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Tissue specificities of tumor induction by aromatic amines

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1. ABSTRACT

Certain aryl compounds that have nitrogen substitutions on their ring structures are, following metabolic conversion to reactive derivatives, able to elicit toxic responses by virtue of their modifications of protein and nucleic acid. This group of compounds is often referred to as aromatic amines (AA), although from a structural perspective, compounds that can be converted metabolically to crucial AA derivatives are also capable of producing the same adverse biological effects. These effects include cellular death, mutagenic events and tumor induction. Importantly, in humans, AA can induce tumors in the urinary tract, and possibly other tissues. This contribution addresses the mechanisms by which AA are likely to produce these carcinogenic consequences.

2. INTRODUCTION

Recognition that occupational exposure to aromatic amines (AA) resulted in the induction of urinary bladder tumors prompted exploration of the abilities of this class of compounds to induce tumors in experimental animals with the objective of identifying the structural features and molecular mechanisms responsible for their carcinogenic properties. In contrast to observations in humans, dependent on the species and the structure of the compound, tumors were sometimes found in the urinary bladder, as well as a wide variety of other tissues and organs (Table 1) (1,2). Here we consider the reasons for these differences with the objective of better understanding the processes responsible for the carcinogenic properties of these compounds.

Table 1. Carcinogenicity of human carcinogenic aromatic amines in other species (1)

	Human	Dog	Rabbit	Rat	Mouse
4-Aminobiphenyl	Bladder	Bladder	Bladder	Mammary gland Intestine Ear duct	Bladder Liver Mammary gland
2-Aminonaphthalene	Bladder	Bladder	Bladder[1]	Bladder[1]	Liver
Chlornaphazine	Bladder			Lung	
Benzidine	Bladder	Bladder[2]	ND*	Mammary gland Ear Duct Intestine	Liver
4,4'-Methylene-bis(2-chloroaniline) (MOCA)	Bladder[1]	Bladder	ND	Liver Lung Mammary gland Ear Duct Blood vessel	Liver Blood vessel
o-Toluidine	Bladder[1]	ND	ND	Bladder Mammary gland	Liver
Phenacetin	Bladder Renal pelvis	ND	ND	Renal pelvis Bladder Nasal cavity	Liver Lung Kidney

[1]The evidence of carcinogenicity is weak. [2]Three of 7 dogs treated with a total dose of 325 g of benzidine developed tumors after a latent period of 7 to 9 years and at a time in a dog's life when spontaneously occurring tumors are common (2). *ND: not done

3. WHAT IS REQUIRED FOR THE INDUCTION OF TUMORS BY CHEMICALS?

A broad range of efforts led to the generally accepted hypothesis that a key event in the development of malignancy involves nucleic acid modification by the carcinogenic agent or process. Much effort has been expended in the identification and evaluation of these events in molecular terms. On introduction into animals, AA, as with many other carcinogens, are incapable of reaction with nucleic acids without undergoing metabolic transformation to reactive derivatives. This requirement for metabolic activation comes from degradation of potentially reactive agents that might have been generated externally prior to introduction into biological systems. However, the instabilities of reactive metabolites produced in target tissues also limit the distance through which their nucleic acid modification potential is likely to be exerted and, consequently, aids in the identification of the metabolic activation pathways that are likely to be involved in the carcinogenic process.

Opportunities to explore the molecular mechanisms by which tumors were induced by AA were facilitated with the development of techniques suitable for the isolation, characterization and use of proteins and nucleic acids. In conjunction with the use of structurally related compounds, insight was provided into the relative importance of the metabolic pathways involved in the modification of tissue components.

Although the production of reactive metabolites in target tissues is a crucial element, a number of other factors influence the production of tumors as depicted in Figure 1. Importantly, there must be transport of suitable AA derivatives that can be activated by the target tissues. With systemic exposures to AA, metabolic activation processes are most frequently facilitated by initial metabolic transformations prior to distribution to the target tissues as seen by the generation of N-oxidized metabolites. Metabolites that are intermediate between the parent compounds and metabolites that are able to modify nucleic acid without further metabolism have been designated as "proximate" carcinogens by some investigators; metabolites that can modify nucleic acid directly have been labeled as "ultimate" carcinogens (3).

