Interactions of Ingested Food, Beverage, and Tobacco Components Involving Human Cytochrome P4501A2, 2A6, 2E1, and 3A4 Enzymes

F. Peter Guengerich,[1] Tsutomu Shimada,[2] Chul-Ho Yun,[1] Hiroshi Yamazaki,[2] Kevin D. Raney,[3] Ricarda Thier,[1] Brian Coles,[3] and Thomas M. Harris[3]

[1]Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee; [2]Osaka Prefectural Institute of Public Health, Osaka, Japan; [3]Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, Tennessee

Human cytochrome P450 (P450) enzymes are involved in the oxidation of natural products found in foods, beverages, and tobacco products and their catalytic activities can also be modulated by components of the materials. The microsomal activation of aflatoxin B_1 to the *exo*-8,9-epoxide is stimlated by flavone and 7,8-benzoflavone, and attenuated by the flavonoid naringenin, a major component of grapefruit. P4502E1 has been demonstrated to play a potentially major role in the activation of a number of very low-molecular weight cancer suspects, including ethyl carbamate (urethan), which is present in alcoholic beverages and particularly stone brandies. The enzyme (P4502E1) is also known to be inducible by ethanol. Tobacco contains a large number of potential carcinogens. In human liver microsomes a significant role for P4501A2 can be demonstrated in the activation of cigarette smoke condensate. Some of the genotoxicity may be due to arylamines. P4501A2 is also inhibited by components of crude cigarette smoke condensate. The tobacco-specific nitrosamines are activated by a number of P450 enzymes. Of those known to be present in human liver, P4501A2, 2A6, and 2E1 can activate these nitrosamines to genotoxic products.—Environ Health Perspect 102(Suppl 9):49–53 (1994)

Key words: cytochrome P450, humans, glutathione *S*-transferase, aflatoxin B_1, urethan, cytochrome P450, arylamines, tobacco, *N*-nitrosamines, naringenin, flavones

Introduction

Many chemicals that are genotoxic or otherwise initiate cancers are not inherently dangerous in themselves but are activated into electrophilic forms through metabolism (*1*), often by the cytochrome P450 (P450) enzymes (*2–4*) (Table 1). A number of lines of epidemiological evidence argue that diet is a major contributor to cancer risk (*5*). Other important variables include societal habits such as smoking, other consumption of tobacco, and overuse of alcoholic beverages (*5,6*).

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Address correspondence to Dr. F. Peter Guengerich, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232-0146. Telephone (615) 322-2261. Fax (615) 322-3141.

There is a considerable body of evidence indicating that alterations in the pool of P450 enzymes can dramatically influence tumor incidence in experimental animals (*7*); in humans, the situation must still be considered hypothetical (*8*). Nevertheless there is ample evidence that *in vivo* disposition of drugs in humans can be influenced by diet (*9*) and some of the dietary effects on tumor incidence may be explainable in the context of our knowledge regarding individual P450 enzymes. However, the analysis of situations involving complex mixtures presents special problems for consideration.

Results and Discussion

Aflatoxin Metabolism and P4503A4

Aflatoxin B_1 is one of the most notorious mycotoxins that contaminate human foodstuffs and poses a problem of serious importance in many parts of the world (*10*). We have investigated various aspects of the metabolism (Figure 1) of this compound in human liver microsomes and have shown that P4503A4 can play a major role in its oxidation to the 8,9-epoxide (*11,12*).

Recently we have found that P450 can catalyze two oxidations of aflatoxin B_1, the 8,9-epoxidation and the 3α-hydroxylation (*12*). The former reaction renders the compound dangerous while the latter (formation of aflatoxin Q_1) is a detoxication step. While both reactions appeared to be attributable to P4503A4, the addition of 7,8-benzoflavone (α-naphthoflavone) was shown to enhance the 8,9-epoxidation, as expected (*11–13*), but to inhibit 3α-hydroxylation. Although these results seemed unusual, the same pattern was demonstrated with the recombinant enzyme (Figure 2). Thus the result has been interpreted in terms of an allosteric effect of the flavone on an extrasubstrate binding site in P4503A4 (*12*). Such effects of flavonoids are not only of academic but also practical interest; flavonoids are commonly found in foods, and Conney and his associates have demonstrated the direct stimulatory effects of such compounds *in vivo* in experimental animals (*14*).

The major stereoisomer of aflatoxin B_1 8,9-epoxide is known to be the *exo* form shown in Figure 1. However, we have recently demonstrated that the *endo* isomer

Table 1. Prooxicants and procarcinogens activated by human P450s [a].

