stimulated cross-sectional epidemiologic investigations to assess relationships between exposure and incidence of HCC. Collectively, studies conducted in subSaharan Africa and Asia between 1965 and 1985 revealed a highly significant association between AFB1 intake, calculated from analysis of foods as consumed, and HCC incidence estimated from cancer registry data.

8.3 Tobacco Products

Evaluation of tobacco carcinogens: a model for environmental carcinogenesis. Tobacco products provide a clear example of cancer causation by a life-style factor involving carcinogen exposure. There are over one billion smokers and hundreds of millions of smokeless tobacco users worldwide. Tobacco use is by far the most widespread link between exposure to known carcinogens and death from cancer, and like the aflatoxin-HCC relationship, can be considered a model for understanding mechanisms of cancer induction by exogenous chemical carcinogens.

Tobacco products and cancer

The IARC Monograph entitled 'Tobacco Smoke and Involuntary Smoking', to be published in 2004, concluded the following based on an evaluation of the world's literature. Cigarette smoking increases the risk of all histological types of lung cancer. It causes cancer of the oral cavity, and this risk is greatly increased by the use of smokeless tobacco or by alcohol consumption in combination with smoking. Cigarette smoking is also causally associated with laryngeal, oropharyngeal and hypopharyngeal cancer, and increases the risks for sinonasal and nasopharyngeal cancer.

Cigarette smoking is causally associated with cancer of the esophagus, both squamous cell carcinoma and adenocarcinoma. Furthermore, cigarette smoking causes cancer of the stomach, liver, and pancreas, as well as transitional cell carcinomas of the bladder, ureter and renal pelvis, and renal cell carcinoma. Finally, cigarette smoking is also a cause of squamous cell cervical carcinoma and myeloid leukaemia, and the risk of colorectal cancer can also be increased by

smoking. Environmental tobacco smoke (ETS) causes lung cancer. Smokeless tobacco products are established causes of oral cavity cancer.

8.4 Tobacco Carcinogens and Cancer

The central role of tobacco carcinogens and their DNA adducts in tobacco-induced cancer. Carcinogens are the key connection between nicotine addiction and cancer. Nicotine addiction is the major reason why people continue to use tobacco products. While nicotine itself is not carcinogenic, each cigarette or dip of smokeless tobacco contains a mixture of carcinogens, tumor promoters, and co-carcinogens. Most tobacco carcinogens require metabolic activation to exert their carcinogenic effects; there are competing detoxification pathways and the balance between metabolic activation and detoxification differs among individuals and affects cancer risk.

Metabolic activation leads to the formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA. DNA adducts are absolutely central to the carcinogenic process. If their formation is inhibited or blocked, so is carcinogenesis. If DNA adducts escape cellular repair mechanisms and persist, they may lead to miscoding, resulting in permanent mutations. Cells with damaged DNA may be removed by apoptosis, or programmed cell death. If a permanent mutation occurs in a critical region of an oncogene or tumor suppressor gene, it can lead to activation of the oncogene or deactivation of the tumor suppressor gene.

Multiple events of this type lead to aberrant cells with loss of normal growth control and ultimately to cancer. The chronic barrage of DNA damage by tobacco carcinogens in people who use tobacco products is completely consistent with the multiple genetic changes observed in tobacco-induced cancers and with our current understanding of the role of genetic aberrations in cancer induction.

The upper track of nicotine and tobacco-specific nitrosamines can bind directly to certain receptors leading to activation of cellular regulatory factors such as AKT. This can result ultimately in decreased apoptosis, increased angiogenesis, and increased cell transformation. These changes may enhance the effects of carcinogens and their DNA adducts. The lower track of the contributions of cofactors such as tumor promoters and co-carcinogens in tobacco products, or irritation and viruses (for example, in the oral cavity) which may enhance the activity of tobacco carcinogens through a variety of mechanisms.

Tobacco products contain a diverse array of chemical carcinogens. More than 60 known carcinogens have been detected in cigarette smoke. Several carcinogens

have been detected only sporadically, but most are routinely found. All of the carcinogens have been formally evaluated by the IARC, and in each case, studies in either laboratory animals or in humans have provided sufficient evidence of carcinogenicity. There is a large range of potencies and concentrations among these carcinogens. In general, the stronger carcinogens such as polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aromatic amines occur in lower amounts in cigarette smoke (1–200 ng per cigarette) than the weaker carcinogens such as acetaldehyde (nearly 1mg per cigarette).

The total amount of carcinogens in cigarette smoke add up to 1–3 mg per cigarette (similar to the amount of nicotine, 0.5–1.5 mg per cigarette), although most of this total is comprised of weaker carcinogens such as acetaldehyde, catechol, and isoprene. Unburned tobacco, including cigarette tobacco, oral snuff, chewing tobacco, and other smokeless tobacco products.

8.5 Evaluating the Role of Specific Carcinogens

When considering the relationship between tobacco carcinogen exposure and specific types of cancer, we have the "advantage" of a known exposure and a known cancer endpoint. We are also aided by the comprehensive characterization of tobacco product chemistry that is available in the literature. Tobacco use is unfortunately the largest voluntary carcinogen exposure experiment in history, and is still ongoing.

The major disadvantage, from the point of view of relating specific carcinogens to tobacco-induced cancer, is that the exposures are always to mixtures of tobacco carcinogens, along with cofactors such as tumor promoters and cocarcinogens. This clearly complicates the task of relating particular tobacco carcinogens to specific cancer types.

Nevertheless, a weight of the evidence approach can be taken the criteria used for evaluation are the presence of the compounds in cigarette smoke, their pulmonary carcinogenicity in laboratory animals, their human uptake, metabolism and adduct formation, their possible role in causing molecular changes in oncogenes or suppressor genes, and other relevant data. Using this approach and assigning a score to each group of compounds is the conclusion of considerable evidence favors PAHs and NNK as major etiological factors in tobacco-induced lung cancer.

PAHs are strong locally-acting carcinogens, and tobacco smoke fractions enriched in these compounds are carcinogenic. PAH-DNA adducts have been detected in human lung samples, and mutations in the Tp53 gene isolated from lung tumors

are similar to those produced in vitro by PAH diol epoxide metabolites. NNK is a strong systemic lung carcinogen in rodents, inducing lung tumors independently of its route of administration. The strength of NNK is particularly great in the rat, in which total doses as low as 6 mg/kg (and 1.8 mg/kg when considered as part of a dose-response trend) have induced a significant incidence of lung tumors. This compares to an estimated 1.1 mg/kg dose of NNK in 40 years of smoking. DNA adducts derived from NNK or the related tobacco-specific nitrosamine NNN are present at a higher level in lung tissue from lung cancer patients than controls, and metabolites of NNK are found in the urine of people who use tobacco products or are exposed to ETS.

Epidemiologic data indicate that a systemic carcinogen causes lung cancer in cigar smokers who do not inhale; this is consistent with the tumorigenic properties of NNK. The changing histology of lung cancer, in which adenocarcinoma has now overtaken squamous cell carcinoma as the most common lung cancer type, is also consistent with the role of NNK, which produces primarily adenocarcinoma in rodents. NNK concentrations in mainstream smoke increased, while those of BaP decreased, as nitrate concentrations in tobacco increased over the period of 1959–1997 due to the use of tobacco blends containing higher levels of air-cured tobacco, use of reconstituted tobacco, and other factors.