These relationships are shown in Figure 1. For example, tumor induction results from direct exposure of susceptible tissues by synthetic N-oxidized derivatives, such as N-hydroxy-2-acetylaminofluorene (N-OH-AAF) in rats, but not by the parent amide. Thus circumventing the requirement for systemic metabolic N-oxidation, oral administration induces tumors of the forestomach (4), bladder tumors on instillation into the urinary bladder (5-7), and tumors on direct injection into the peritoneal cavity (8-10) or mammary gland (11-12). These observations directly implicate a non-reactive N-oxidized compound in the transformation process within the target tissue by a putative metabolic activation event.

In contrast, diversion of potential candidates for metabolic activation can decrease tumor formation through their removal via pathways that preclude conversion to reactive metabolites in potential target tissues. For example, the formation of phenols usually decreases the carcinogenic potential of AA. In addition, both activation and metabolic diversion pathways may be regulated by hormonal and genetic factors, thereby providing opportunities for identifying and manipulation of pathways in the carcinogenic process (13,14). Metabolic activation and deactivation of AA are depicted in Figure 2.

3.1. Modification of protein by AA

Initial experiments with protein disclosed that quinone imines generated from the enzymatic or chemical oxidation of aminophenols in the presence of protein led to the formation of protein adducts and catalytic inactivation in the case of enzymes (15). Subsequent studies showed that analogous adduct formation with nucleic acids did not occur (16). These observations, and the failure of phenolic derivatives of AA to demonstrate significant toxicity or carcinogenicity, suggest that quinone imines would not be involved in the modification of nucleic acids of target

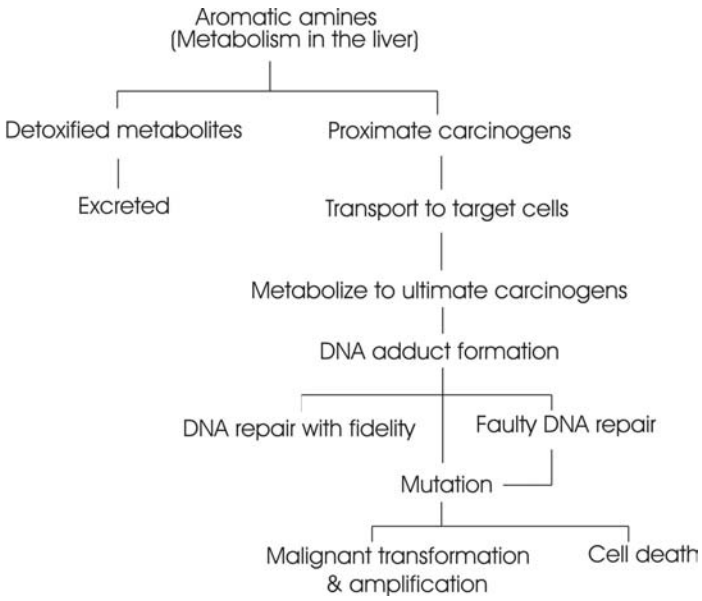

Figure 1. Proposed mechanism of malignant transformation by aromatic amines.

