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MEDICAL PROGRESS

CHEMICAL CARCINOGENESIS

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THE perception of cancer as a disease primarily related to the environment has been progressively strengthened since the publication of a Medical Progress article on chemical carcinogenesis in 1971.[1] By the mid-1960s, the view of the cause and pathogenesis of cancer had already begun to change radically from that of previous decades, during which no clear perception of the possible role of the environment in the genesis of most of the major forms of cancer was evident. Although the relation of cancer to environmental hazards in the work place and to a few unusual cultural patterns around the world was established, the examples were considered exceptional.

The general acceptance of cigarette smoking as a major factor in the development of lung cancer and the increasing number of examples of major shifts in the organ or tissue distribution of primary cancers with migration of distinctive ethnic groups from one geographic location to another were important in changing the whole perception of the genesis of cancer.[2,3]

This radical change has encouraged increasing attention to the nature of the environmental influences. The available epidemiologic evidence indicates the likelihood of several (if not many) components in this environmental outlook. Diet, carcinogenic chemicals, radiation, and viruses are among the major factors that appear to be involved. Of these, chemicals as carcinogens have been receiving increasing attention as having the greatest role in the genesis of cancer. How justified is this perception of the causation of cancer?

About one third of the cancer in North America and Europe is related to the use of cigarettes or other tobacco products. There is incontrovertible evidence that chemicals related to the work place or occupation are responsible for a segment of cancer in human beings (Table 1). To these must be added a growing list of naturally occurring chemicals and of drugs used in medicine. All these known agents, together with ultraviolet light as the single most common cause of cancer, can easily account for over 50 per cent of the cases

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can be seen during the long so-called latent period. Often, new cell populations appear that probably represent stages or steps in the cellular evolution from normal cells through initiated, preneoplastic, and premalignant cells to highly malignant neoplastic cells.[15-17] Research in laboratory animals has concentrated heavily on the early events and has suggested the existence of common patterns of early preneoplastic changes in several organ systems and in several species.[17] Research in human beings has concentrated largely on the later events and has confirmed the occurrence of atypical hyperplasias, dysplasia, and carcinoma in situ as probable precancerous steps in several organs late in the process.[17-19] However, the critical properties of each different cell population that relate to the possible roles of these cells in cancer development and to the manner in which the two aspects of carcinogenesis (the experimental and the human) fit together into some biologically meaningful pattern remain as challenges.[17]

The conceptual advances made in the late 1930s and 1940s concerning the first two early steps in chemical carcinogenesis, initiation and promotion, are well known.[15-17,20] However, their mechanistic bases are only now unfolding, and these will be topics for review. The basic validity of these concepts for cancer development in human beings has been established in a few instances and as such is reassuring both for the physician and for the scientist. For example, a relatively brief exposure to diethylstilbestrol during pregnancy may be associated with the development of vaginal neoplasia some 15 to 25 years later in the exposed person's daughters.[21] Parenthetically, it should be mentioned that it is not known whether diethylstilbestrol acts in this situation as a chemical carcinogen or whether the effect is predominantly hormonal. Again, a limited exposure for only a few months to a known chemical hazard, such as vinyl chloride, may lead many years later to the appearance of angiosarcoma of the liver.[22] Nonneoplastic cellular and tissue changes are seen during the apparent latent period.

Initiators and Initiation

Initiators

Until fairly recently, one of the most puzzling and confusing aspects of chemical carcinogenesis was the diversity in the chemical structure of carcinogens. This problem has now been largely resolved by the discovery of metabolic conversion of many carcinogens to highly reactive metabolites.[20] The best-known form of reactive moieties, generated from several different types of carcinogens, is the "electrophilic reactant," which is a positively charged molecule that reacts well with sites of electron densities in many different cellular components, including DNA, RNA, protein, glutathione, and probably also polysaccharides.[20] A minority of chemical carcinogens, such as some nitrosamides (e.g., alkyl nitrosourea), bis(chloromethyl) ether, and nitrogen mustard, are active by themselves and do not seem to require any metabolic conversion to more reactive metabolites.