P-4501A1	Benzo[*a*]pyrene and other polyclyclic hydrocarbons	2-Amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine (PhIP)
P-4501A2	2-Acetylaminofluorene 2-Aminofluorene 2-Aminoanthracene 2-Amino-3-methylimiazo[4,5-*f*]quinoline (IQ) 2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoline (MeIQ) 2-Amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx) 2-Amino-3,4,8-trimethylimidazo-[4,5-*f*]quinoxaline (DiMeIQx) 2-Amino-6-methyldipyrido-[1,2-*a*:3′,2′-*d*]imidazole (Glu P-1)	3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp P-2) 2-Aminopyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu P-2) 2-Naphthylamine 4-Aminobiphenyl 2-Amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP) Acetaminophen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) 6-Nitrochrysene
P-4502A6	*N*-Nitrosodimethylamine *N*-Nitrosodiethylamine *N*-Nitrosonornicotine *N*-Nitrosonornicotine (NNN)	4-(Methylnitrosamino)-1-(3-pyridyl) 1-Butanone (NNK) 4,4′-Methylene-bis(2-chloroaniline) (MOCA) 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)
P-4502E1	*N*-Nitrosodimethylamine *N*-Nitrosodiethylamine *N*-Methyl-*N*-benzylnitrosamine *N*-Nitrosnornicotine (NNN) 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) Chloroform Methylene chloride Trichloroethylene Ethylene dichloride Ethylene dibromide 1,2-Dichloropropane	*N*-Methyl-*N*-butyl-1-nitrosamine Benzene Carbon tetrachloride Styrene 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) Vinyl chloride Vinyl bromide Acrylonitrile Ethyl carbamate Vinyl carbamate Acetaminophen
P-4503A4	Aflatoxin B_1 Aflatoxin G_1 Sterigmatocystin Senecionine 7,8-Dihydroxy-7,8-dihydrobenzo[a]pyrene 9,10-Dihydroxy-9,10-dihydroxobenzo[b]-fluoranthene	3,4-Dihydroxy-3,4-dihydro-7,12-Dimethylbenz[*a*]anthracene 6-Aminochrysene *tris*-(2,3-Dibromopropyl)phosphate 1-Nitropyrene 17b-Estradiol

[a] The purpose of this table is to summarize evidence for a major role of a single (human liver) P450 in the activation of each chemical. Of course, other P450s may also contribute to some extent, particularly in some individuals where the major enzyme is deficient. In some cases a chemical appears in ≥2 categories if evidence exists for substantial roles in these cases. For further discussion see the text and Guengerich and Shimada (*4*).

is formed as a minor product in chemical oxidations and in microsomes (*15*). Mouse liver cytosol glutathione (GSH) S-tranferases react essentially only with the *exo* isomer, while rat liver GSH S-tranferases actually show greater efficiency in the reaction with the *endo* form (*16,17*). We have found that rat and human liver microsomal P450s form very little of the *endo* epoxide (<1%) but in some human liver microsomal preparations the extent of *endo*-8,9-epoxide formation is sometimes as much as that of *exo* (Figure 3). The patterns indicate that P4503A4 is probably not the enzyme forming the *endo* epoxide; another (unknown) P450(s) apparently transfers oxygen to this face of the olefin. The *endo* epoxide appears not to be capable of forming DNA adducts and is not genotoxic in *Salmonella typhimurium* TA100 or in the *umu* test (<2% activity of the *exo* epoxide). Thus, the stereochemistry can lead to a remarkable difference in biological activity; however, the ready formation of the *endo* epoxide and its GSH conjugate by some human liver enzymes suggests caution in the use of biomarkers such as GSH conjugates and mercapturic acids in considerations of risk.

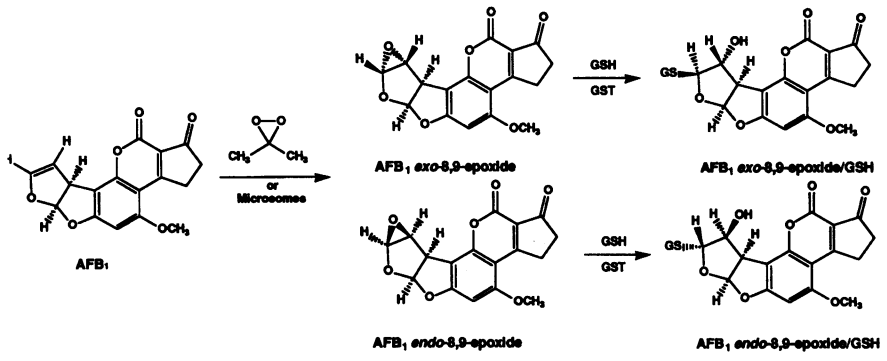

Figure 1. Oxidation of aflatoxin B_1 to *exo* and *endo*-8,9-epoxides and conjugation with GSH.

