Metabolism of chemical carcinogens

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The transformation of chemicals is important in carcinogenesis, both in bioactivation and detoxification. Major advances in the past 20 years include appreciation of the migration of reactive electrophiles, the ability of Phase II conjugating enzymes to activate chemicals, understanding of the human enzymes, the realization that DNA modification can result from endogenous chemicals, and the demonstration that cancers can result from the metabolism of chemicals to non-covalently bound products. Pathways of transformation in which major insight was gained during the past 20 years include nitropolycyclic hydrocarbons, polycyclic hydrocarbons and their diols, vinyl halides and dihaloalkanes. Advances in analytical methods and recombinant DNA technology contributed greatly to the study of metabolism of chemical carcinogens. Major advances have been made in the assignment of roles of individual enzymes in reactions. The knowledge developed in this field has contributed to growth in the areas of chemoprevention, molecular epidemiology and species comparisons of risk. Some of the areas in which future development relevant to carcinogen metabolism is expected involve pathways of transformation of certain chemicals, regulation of genes coding for many of the enzymes under consideration and genomics.

Introduction

The discussion of the metabolism of carcinogens will be focused largely on the enzymes involved in the activation and detoxification of these chemicals. The basis of chemical reactivity of activated carcinogens with DNA will not be discussed per se. Most of the basic enzymatic transformations of chemical carcinogens were already known in 1980 (1–3). However, most of the enzyme systems were not well characterized and very little information existed regarding individual enzymes within groups.

Highlights of metabolism research, 1980–2000

General concepts

Many of the general concepts regarding bioactivation, detoxification and genotoxicity had already been developed by Miller and others (4,5). However, at least four major new concepts regarding metabolism were developed during the past 20 years.

(i) ‘Reactive intermediates’ do have finite stability and can travel limited distances to alkylate DNA. In the 1970s a view had developed that ‘activated carcinogens’ were so reactive that they could not diffuse very far. This view led to the hypothesis that only nuclear enzymes could be involved in the activation of carcinogens (6). Subsequent work showed that products reactive with DNA could be generated in hepatocytes and be trapped outside the cells, e.g. with polycyclic hydrocarbons, nitrosamines and vinyl halides as the substrates (7–9). Furthermore, i.p. injection of a benzo[a]pyrene diol epoxide into pre-weanling mice generated lung tumors (10), arguing that distribution could be widespread even with a compound having a 30 s half-life.

(ii) ‘Phase II’ enzymes involved in conjugates are not only protective but also activate chemical carcinogens. An example is glutathione (GSH) transferase, which activates 1,2-dihaloethanes (11,12). These enzymes can also activate other chemicals (13,14). Examples of roles of bioactivation are also known for N-acetyltransferase (15), UDP-glucuronosyltransferase (16), sulfotransferase (17) and other Phase II reactions. During the past 20 years evidence has been obtained for roles of kinases in activation of chemical carcinogens (18).

(iii) Humans generally form the same DNA (and RNA and protein) adducts as animal models. Twenty years ago this was still a hypothesis but has now been clearly demonstrated in many cases (19). Further review is beyond the scope of this review and more relevant to another (20). The point is that the same metabolic pathways are also important in humans and animals; generally the major differences are quantitative.

(iv) DNA adducts can be generated by the metabolism of ‘endogenous’ chemicals. Key examples here are the generation of DNA adducts from products of lipid peroxidation (21,22), estrogens (23,24) and some other ‘endogenous’ materials.

(v) Metabolism of chemicals to unreactive, non-genotoxic products can be an important issue in tumorigenesis, at least in animal models. A classic example is the oxidation of 2,2,4-trimethylpentane to an alcohol, which is stable but is complexed with α2u-globulin to produce male rat kidney tumors (25).

Development of pathways of metabolism of carcinogens

Many of the pathways we accept today were already established by 1980. For instance, the bay-region diol epoxide pathway for polycyclic hydrocarbons, the N-hydroxylation of arylamines followed by addition of a leaving group, and the α-hydroxylation of N-nitrosamines were generally accepted pathways (26). However, some reactions had not been established yet. One important group of carcinogens, the heterocyclic amines, has been studied extensively only in the past 20 years, but the pathways of metabolism are largely identical to those established for aryl amines (1). Some of the newer pathways for carcinogen metabolism include the following.

Nitropolycyclic hydrocarbons

These are of considerable interest because of their unusually high bacterial mutagenicity (27) and potential activity in some animal tumor models (28). Activation involves both diol epoxide pathways and reduction to aryl hydroxylamines (29,30). Redox cycling can occur with some of the nitro...
compounds, at least monocyclic, to yield nitro anion radicals and superoxide (31). P450s are involved in the oxygenation reactions but a number of redox-active enzymes can participate in activation (and detoxification) of nitro groups via reduction (32,33).