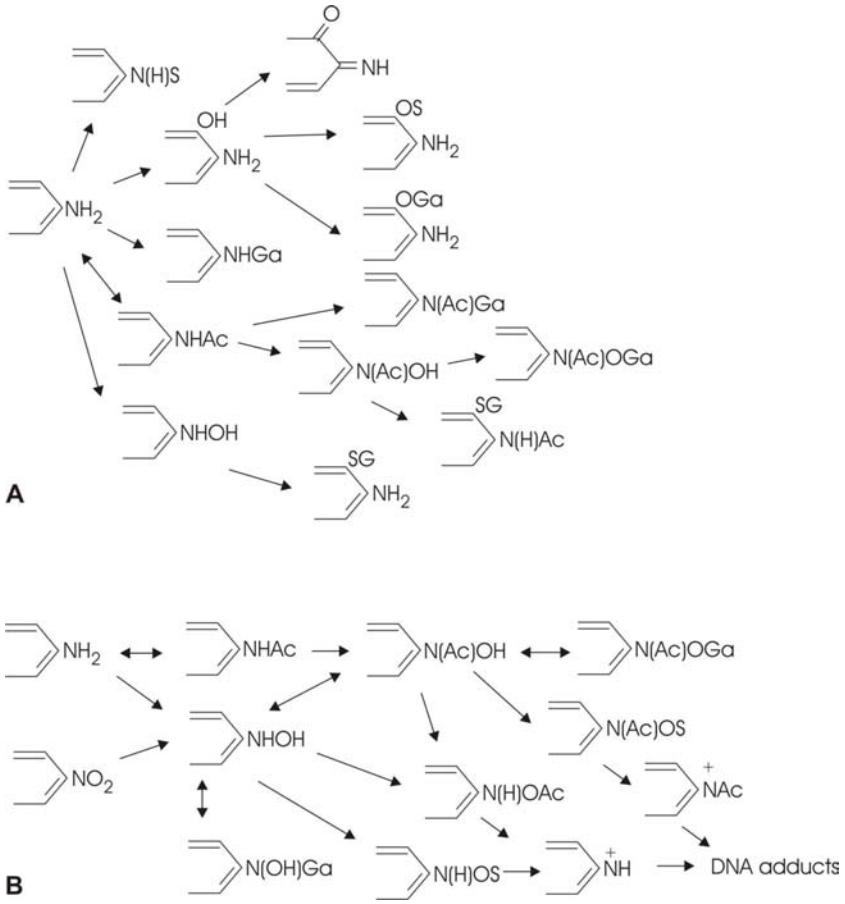

Figure 2. Metabolic pathways leading to the deactivation (A) and activation (B) of aromatic amines. O-Glucuronic acid conjugates of hydroxamic acids may be excreted as detoxified metabolites, but they can also be hydrolyzed and subsequently activated. They may, therefore, be regarded as a potential reservoir of N-oxidized AA that require terminal activation in target tissues, thereby simplifying the metabolic capacities required by target tissues for nucleic acid modification. Ga, S and G represent glucuronic acid, sulfate and glutathione, respectively.

tissues. They also point out that the ability of an AA metabolite to react with protein is not necessarily synonymous with their capacity to react with nucleic acid and, therefore, protein adduct formation is not an appropriate surrogate for the exploration of potentials of nucleic acid modification by AA.

3.2. Modification of nucleic acids by AA

What are the metabolites with the greatest potential for modification of the nucleic acid of target tissues? The most probable candidates for the ultimate carcinogens of AA are N-acetoxy or N-sulfate derivatives of arylhydroxylamines. The formation of the reactive sulfate conjugate of a hydroxamic acid, N-OH-AAF, an N-acetylated hydroxylamine, has been demonstrated, but this reaction may be limited primarily to the liver of some rats (17-19). Evidence for the enzymatic formation of analogous N,O-diacetyl derivatives of arylhydroxylamines is lacking.

3.3. Reactive nitrenium derivatives

3.3.1. Formation by cytosolic acyltransferases

Formation of reactive nitrenium derivatives from arylhydroxylamines can result from incubation in acidic solutions and by enzymatic O-acetylation. Though the acidic generation of reactive nitrenium species has been demonstrated (20,21), and has been useful in studying the modification of nucleic acid by AA, the reaction of hydroxylamines with cellular nucleic acids at physiological pHs is not convincing.

If one accepts the need for metabolic activation of arylhydroxylamines as a prerequisite for the tumorigenic potential of AA, the most striking example of the requirement for metabolic activation of arylhydroxylamines may be their modest mutagenicity in Salmonella systems that are not capable of enhancing their mutagenicity through O-acetylation (22).