Not all flavones stimulate aflatoxin epoxidation (*13*). Recently Bailey et al. (*18*) have demonstrated a remarkably strong inhibition of *in vivo* oxidation of the dihydropyridine drugs nifedipine and felodipine by relatively modest amounts of grapefruit juice (but not orange juice). The major natural product in grapefruit is naringin (Figure 4), which can constitute up to 10% of the dry weight and is responsible for the bitter taste. When ingested it is readily hydrolyzed to naringenin (Figure 4), which has been shown to inhibit the oxidations of both nifedipine and aflatoxin B_1 in human liver microsomes (*20*). The potency of naringenin for P4503A4 inhibition appears similar to that of cimetidine (*21*); the questions of whether naringenin is responsible for the observed *in vivo* effects and whether aflatoxin metabolism can be modulated *in vivo* remain unanswered.

P4502E1, Ethanol, and Ethyl Carbamate

Early studies with rodents suggested that liver microsomal P450s might be involved in ethanol oxidation and also be induced by ethanol consumption (*22–24*). Ultimately research in this area led to the discovery of P4502E1 (*25,26*). Whether or not the oxidation of ethanol by P4502E1 is a major factor in its disposition has not been clearly demonstrated but it is clear that ingestion of ethanol (or certain other compounds) can lead to increases in the level of the enzyme, even in humans (*27*). However, ethanol is also an inhibitor of the enzyme, so experiments on induction are complex and must be interpreted carefully.

It is of interest that P4502E1 has been shown to contribute to the oxidation of numerous small chemicals, many of which are cancer suspects, in human liver microsomes (*26,28*). These interactions are probably of significance in that numerous interactions with ethanol in experimental

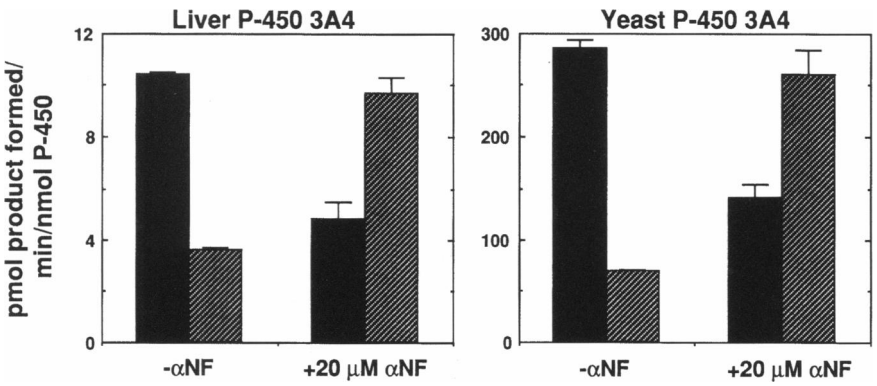

Figure 2. Effect of 7,8-benzofluorene (α-naphthoflavone, αNF) on the oxidation of aflatoxin B_1 by human P4503A4. P4503A4 was purified from human liver microsomes (Liver P4503A4) or microsomes prepared from *Saccharomyces cereresiae* expressing the cDNA clone NF 25 (yeast P4503A4) and reconstituted with rabbit NADPH-P450 reductase and L-a-1,2-dilauroyl-*sn*-glycero-3-phosphocholine, with a 50 μM concentration of the substrate aflatoxin B_1. Aflatoxin Q1 (solid black bar) and aflatoxin B_1 8,9-oxide (diagonal-lined gray bar) were quantified, with the latter being trapped as the GSH conjugate with the use of mouse liver cytosol in the incubation mixture (*12*).

