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. Harris3

1Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee; 2Osaka Prefectural Institute of Public Health, Osaka, Japan; 3Department 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 B1 to the exo-8,9-epoxide is stimulated 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 nitroasamines are activated by a number of P450 enzymes. Of those known to be present in human liver, P4501A2, 2A6, and 2E1 can activate these nitroasamines to genotoxic products.—Environ Health Perspect 102(Suppl 9):49–53 (1994)

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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 argues 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).

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 B1 is one of the most notorious mycotoxins that contaminate human food-stuffs 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 B1, the 8,9-epoxidation and the 3α-hydroxylation (12). The former reaction renders the compound dangerous while the latter (formation of aflatoxin Q1) is a detoxication step. While both reactions appeared to be attributable to P4503A4, the addition of 7,8-benzo-2-alkylflavone (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 extracellular 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 B1 8,9-epoxide is known to be the exo form shown in Figure 1. However, we have recently demonstrated that the endo isomer
is formed as a minor product in chemical oxidations and in microsomes (15). Mouse liver cytosol glutathione (GSH) S-transferases react essentially only with the exo isomer, while rat liver GSH S-transferases 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 site 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.

Not all flavones stimulate aflatoxin epoxidation (13). Recently Bailey et al. (18) have demonstrated a remarkably strong inhibition of in vivo oxidation of the dihydropriaplyridine 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 B1 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

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Table 1. Proximates and procarcinogens activated by human P450s

| P4502A6 | N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
|--------|-----------------------|--------------------------------------------------|
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
| N'-Nitrosodimethylamine | 4-(Methylisatinraino)-1-(3-pyridyl)-1-butanol (NNAL) |
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 many 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 dihydroxides, 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.
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 arylnitrosamines 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).

We utilized this system in the examination of a series of nitrosamines, including *N*-nitrosothymylamine, *N*-nitrosodiethylyamine, NNK, 4-(methylamino)-1-(3-pyridyl)-1-butanol (NNAL), and *N*-nitrosornornicotine, 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 B1) 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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