The initial report of the enzymatic activation of an AA derivative to a metabolite capable of reaction of nucleic acid came from incubation of N-hydroxy-2-acetylaminofluorene (N-OH-AAF) with cytosols of rat liver (23). In the presence of nucleic acid, added to serve as a scavenger for activated products of the hydroxamic acid, enzyme-dependent adduct formation with loss of the acetyl moiety of the substrate was observed. Subsequent studies demonstrated the wide distribution of this enzyme activity in most tissues of humans and experimental animals in which AA induce tumors (24). Mechanistic investigations supported the conclusion that the production of adducts by this enzyme came from transfer of the N-acetyl group to the oxygen of the hydoxylamine, i.e. the production of an N-acetoxyarylamine derivative capable of reacting with nucleic acid. In addition, the enzyme could transfer the N-acetyl group to primary AA, i.e. the hydroxamic acid substrate was capable of serving as an acetyl donor as in systems that utilized acetyl-CoA as a donor (25). 3,5-Dimethylaniline, a suspected human bladder carcinogen, is probably activated by the same mechanism (26).

Carcinogenesis experiments demonstrated that direct injection of arylhydroxamic acids into rat mammary glands yielded mammary tumors, thereby avoiding systemic circulation and implicating tumor formation that was not dependent on metabolites produced in other tissues (11-12). Control experiments demonstrated that arylacetamides were not carcinogenic in this system. By varying the N-acyl group, such as compounds with N-formyl and N-propionyl groups, it was shown that compounds other than those carrying N-acetyl moieties also yielded nucleic acid adducts on incubation with enzymatic preparations (8,12). Tumor induction studies showed that different acyl-substituted arylhydroxamic acids were carcinogenic and that they exhibited different tumorigenic potentials (9,10).

Molecular biological approaches have elucidated the DNA sequence of rat arylhydroxamic acid acyltransferase. The nucleic acid sequence of the rat enzyme that activates arylhydroxamic acids is the same as the enzyme that can transfer acetyl groups from acetyl-CoA to the primary amino groups of many compounds. Expression in cellular systems demonstrated, as was the case with tissue-derived preparations, that the arylhydroxamic acid acyltransferase had the ability to transfer the N-acetyl group of the hydroxamic acid to primary arylamines as well as using acetyl-coA as an acetyl donor for the same reactions (27-30). Thus, designation as an N-acetyltransferase, or NAT as it is frequently abbreviated, is correct, but limited in that this nomenclature does not acknowledge the enzyme's abilities to utilize acyl substrates other than the acetyl group of CoA as acyl donors. Moreover, this nomenclature is incomplete in that it also fails to identify the enzyme's important ability as an arylhydroxamic acid acyltransferase in the modification of genetic components in the production of tumor cells by AA. Failure to recognize this enzymatic potential may well confuse attempts to assign the role of the enzyme in the carcinogenic process.

Nucleic acid modification by the products of activation by arylhydroxamic acid acyltransferase are likely to be involved in inducing tumors by AA, in that the predominant nucleic acid products are consistent with activation by this mechanism. Moreover, these adducts have been shown capable of generating mutations (31,32).

Exploration of the production of transcripts of arylhydroxamic acid acyltransferase in rat tissues by *in situ* hybridization revealed that it was widely distributed in organs often associated with carcinogenesis studies with AA, such as the liver, gastrointestinal tract, mammary gland, Zymbal gland of the ear duct, bladder, lung, kidney, ovary, uterus, fallopian tube, testes, epididymis, adrenal gland, and skin (33). In addition, evidence of transcripts in nasal turbinates, cornea, ciliary process, exorbital lacrimal gland, cerebral cortex, hypothalamus pineal gland, pituitary gland leptomeningeal membranes were also observed.

Demonstration of arylhydroxamic acid acyltransferase in such diverse tissues raises the possibility that, in addition to it's role in carcinogenesis, the enzyme

may play a role in hormonal regulation and neurotransmission. These possibilities were bolstered by the abilities of the expressed enzymes to N-acetylate tryptamine, 5-hydroxytryptamine and 5-methoxytryptamine (33,34).

Studies with human tissues have shown that arylhydroxamic acyltransferase can be demonstrated in liver, breast, prostate and urothelium, crucial observations if this mechanism of activation is to be regarded as a candidate for the carcinogenic process by AA (34,35).