Activation and Metabolism of Procarcinogens

The first major type of metabolic activation discovered was the conversion of an aromatic amine, 2-acetylaminofluorene, by *N*-hydroxylation to an N-OH derivative.[20] This type of metabolic product is seen with several aromatic amines, including the human bladder carcinogen 2-naphthylamine (β-naphthylamine).

More recently, much effort has been spent on the clarification of the activation of benzo(*a*)pyrene and the many other polycyclic aromatic hydrocarbons. Many of them are easily generated by burning (pyrolysis), are widely distributed in our environment, and are thought to be partly responsible for the carcinogenicity of coal-tar products, oils, and tars. These compounds undergo epoxidation to form reactive epoxides, some forms of which are considered to be ultimate carcinogens.[23] In the case of benzo(*a*)pyrene (and probably other polycyclic aromatic hydrocarbons), the initial site of epoxidation may undergo hydration to form a dihydrodiol in a reaction catalyzed by epoxide hydrolase. This inactive derivative, in turn, can be converted to another epoxide at a second site to form a dihydrodiol epoxide. These are considered to be the most likely ultimate carcinogens for at least some polycyclic aromatic hydrocarbons. This series of reactions for benzo(*a*)pyrene is illustrated in Figure 1. Another potentially important carcinogen for human beings that is subject to activation through epoxidation is aflatoxin B_1.[20] This substance undergoes oxidation at its 2,3-position, and this derivative, the 2,3-oxide, appears to be one form of ultimate carcinogen of this mycotoxin. Vinyl chloride is also activated through epoxidation.[20]

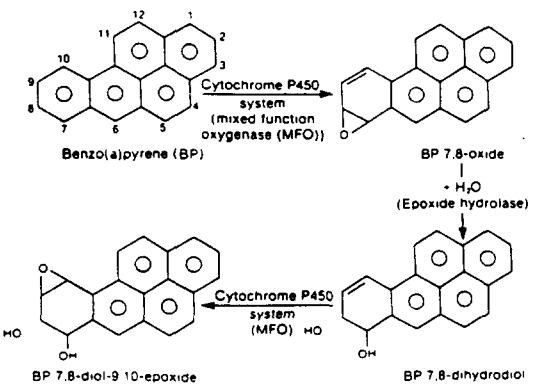

Figure 1. Current View of the Activation of Benzo(*a*)pyrene (BP) to Benzo(*a*)pyrene 7,8-Dihydrodiol,9,10 Epoxide through the Mixed-Function Oxygenase (MFO) System (Cytochrome P-450 System) and Epoxide Hydrolase.

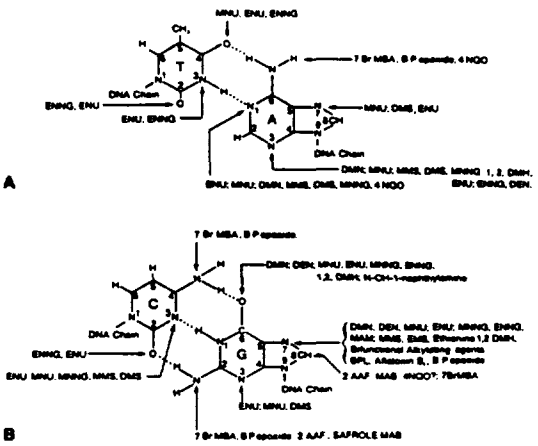

Figure 2. Diagrammatic Representation of Sites of Interactions of Activated Forms of Carcinogens with the Four Bases of DNA.