**Oxidation of polycyclic hydrocarbon diols**

One transformation that was not recognized until the 1980s was the oxidation of trans-dihydriodials of aromatic hydrocarbons to o-catechols by dihydriodil dehydrogenase [EC 1.3.1.20] (34). These enzymes are members of an aldo-keto reductase superfamily and also have activities toward hydroxy steroids. The oxidation suppresses the formation of carcinogenic dihydriodil epoxides; conversely, the catechols may oxidize (enzymatically or non-enzymatically) to o-quinones and cause damage by reaction with macromolecules or through generation of reactive oxygen species (35,36).

**Vinyl halides**

In 1980 the metabolism of vinyl halides was understood only in terms of epoxidation. Studies in this laboratory with trichloroethylene (37) and vinylidene chloride (38) established stepwise oxidation and 1,2-shifts as an explanation for the products. This mechanism, with hydride migration, is generally viewed as a part of the oxidation of terminal olefins (with an aldehyde product) (39,40). Reactive haloacyl halides are produced (41); the relevance of these and the epoxides themselves to genotoxicity remains to be established.

**Dihaloalkanes**

In 1978 Rannug reported the GSH-dependent activation of 1,2-dichloroethane (11). This pathway is now well-established, with an epilsulfonylion intermediate (42) and several characterized DNA adducts (43). The general pathway has been extended to dihalomethanes, although very limited information is available about DNA adducts (44). The same pathway appears to apply to trihalomethanes (45).

**Development of analytical methods**

Although many of the basic methods existed in 1980, the past two decades have seen an explosion of capability in two major areas.

**Analytical chemistry**

The first area involves chromatography and spectroscopy. HPLC has been developed as a necessary technique in the area. Capillary electrophoresis and other new separation techniques have potential. NMR and mass spectrometry were already very important in 1980 but today both methods have much greater resolution and sensitivity and are coupled on-line with HPLC. Other spectroscopic methods such as fluorescence (46) have been applied to DNA adduct studies. ³²P-post-labeling was developed as a method of DNA adduct analysis by Randerath (47) and has been integrated into metabolic studies, although this method may ultimately be replaced by mass spectrometry methods.

**Recombinant DNA technology**

These approaches were in their infancy in 1980. In 1982 the first P450 cdNA sequence was published (48), and the characterization of the enzymes involved in carcinogen metabolism would have been impossible without these methods. Scarce proteins can be identified, characterized, and produced in large amounts.

As an aside, it should be emphasized that human tissue samples are now much more readily available than in 1980 and have been invaluable in extending work from animal models to humans.

**Characterization of roles of individual enzymes in carcinogen metabolism**

By 1980 many of the general concepts about the significance of enzymes in carcinogen metabolism had been developed, at least in principle. For instance, enzymes such as P450 were known to be inducible (49), enzymatic differences could be used to explain variable susceptibility of individual animals to carcinogens (50), and some evidence for P450 polymorphism as a risk factor in lung cancer had been presented (51). Enzymology had been used successfully to address issues regarding the existence of multiple enzyme forms within some multi-gene families (52–56).

Protein chemistry and, later, molecular biology techniques were developed to characterize the enzymes involved in carcinogen metabolism, both in terms of the enzymology and also regulation of gene expression. This work was done with experimental animal models and, most importantly, with humans. Today there is a reasonably good understanding of many individual enzymes (e.g. individual P450s, GSH transferases, etc.) in terms of their concentrations in various human tissues, the extent of variability among humans, and the involvement of these enzymes in particular steps in metabolism of chemical carcinogens (57). The approaches are used rather routinely and many have been relatively straightforward, although the overall significance of a particular reaction to tumorigenesis may not always be clear. In the past few years, animals in which particular enzymes have been ‘knocked out’ have been used in cancer studies to define the roles of individual proteins in the overall processes of toxicity and carcinogenesis (58,59).

Inter-individual variation in the enzymes involved in carcinogen metabolism is now extensively studied. To date there has been impressive success in the application of information about human P450s in the development of new drugs in the pharmaceutical industry (57). There is optimism that such approaches will also be productive in the prediction of cancer risks due to inter-individual variations in enzymes. The National Institute of Environmental Health Sciences has begun an Environmental Genome Project, in which the long-term goal is to associate risks with polymorphisms of the genes involved in carcinogen metabolism, as well as others (60). The overall appreciation, characterization and organization of information about individual genes has developed dramatically since 1980.