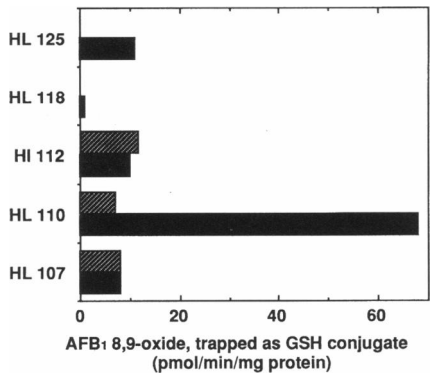

Figure 3. Rates of oxidation of aflatoxin B_1 to *exo* and *endo* epoxides by microsomes prepared from different human liver (HL) samples. The substrate concentration was 16 μM and the diastereometric GSH conjugates were quantitatively trapped in the presence of mouse (*exo*) or rat (*endo*) liver cytosol and separated by HPLC as described elsewhere (*12*).

animals can be explained in this way (*26*). One substrate of P4502E1 is ethyl carbamate (urethan), which is of interest because of its widespread use as an injection vehicle for patients in Japan and its presence in alcoholic beverages, especially the so-called stone brandies (*29*). Recently we have been able to demonstrate the *in vitro* formation of vinyl carbamate from ethyl carbamate (*30*), thus providing support for the pathway originally proposed by Dahl et al. (*31*) (Figure 5). The carcinogenicity of ethyl carbamate in humans is still unproven and the impact of this pathway is yet unknown. However, the research in this area has shown the potential for ethanol as an inducer and inhibitor of P450s and the genetic potential of chemicals in alcoholic beverages that require activation by P450 modulated by ethanol.

Tobacco Components and P4501A2, 2A6, and 2E1

Obviously tobacco has been a target of interest because of the extensive epidemiology supporting its role in the production of several human cancers. However, there are thousands of chemicals present in tobacco and tobacco smoke, and most major classes of chemical carcinogens are represented (*6*). Determining which are most significant and the specific enzymes that contribute to activation processes have been very challenging tasks.

We carried out a series of studies with a standard cigarette smoke condensate and human liver microsomes (*32*). It was found that (unknown) components of crude cigarette condensate were quite inhibitory to P4501A2, more dramatically than to other P450s. Fractionation of the condensate with "blue cotton" (copper phthalocyanine cellulose) removed may of the inhibiting components and yielded material which could be activated by human liver microsomes to products genotoxic in the *S. typhimurium umu* assay. We found that rat, rabbit, and human liver P4501A2 enzymes were all rather effective in this activation and that some other human liver P450s were not. P4501A2 has also been demonstrated to activate many individual carcinogenic arylamines (*19*). Many of these are prominent in tobacco, as well as in pyrolyzed meat, and the possibility can be considered that they are major contributors to the genotoxicity of tobacco, after activation by P4501A2 and other enzymes. Indeed, both cigarette smoking and several industrial arylamines have been implicated in human bladder cancer.

Another class of known carcinogens found in tobacco is the nitrosamines. The metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is complex and a brief outline is presented in Figure 6 (*33*). At least three different reactions yield reactive alkyl diahydroxides, and elucidation of precise roles of particular chemicals and enzymes is difficult, even starting with a single compound and the apparent absence of issues of stereochemistry. In a series of studies done in collaboration with Prof C-S. Yang and others found that human P4501A2 played a role in the oxidation of NNK to the specific stable end products shown in Figure 6 (*33*). However, it is not known with certainty which of these particular entities is most genotoxic in any of the various assays or in tumor bioassays.

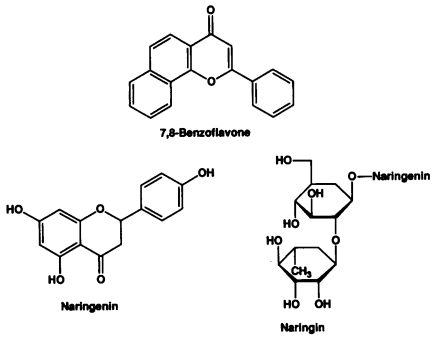

Figure 4. Structures of flavones shown to modulate the oxidation of aflatoxin B_1 by human P4503A4 (*11–13,19,20*).

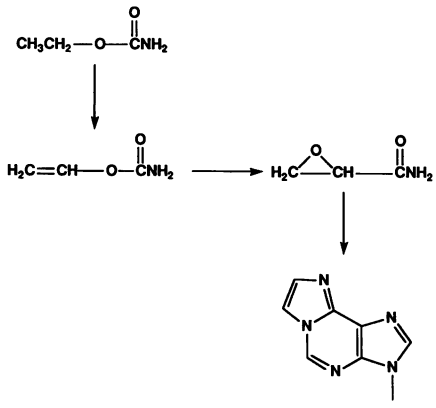

Figure 5. Postulated scheme of activation of ethyl carbamate and reaction of vinyl carbamate epoxide with adenine residues to yield 1,N^6-ε-adenine derivatives (*30,31*).