3.3.2. Formation by microsomal acyltransferases

A second enzymatic system that can generate reactive O-acetylated hydroxylamines may also be important in the generation of tumors by AA. Early efforts that explored the physical characteristics of rat arylhydroxamic acyltransferase from rat tissue cytosols identified a second enzyme, initially in cytosols from the small intestine, that was also capable of activating arylhydroxamic acids (36). These two enzymes differed in their molecular weights and in their inhibition by organophosphates, compounds that have been used extensively to study esterases. The enzyme identified initially in rat liver cytosols was smaller and refractory to organophosphates; the larger acyltransferase from intestinal preparations was inhibited by organophosphates. The enzymes could also be differentiated by the failure of the paraoxon-sensitive enzyme to be precipitated with antibodies produced in rabbits by injection of rat liver arylhydroxamic actyltransferease.

Subsequent efforts explored metabolic activation systems in the dog (37,38), a species susceptible to urinary bladder tumor induction by AA that does not have the cytosolic acyltransferase described above. These experiments focused on the possibility that dogs might possess a paraoxon-sensitive acyltransferase that provided a biochemical rationale for the activation of AA by acyltransfer in the absence of the cytosolic enzyme. Three different enzymes were identified in preparations of dog liver micosomes. These enzymes were capable of the deacylation of amides, N-arylhydroxamic acids and carboxylesters, the acyltransfer of arylhdroxamic acids and the N-acetylation of AA. Monoclonal antibodies prepared against the dog enzyme that was homologous to carboxylesterase 1 of rabbit liver cross-reacted with pI 6.0 carboxylesterase of rat liver microsomes. Conversely, a polyclonal antibody raised against the rat esterase reacted with the dog enzyme. Immunohistochemical analyses demonstrated the presence of this enzyme in the epithelium of dog liver and urinary bladder, human liver and rat liver, esophagus, forestomach, glandular stomach, small and large intestines, renal tubules, trachea, prostate and alveolar cells of lung.

Experiments with solubilized guinea pig liver microsomes, a source of paraoxon-sensitive esterase, showed that they could activate arylhydroxamic acids, including that derived from phenacetin, an arylamide known to produce urinary tract tumors in humans (39).

3.3.3. Formation by cytosolic sulfotransferase

In contrast to arylhydroxylamines, arylhydroxamic acids, often identified as metabolites of carcinogenic AA, are more stable than arylhydroxylamines. and, therefore, capable of systemic transport to target tissues from the most likely organ of their formation, the liver. Chemical conjugation of the hydroxamic acid function with either sulfate or acetyl moieties yields products capable of reacting with nucleic acid. However, enzymatic O-acetylation of a hydroxamic acid has not been demonstrated. Since the enzymatic conjugation of arylhydroxamic acids with sulfate appears to be limited to the liver of certain rats, and the sulfate conjugates are sufficiently reactive as to limit transport from the liver, this pathway appears unlikely to participate in nucleic acid alteration in tissues outside of this organ (17,18). Even in tissues with the greatest capacities for activation through sulfate conjugation, the predominant DNA adduct is consistent with the activation of an arylhydroxylamine through O-acetylation, not through conjugation of an arylhydroxamic acid with sulfate. Many investigators, therefore, believe that the induction of liver tumors by AA compounds in tissues that can synthesize reactive sulfate conjugates from arylhydroxamic acids reflects cellular toxicities that serve to enhance genetic modifications elicited by AA. This potential toxicity is subject to gender differences, e.g. out-bred Sprague-Dawley male rats have high levels of a sulfotransferase capable of activating N-OH-AAF whereas females of this strain do not; liver tumors in Sprague-Dawley rats are essentially observed only in males (17). Hepatotoxicity is usually seen only in males of this strain. In contrast, inbred Fischer 344 rats of both genders have significant abilities to activate suitable substrates by sulfate conjugation and substrates capable of activation in Fischer rat liver are capable of inducing liver tumors in both males and females, and both exhibit hepatoxicity in response to N-OH-AAF (19). Neither gender of the Fischer rat is very susceptible to mammary tumor formation. In contrast, mammary tumors are readily induced in female Sprague-Dawley rats, but are rarely seen in male Sprague-Dawley animals. Thus, it can be argued that the Fischer female rat exhibits enzymatic characteristics of their male counterparts. Such dissimilar carcinogenic responses may reflect differences in the hormonal status that favors tumor formation in the livers of female Fischer animals and not in their mammary glands because they are exhibiting a male pattern of tumor induction.