**Development of approaches to chemoprevention**

Chemoprevention is discussed in another article in this issue (61). The strategy of inducing ‘Phase II’ conjugating enzymes was already in place before 1980 (62), but knowledge about the regulation of conjugating enzymes (63) and the inhibition of P450s (64–66) has been integrated. Also of interest, however, are reports that some of the chemopreventive agents can increase the toxicity, or at least DNA adduct formation, by inducing the conjugating enzymes (67,68).

**Development of in vitro genotoxicity assays**

These cellular systems are very useful both in basic studies on roles of enzymes in activation/detoxification and in practical work on new chemicals, etc. The majority of the major systems used today was done before 1980. During the past decade many
improvements in the systems have been made, particularly in terms of integration with enzyme systems of mammalian origin. Purified enzymes and human microsomes have been added to \textit{Salmonella typhimurium}-based systems, both in the classic Ames’ reversion assay (69) and an SOS response-based colorimetric assay (70), in order to define roles of individual enzymes. Another approach is to express individual enzymes, or sets of enzymes, and reporter genes within cells. Such approaches have been utilized with mammalian cells (71) and bacteria (72). Both \textit{S.typhimurium} and \textit{Escherichia coli}-based systems with P450s have been used (73–75).

**Development of predictions for humans based upon species comparisons**

One of the problems with \textit{in vitro} results obtained in some of the systems described above is that the relevance of metabolism in different tissues may be difficult to relate to \textit{in vivo} problems, e.g. cancer. One approach is the use of PBPK models, which have been developed mainly in the past 20 years. A common strategy is to: (i) define specific enzymatic steps of major relevance to bioactivation and detoxification; (ii) identify the most important tissues, in terms of metabolism and as targets for tumor development; (iii) collect data on rates of all relevant enzymatic transformations \textit{in vitro}, with tissues of interest both from humans and experiment models; (iv) if possible (i.e. with reasonably weak cancer suspects such as industrial solvents), validate the model in humans with low exposures; and (v) on the basis of comparisons and predictions about the effective concentration of the activated carcinogen delivered at the target site in humans and animals, compare the risk of concentrations of the chemical to humans to those known to produce tumors in the experimental animal models (76,77). This strategy has been used with information about lung and liver measurements of CH$_2$Cl$_2$ metabolism by P450 2E1 and GSH transferase T1 (78), and as a consequence the Environmental Protection Agency revised its exposure limit upwards.

**Current and future issues in metabolism of chemical carcinogens**

Although much progress has been made in the past 20 years and the field has matured, there are a number of remaining challenges that must be considered. These involve the general significance of the field and the basis for development of related areas, such as genomics.

Relevant pathways need to be established for more chemicals Despite all of the knowledge of pathways obtained in the past half-century or more (79), the reactions remain to be established in many cases. Some notable examples include the GSH conjugation of trihalomethanes found in drinking water (45) and the oxidation products of trichloroethylene, at least those that are involved in reactions with macromolecules (9) [a separate issue is the contribution of an alternate, GSH-dependent pathway to activation (80,81)]. Even after many years of research on polycyclic hydrocarbons, controversies exist about the contribution of a radical pathway (82,83) and suggestions of ‘poly’ oxidized products have been proposed (84). Another issue involves the chemical characterization of the DNA adducts generated by oxidations of several compounds by peroxidases (e.g. myeloperoxidase, cyclooxygenase, etc.) (85).

**Basic enzymology**

Understanding mechanistic details of one transformation is critical in making predictions about others. Much remains to be understood about most of the enzymes under consideration here (for recent review on the enzymes of interest, see ref. 86). The questions relate to both structure and catalysis (which are not unrelated). With some of the enzymes, the availability of crystal structures has facilitated progress (87,88); more are needed. Enzymology issues with practical implications include the question of whether P450s can catalyze 1-electron oxidation of polycyclic aromatic hydrocarbons (82). This hypothesis seems reasonable but has been difficult to address (89). The meaning of $K_m$ in most of the enzymatic transformations is not well understood (90–92); what does this value mean when incorporated into PBPK models (93)?

**Genomics**

Genomics is a popular field today, and in the area of carcinogen metabolism there is much anticipation that genetic differences in the enzymes of interest will be associated with major differences in cancer risk. Two major issues must be addressed in the course of a massive study. The first is the identification of the major allelic variants of the genes (that code for the enzymes under consideration). This effort will require large-scale sequencing efforts. In some enzyme families not all of the individual genes have been identified yet (94,95).