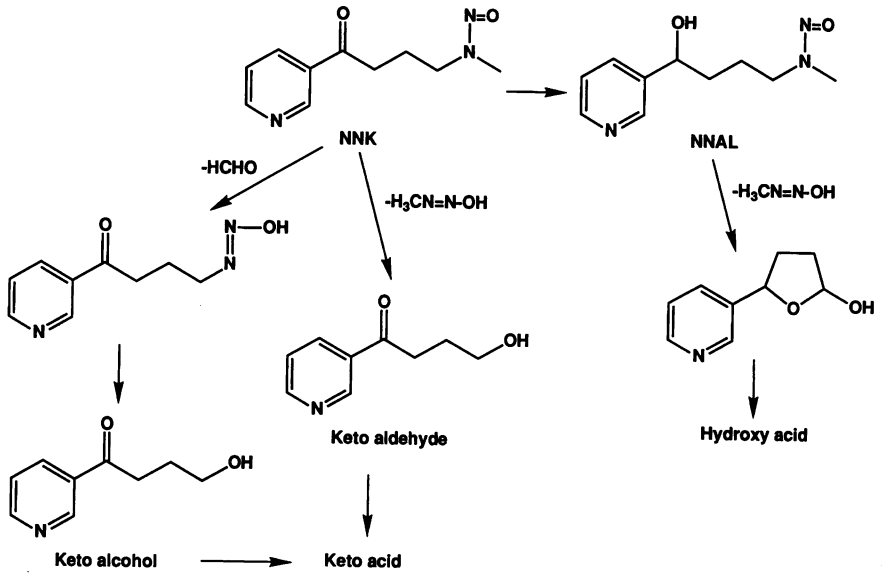

Figure 6. Major pathways postulated for the metabolism of the tobacco-specific nitrosamine NNK (*33*).

In general, the genotoxic response to nitrosamines in *in vitro* assays is poor, and it does not appear that the problem is a deficiency in enzymatic oxidation. Indeed, in our early work with the *S. typhimurium umu* assays we did not find an appreciable response to any of the nitrosamines we examined (*19*). Recently we have found that overexpression of bacterial acetyl transfer activity in the bacterium (*S. typhimurium* NM2009) renders it not only much more sensitive to *N*-hydroxy arylamines but to oxidized nitrosamines as well (*34*). We feel that the most likely explanation is the acetylation of the alkyl diazohydroxide (Figure 7). In support of this view, the acetyl derivative (of methyl diazohydroxide), when activated by an esterase preparation, also yielded an enhanced response in the overexpressing strain (*34*).

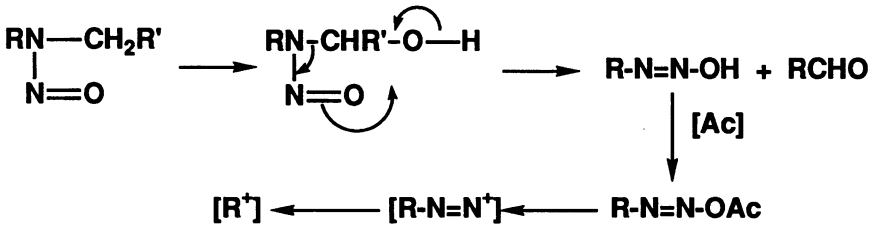

Figure 7. Scheme depicting postulated enhancement of nitrosamine genotoxicity by acetyltransferase in *S. typhimurium* (*34*).

We utilized this system in the examination of a series of nitrosamines, including *N*-nitrosdimethylamine, *N*-nitrosodiethylamine, NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and *N*-nitrosonornicotine, the latter three being particular to tobacco. Experiments involved the use of purified human P450 enzymes, correlation of catalytic activities in different human liver microsomes, and chemical and antibody inhibition of activity (*umu* test) with human liver microsomes (*35*). The results indicate that with all five nitrosamines P4502E1 and P4502A6 appear to have significant roles in activation; P4501A2 also appeared to make some contribution, but no evidence for roles of P4503A4 and P4502C enzymes was found (*35*).

Conclusions

The possible interactions involving substrates, inhibitors, and inducers in complex mixtures are considerable. Such is the case with foodstuffs, tobacco, and alcoholic beverages. Even with a single compound (e.g., aflatoxin B_1) there is potential for different enzymes to generate stereoisomers (of an epoxide) with dramatically different biological activities. Although these may be many chemicals and enzymes to consider in understanding mixtures, it may still be possible to develop rational plans to understand interactions. Some of the approaches we have used in our own work include *in vitro* assays, simplified biological end points even if the chemical basis for the response is not yet known in every case, and strategies designed to identify the most important microsomal and cytosolic enzymes contributing to an *in vitro* response.

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