3.3.4. Circumventing the need for enzymatic N-oxidation

N-Oxidized derivatives, crucial to the production of nitrenium ions, can also come from the reduction of arylnitro compounds, thereby circumventing the need for N-oxidation required for the activation of AA. For example, aryl compounds carrying nitro groups, being relatively stable, can avoid the requirement for N-oxidation and potentially be distributed in biological systems without degradation, where they can then be reduced to metabolites

capable of activation (40). This class of compounds is found in particulates generated by internal combustion engines and have very high carcinogenic potentials when injected intraperitoneally into rats (41-43). Such experiments demonstrated the direct effects of these nitrocompounds on target tissues. In mutagenicity studies with salmonella systems, it was shown that the mutagenicity of arylnitrocompounds was greatly enhanced in strains that had O-acetylation capabilities capable of activating arylhydroxylamines (44). These observations suggest that the carcinogenic properties of nitro-substituted arylcompounds reflect their reduction to hydroxylamines and subsequent activation by O-acetylation and further supports the idea that this pathway is causally involved in the carcinogenicity of AA.

3.4. Activation by cyclooxygenases

The activation of benzidine by prostaglandin H synthase (cyclooxygenases) may represent an example of an AA activation that does not require the production of an N-oxidized intermediate (45). However, this potential appears to be limited, in that substrates that were not diamines were essentially not activated by this metabolic system (46). Most of the oxidized benzidine binds to protein instead of DNA (47). The DNA-binding level of 4,4'-Methylene-bis(2-chloroaniline) is much greater than that of 4-aminobiphenyl when oxidized by a cyclooxygenase-mediated reaction (47), yet 4-aminobiphenyl is a much stronger carcinogen than 4,4'-Methylene-bis(2-chloroaniline) (1). Tumor induction mediated by cyclooxygenases has yet to be demonstrated.

3.5. What are the criteria for the production of tumors in humans?

3.5.1. Distribution of compounds that are precursors suitable for metabolic activation by the target tissue

AA have been shown to produce urinary tract tumors in humans, whereas AA in experimental animals have more frequently yielded tumors in the gastrointestinal tract and other organs. This may reflect species differences in thresholds for urinary and bile excretion, i.e. the urinary excretion of larger molecules being more readily accomplished in humans than in experimental animals. Statements have been made that humans only develop bladder tumors on exposure to AA. It is also possible that tissues not associated with tumor induction in other organs are not as readily evident because of exposures to a multiplicity of related compounds, some of which are excreted via urinary and/or biliary routes. Size differences of the AA may be one reason why some of the larger food-derived heterocyclic amines yield tumors of the gastrointestinal tract of experimental animals more frequently than in the urinary tract.

Tumor development in humans as a consequence of exposure to chemicals is usually identified with high exposures to a restricted number of compounds. However, given that tumor development usually takes place over a long period of time to a multiplicity of carcinogenic compounds, identification of the causative agent is made more difficult. A notable exception to the time from first exposure to tumor identification was found in exposures to chlornapthazine, a 2-naphthylamine derivative used to treat polycytemia vera, where tumors of the urinary tract were seen in as little time as 2.5 years with total amounts of the drug as low as 4 g (48). In contrast, exposure to low doses of a multiplicity of carcinogenic AA over a long period of time, such as with food-derived heterocyclic amines that can be activated by N-acetoxyarylamine formation by human tissues and, consequently, induce tumors, may obscure identification of the carcinogen because of the multiplicity of exposures and the extended time period involved in the induction of tumors. Conversely, the ambiguity of the agent and the extent of exposure also make more difficult the identification of just what tumors are induced by AA.