T denotes thymine; A, adenine; C, cytosine; G, guanine; MNU, *N*-methyl-*N*-nitrosourea; ENU, *N*-ethyl-*N*-nitrosourea; ENNG, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine; 7 Br MBA, 7-bromothyl-benzo(a)anthracene; BP epoxide, benzo(a)pyrene 7,8-dihydrodiol,0,10 epoxide; 4NQO, 4-nitroquinoline-*N*-oxide; DMS, dimethylsulfate; DMN, dimethylnitrosamine; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; 1,2.DMH, 1,2-dimethylhydrazine; DEN, diethylnitrosamine; N-OH-1-naphthylamine, *N*-hydroxy-1-naphthylamine; 2 AAF, 2-acetylaminofluorene; MAM, methylazoxymethanol; EMS, ethyl methane sulfonate; BPL, β-propiolactone; and MAB, 4-methylaminoazobenzene.

studies of susceptibility to nucleases, separation of transcriptionally active from inactive DNA, or isolation of linker and nucleosome regions in chromatin, some of the interactions of carcinogens with DNA have been found to be nonrandom. However, no single region of high affinity has been found.[36] The use of DNA cloning for specific genes in biologically well-defined preneoplastic and early neoplastic populations should be profitable.

DNA Repair

In view of the essentially irreversible nature of initiation with chemicals and the apparent focal nature of the initiation process, major emphasis at the molecular level is given to DNA as a probable target in initiation. However, the evidence is largely circumstantial.

The bulk of evidence comes from studies in patients with xeroderma pigmentosum, who have a high incidence of skin cancers. The etiologic agent is ultraviolet light, and the vast majority of patients have a defect in their ability to repair the damage inflicted on DNA by this form of radiation. Such patients can be protected from skin cancer by careful avoidance of exposure to ultraviolet light.[40] Fibroblast cultures from these patients have increased sensitivity not only to ultraviolet light but also to some chemical carcinogens and mutagens.[41] Other diseases (e.g., ataxia-telangiectasia and Fanconi's anemia) also involve deficiencies in DNA repair as well as an increased risk of neoplasia.[42]

Experimentally, there is a correlation under some conditions between the formation of specific DNA adducts (such as O^6-methylguanine in the brain), persistence of this biochemical lesion, and the ultimate appearance of gliomas.[43] Other studies indicate that the genesis and persistence of O^6-methylguanine may be important but is insufficient to account for cancer production with some nitrosamines or nitrosamides[44] or 1,2-dimethylhydrazine.[45]

The major protection against cancer may reside in the efficiency with which we repair DNA that has been damaged by mutagens and carcinogens. As pointed out by German[11] and by Cairns,[12] since fibroblasts from patients with xeroderma pigmentosum show a defect in the repair of DNA damage by some chemicals as well as by ultraviolet light, such patients should have an elevated risk for cancer in organs or tissues other than the skin if chemical carcinogens are important in the genesis of many forms of cancer. Yet the available data show no apparent increase in the risk of cancers other than those of the skin in patients with xeroderma pigmentosum.[12] Patients with Bloom's syndrome do have an elevated risk. Such observations are interpreted as evidence against a role for the many environmental chemical hazards in the causation of human cancers generally, except in well-established instances such as respiratory-tract cancer in smokers and in persons with occupational exposures.[12] An alternative hypothesis must be entertained: that the role of repair may be quite different in the dynamics of cancer induction by chemicals, as compared with induction by ultraviolet light.

The past 10 years have seen a large expansion of studies of DNA repair, largely in vitro. These studies should lead to a much clearer delineation of the types of repair that may occur in carcinogenesis. What does happen remains poorly understood. Processes of repair by base excision, by "long-patch versus short-patch" removal, by possible recombination or other forms of post-replication repair, by removal of inter-strand cross-links or other more complex forms of damage, and by other mechanisms are slowly being studied in many different systems in vitro and to a much lesser degree in vivo.[36,41] The enzymology, although difficult, is also slowly becoming clear. In the case of methylating carcinogens, an interesting recent finding is the discovery of an enzyme in bacteria that removes the methyl group from the O^6 position of guanine in DNA by transmethylation with its transfer to a sulfur-containing moiety in protein.[46] This raises the question of whether some of the enzymes involved in repair of DNA damage perform physiologic functions other than repair that subserve normal cellular requirements.