The second issue is functional analysis. One approach to assessing the significance of genetic variations is to go directly to epidemiological correlations. However, the danger of going directly to such associations in the absence of basic scientific information has been demonstrated in the work with P450 2D6 (96,97). A logical approach is development of functional analysis systems, in which allelic variants of enzymes can be expressed and examined for parameters relevant to metabolism of relevant carcinogens. (For further discussion of the complexity and relevant issues, see ref. 60.)

**Regulation of expression of enzymes**

In a sense, most of the progress made in the regulation of the enzymes of interest has been made in the past 20 years, primarily because of the development of useful methodology. Today, much is known about the systems, but even more questions remain. In many cases, even the primary responsive elements (receptors) remain to be identified and fully characterized [e.g. antioxidant response element system (98–100)]. Recent progress in the characterization of barbiturate and other drug-binding receptors has been promising (101,102). However, as work has proceeded with the elucidation of elements involved in gene regulation, so has the appreciation of the complexity of the systems. As an example, consider the \textit{Ah} locus. The \textit{Ah} receptor has now been extensively characterized but the list of involved partners continues to grow (103–107) and definition of the role of each component remains challenging.

A need exists for more understanding of mechanisms of regulation of enzymes involved in carcinogen metabolism. Just as important is an understanding of the influence of modulation on carcinogenesis and toxicity. For instance, we are still not in a position to provide an explanation for the carcinogenicity and toxicity of dioxins (108). Without such understanding, efforts to compare risk among species (e.g. humans) are deficient (109). Does induction of \textit{Ah}-linked genes really imply increased risk from a chemical? Although regulatory agencies are concerned because of the example of dioxin, the answers still remain equivocal or, at the least, dependent upon the particular model under investigation (110,111). Concerns
also exist about the significance of induction by barbiturates and peroxisome proliferators to carcinogenesis (112).

Relevance to biomarkers and molecular epidemiology

The basic question is whether high or low activity of a particular enzyme is related to a change in risk. This type of work has become popular and there is good precedent for success in the paradigms of drug metabolism and P450 (57). Programs such as the Environmental Genome Project have been set up to evaluate the contribution of gene polymorphisms to various risks. Many issues need to be addressed, and there is considerable room for innovative approaches. These include better technology for high-throughput screening and also ‘functional genomics’ (i.e. approaches to determining the effect of a genetic change on the enzymatic or other function of the involved proteins) (60,113,114). Another issue in this area is that of tissue localization and relevance to exposure. For instance, we have made the point that the epoxidation of aflatoxin B1 in the small intestine should be considered a detoxification event, in that it diverts the mycotoxin (from the liver) to cells that are readily sloughed (115). The issue of tissue localization is a problem in that it shifts the field to measurement of RNA and proteins, which must be excised from humans in order to make analyses. A related issue with polymorphisms is with genes that are not expressed in target tissues. Consideration of distribution kinetics of carcinogens and active metabolites and PBPK modes is needed (vide supra).

Assays with mixtures

Almost all of the experiments in the chemical carcinogenesis literature have been done with single compounds. This reductionist approach is not inappropriate and is certainly commended for understanding basic mechanisms. However, real exposure situations all involve mixtures and the approaches to dealing with these are difficult. As an example from our own recent work, consider work on the genotoxicity of tobacco smoke condensate components (116). The work was difficult because of the extremely inhibitory effect of tar components on P450 enzymes. What does this mean in terms of issues with smoking? The literature contains considerations of strategies. More insight will be needed in this area, particularly as scientists try to relate work on carcinogen metabolism to molecular epidemiology and practical human issues.

General relevance of carcinogen metabolism to cancer

This issue was addressed in a 1988 review (2). There is ample evidence in experimental animal models that altered carcinogen metabolism can have dramatic effects on tumor incidence (111,117). The concept that enzyme differences in humans can alter xenobiotic disposition and effects has very good precedence in the pharmaceutical industry, as mentioned earlier. Therefore, there is great hope that similar effects will be seen with cancer. However, the epidemiology results to date have not been strong. Although the association of P450 2D6 with lung cancer has been studied for many years, neither consistent epidemiology nor an experimental basis of a causal relationship have resulted (96,97,118). Similar problems have been encountered with GST transferases M1 and P1 (119,120). Although there have been some reports of altered cancer incidence related to P450s 1A1 and 1B1 (121,122), the results cannot be interpreted in terms of any dramatic experimental differences between the allelic gene products (123–125). The evidence regarding N-acetyltransferase may be more compelling (126,127). One of the major problems in doing epidemiology studies of this type is assessment of the actual chemical exposure, whether an endogenous or xenobiotic chemical. Linking variations in enzyme expression and catalytic activity to the endpoint of human cancer (128) will remain a challenge for at least part of the next 20 years.

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