3.5.2 Metabolic activation

Given knowledge of the chemical reactivities and enzymatic systems capable of producing metabolites capable of modification of DNA described above, it is important to evaluate the capacities of human tissues to carry out these reactions and, hopefully, to establish their role in tumor induction in human tissues. Efforts in pathway identification and how these pathways may be involved in the carcinogenic process have taken many approaches, including the use of cell-free enzyme preparations, intact cells, tissue slices and intact animals. The techniques employed have utilized radioisotope and immunochemical-labeled compounds, structural analogues, histological approaches, tumor induction, unscheduled DNA synthesis, nucleic acid adducts, treatment *in situ*, mutagenicity and a host of other approaches. In the case of experimental animals the techniques employed can be more comprehensive than is possible with human tissues. However, with knowledge derived from metabolic capabilities related to nucleic acid modification and tumor induction in experimental animals, the presence of candidate pathways of metabolic activation in human tissues can often be evaluated using less invasive techniques.

In the case of AA, evidence of such pathways is greatest for those that are able to generate N-acetoxyarylamines. Given that two dissimilar enzyme systems (i.e. cytosolic paraoxon-resistant and microsomal paraoxon-sensitive enzymes) exhibit potentials for this type of activation and may be involved, their expressions in target tissues are unlikely to be reflected by the expression of a single enzyme that accounts for tumor production by AA in all tissues. Furthermore, it can be envisioned that a higher expression of one enzyme may actually moderate the biological effects of the other. For example, a higher capacity for acetylation may, through increased production of *N*-acetyl derivatives, reduce the levels of hydroxylamine substrate available for *O*-acetylation. Enhanced levels of phenol formation could decrease the levels of arylhydroxamic acid available for activation in the target tissues, essentially independent of the levels of enzymes capable of activation. Thus, studies to establish simple relationships between enzyme expression and tumor induction are problematic.

3.5.3. Fate of modified nucleic acid

Once metabolic activation has occurred, there are several possibilities that are likely to influence the

consequences of nucleic acid modification. Repair of a carcinogen-modified DNA structure could remove the compromised lesion, with fidelity, thereby restoring the genetic functionality and presumably eliminating the prospect of tumor induction.

Should DNA repair take place without retention of the normal genetic information, subsequent template authenticity could be compromised so as to lead to malignant transformation of the cell during subsequent replications. Similarly, replication of modified DNA from which the carcinogen-modified structures had not been removed could also result in the production of faulty DNA sequences that produced tumor cells. The neoplastic consequences of the failure to repair modified DNA has been brought home in the case of increased tumor formation in individuals with xeroderma pigmentosum.

Malignant transformation by chemical carcinogens as a consequence of genetic modification may involve mutation as well as gene expression such as p53 oncogene and cyclooxygenases (31,49,50), i.e. either loss of function or increased function of genetic elements.

3.5.4. Amplification of altered DNA templates

A key step in the malignant transformation of a cell is the amplification of cells with functionally-modified DNA templates that results in the loss of cellular growth controls. It can be envisioned that alterations in key biological processes might occur in cells that normally undergo replication, such as, for example, the mucosal lining of the gastrointestinal tract. This mechanism may well account for tumor formation in the gastrointestinal tract of rodents exposed to carcinogenic heterocyclic amines.

Other alternatives that might result in similar changes are cellular replications that come from hormonal stimuli. One example that this mechanism can be effective has been demonstrated in weanling female rats. Direct injection of suitable AA derivatives into the mammary gland at an appropriate time in the development of female rats leads to the development of mammary tumors. Such experiments have convincingly demonstrated that arylhydroxamic acids are the most effective AA-derived structures using this technique (10-12). One advantage of this technique is that neither tumor induction nor metabolism of the test compound is dependent on systemic effects.