Obvious failings in many of the attempts to correlate patterns of DNA repair with carcinogenicity include the wide gap between the chemistry and the bi-

crosis induced by viruses, toxic agents, parasites, or dietary deficiencies, followed by cell regeneration, could be a major determinant of cancer initiation in many sites. In addition, the presence of many proliferating cells is probably one basis for the susceptibility of the fetus and neonate to many chemical carcinogens[78] and could account for the peak in cancer incidence in the first decade of life. The human fetus, unlike the rodent fetus, acquires the capability of activation of some carcinogens early in development[79,80] and thus may be at greater risk than some laboratory animals for cancer development with chemicals.

A most important question about initiation concerns the essential biologic nature of the initiated cells. What properties have they acquired that allows them, as a group, to be precursors for the ultimate development of cancer? The available evidence is against any conclusion that the initiated cells have acquired any autonomy of growth.[15,17]

In most systems, the properties critical to initiation remain unknown. In two continuously proliferating tissues, the skin[81] and the colon,[82] an early property of carcinogen-altered cells may be some disturbance in programming or control, such that the cells do not show the normal progression of differentiated properties. In the liver, the cell with acquired resistance to the inhibitory effects of carcinogens on cell proliferation has been shown to be one type of initiated cell.[17,83]

Although a majority of chemical carcinogens fall well within the current paradigm in which initiating effects are related to some form of DNA damage, there are known carcinogens that appear to be exceptions. A growing list of hypolipidemic agents[84] and several pesticides, herbicides, and other xenobiotics[85] have not been shown to generate mutagenicity or other DNA-damaging effects. Is this merely a reflection of deficiencies in our technology, or are there pathways to cancer that do not involve DNA damage of exogenous origin as essential early steps in the process?

Promoters and Promotion

The term "promotion" is often used for the process whereby neoplastic development, tumor formation, or cancer development is accelerated or encouraged in a tissue that has been exposed to an initiating dose or doses of a carcinogen.

Promoters

Early in the recent history of chemical carcinogenesis it was found that a noncarcinogen, croton oil, could stimulate tumor formation in the skin after a brief initial exposure to a carcinogen. This naturally encouraged a chemical "attack" on croton oil Hecker[86] and Van Duuren[87] discovered that esters of the diterpene phorbol, isolated from croton oil, have potent promoting effects on mouse skin. Many biochemical, metabolic, and biologic effects are induced in many different normal cells by the phorbol esters and related compounds; in the vast majority of cases, the intensity of the particular effect closely parallels the efficacy of the compounds as promoters of skin papillomas in mice after exposure to a single dose of dimethylbenzanthracene or a similar carcinogen.[88,89] These effects include changes in morphology, microtubule polymerization, cell proliferation, enzyme induction, polyamine synthesis, phospholipid synthesis, membrane structure and function, ATPase, release of prostaglandins, inhibition and sometimes stimulation of differentiation, and many others in a wide variety of normal cells and cell lines. Many of the effects are considered pleiotropic.

Recently, with the use of a less active phorbol ester, ^{3}H-phorbol dibutyrate, membranes of fibroblast cultures and mouse epidermal cells were found to contain a high-affinity receptor for many phorbol esters, including one of the most active, 12-*O*-tetradecanoylphorbol-13-acetate.[88,90] The "natural" metabolites for the receptors have not been identified. Given the wide diversity and large number of effects seen in treated cells, it may be that more than one type of receptor is involved.

At present it is impossible to relate the findings in vitro to chemical carcinogenesis in vivo. Given the array of phenomena induced by the phorbol esters, how does one select relevance to promotion?

A major problem concerns the matching of the target cells in vivo and in vitro. In the intact animal, an active promoter does not induce focal proliferations (such as papillomas) in the normal skin, but does so only after initiation. However, many skin promoters do induce general hyperplasia of the epidermis in animals in which initiation has not taken place. Studies in several laboratories have shown that a general stimulation of cell proliferation is insufficient for the selective or differential stimulation of initiated skin to form papillomas.[91]

Promotion

A question that requires an early answer is whether the differential effect of a promoting environment on initiated tissues is directed primarily to the initiated cells or to the surrounding cells.[92] There is considerable circumstantial evidence to suggest that the first major phenomenon in promotion is the selection of an appropriately altered cell to produce a focal proliferation, and that promoting environments create differential effects on the initiated cells and on the surrounding cells.[17,83,92] Cancer frequently arises in an atrophic tissue or organ, not a hypertrophic one. Is this the consequence of a change in the local environment, favoring growth of the rare initiated cell?