A similar technique that involves hormonal modulation to increase tumor development has also been demonstrated in the study of prostate cancer induction in rats. However, in this case, the normally quiescent prostate gland fails to respond to treatment with 3,2'-dimethyl-4-aminobiphenyl, presumably because it did not normally undergo cellular replication in conventional carcinogen treatment protocols. In an attempt to elicit a carcinogenic response, Shirai *et al.* (51) showed that if the prostate gland were treated with estrogen, on cessation of estrogen-induced involution and treatment with the carcinogen during the period that the prostate returned to a normal differentiated state, prostate tumors were induced.

Experiments have also shown that female Sprague-Dawley rats undergoing partial hepatectomies developed neoplastic hepatic lesions when treated with a hydroxamic acid metabolite of 4-aminobiphenyl during the period that the liver was undergoing cellular division (9). These observations are consistent with the enhancement of hepatocarcinogenic effects of arylhydroxamic acid sulfate conjugates addressed above.

One can argue that the enhancing effect of carcinogenicity in these systems came from increased cell division following modification by the carcinogen, thereby reducing the time available for repair of the modified DNA of the carcinogen-treated cells.

3.5.5. Epidemiological considerations

Attempts to relate tumorigenic responses to the levels of expression of specific metabolic pathways must account for the levels of exposure to potentially damaging compounds as well as those pathways that decrease DNA damage. For example, low levels of N-oxidation, or higher levels of conjugation enzymes that decrease the distribution of precursors of metabolites that can be activated by target tissues, may be expected to decrease tumor formation. Given the importance of the generation of reactive nitrenium derivatives data detailed above, demonstration of the expression of N,O-arylhydroxamic acid acyltransferase in human bladder mucosa, prostate and mammary gland makes possible AA-induced DNA damage in these tissues. The expression of N,O-acyltransferase in these tissues and evidence of DNA adducts in human prostate tissue are consistent with activation of hydroxamic acids by N,O-acyltransfer and also support the involvement of this metabolic activation system in the induction of tumors by AA.

Many studies have been conducted to associate the induction of human cancers and with the specific metabolic capacity for the activation of carcinogens. Slow genotypes of NAT2 that carry a low capability of N-acetylation have been suggested to be associated with bladder cancer (52). On the other hand, rapid N-acetylation was associated with bladder cancer induced by benzidine (53). Carcinogenic heterocyclic amines that are resistant to N-acetylation (54-55) are activated by NAT2 (56). Thus, the chemical nature of the carcinogen must be considered when one attempts to elucidate its role in the carcinogenic process.

4. SUMMARY AND PERSPECTIVES

AA are likely to induce tumors as a consequence of the modification of nucleic acids of target cells. This modification comes from enzymatic conversion, within the target cells, of hydroxylamine derivatives that produce reactive N-acetoxy metabolites that combine with bases of DNA. Depending on the capacity and fidelity of DNA repair in these cells, and the interval between DNA damage and replication, cells with modified DNA can be produced. When amplified, these genomic alterations are responsible for the generation of cells that are no longer under normal

growth control mechanisms; they have undergone neoplastic conversion.

The effectiveness of this process can be altered by genetic factors that increase, or decrease, the generation of reactive metabolites crucial to tumor induction. These factors can reflect variations in the activation reactions in the target cell, or they can be metabolic capabilities that regulate the availability of suitable precursors for activation in the cell. Even the presence of non-AA compounds may be expected to have effects through their alteration of enzyme levels and competition with AA derivatives.

Further complicating the carcinogenic transformation process are the roles played by hormonal systems, which themselves are subject to genetic controls. The hormonal status of an animal can alter its metabolic capabilities, facilitate the initial replication of the modified DNA template to "stabilize" lesions introduced by the carcinogen and, from a longer term perspective, can provide the driving force for continued cell replication that will permit expression of a malignant phenotype. Other roles can be envisioned.

Regulations of these complex systems are under multiple genetic controls that seem unlikely to permit identification of a single, crucial metabolic step in the carcinogenic process. Although the controls that are possible in animals may be more amenable for analysis, attempts to assign a single unique role to any one of them in humans will be most difficult.

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Abbreviations: AA: aromatic amines; AAF: 2-acetylaminofluorene; N-OH: N-hydroxy; NAT: N-acetyltransferases

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