As observed in vivo in experimental models in common use today, cell proliferation is an essential phenomenon in promotion. It is therefore to be anticipated that many biochemical changes that are seen in the different phases of the cell cycle,[93] such as an increase in ornithine decarboxylase activity, will be seen in promotion. Determining whether one or more of such changes in enzymes, the cell membrane, DNA organization, or other factors will have a special role in pro-

some patients the risk of second primary tumors varies from 7 to 12 per cent.[115] These figures include some risk factors aside from the therapy, that are possibly genetic and favor second or multiple primary tumors. However, there is an additional risk from the chemotherapy itself. This risk will increase in volume as current types of chemotherapy for cancer become more successful. However, as mechanisms by which chemicals induce cancer and kill cancer cells are clarified, ways will be found to prevent the carcinogenic effects without interfering with the drugs' efficacy in treating cancer.

A common problem in cancer chemotherapy is the appearance of resistant cancer cells. Since resistant cells are also produced by carcinogens during carcinogenesis, and since such resistance constitutes one form of initiation,[17,83] it is conceivable that the induction of resistance to therapy and that of cancer are related.

New Bioassays for Carcinogens and Promoters

The basic knowledge that has been generated during the past 20 years or so has spawned a large array of short-term tests (mostly in vitro) for potential carcinogens, and more recently for promoters.[116] These tests generally fall into two broad groups: those that use some correlate of DNA damage or chromosomal damage as end points and those that use cell transformation as the end point.[116-118] The majority require the inclusion of an appropriate activation system.

This development is an essential requirement for rapid monitoring of our environment. In addition, these tests are being used increasingly as rapid assays for some more basic aspects of chemical carcinogenesis, such as patterns of activation in tissues and the endogenous production of carcinogens or mutagens, to name but two of many examples.

Conclusions

The past decade has produced new insights into the need for metabolic activation, the nature of the active molecules, the enzymology of activation, and the array of products generated, including major ultimate carcinogens. The interactions of such derivatives with DNA and other cell constituents and the possible toxicities and other effects on cells are becoming clearer. There is a general belief that DNA damage is involved in cancer initiation in most instances. The basic studies have generated over 100 short-term assays for possible carcinogens, with many of the assays reflecting damage to DNA or chromosomes.

The importance of the modulation of metabolic activation by enzyme induction with hormones, diet, drugs, and chemicals (including many environmental contaminants such as pesticides, herbicides, and polychlorinated biphenyls) is appreciated. How this ubiquitous exposure may influence cancer development in human beings is not known.

The repair of lesions in DNA is receiving increasing emphasis. The majority of such studies are in relatively simple in vitro systems and are generating insights into what may happen. The study of what does happen remains a major challenge. It may be aided by the development of ultrasensitive enzymatic radioimmunoassays for carcinogen-DNA adducts.

There is an increasing realization that many post-initiation phenomena, including promotion, are of major importance in cancer development. Alterations in diet, hormones, drugs, and xenobiotic agents have major influences through effects on promotion and other later steps. The reversibility of many of the precancerous steps is being studied with vitamin A and its analogues and with other dietary components.

Despite the major advances, the study of the later phases of chemical carcinogenesis lags far behind that of the early biochemical events. A nagging uncertainty in this field in general concerns the role of chemical carcinogens of either exogenous or endogenous origin in the causation and pathogenesis of some of the major forms of cancer. There is a general agreement that cigarette smoking and ultraviolet light are two major factors in the genesis of several important types of cancers. There is also incontrovertible evidence that many chemicals to which persons are exposed in the work place or its environs cause cancer in several organ systems. However, the relative quantitative importance of these considerations in the majority of patients with cancer not related to smoking is unclear. The resolution of this problem, although no doubt difficult, will almost certainly have a major impact on our approaches to cancer prevention in coming decades.

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