ABSTRACT

The capacity of human liver microsomes from 28 individuals to metabolize debrisoquine and bufuralol, two drugs oxidized polymorphically in humans, as well as the carcinogen 2-acetylaminofluorene (AAF), was determined. In addition, the cytochrome P-450 content and the capacity of these microsomes to carry out the epoxidation of aldrin were measured. Interindividual differences in debrisoquine 4-hydroxylation, bufuralol 1-hydroxylation, and aldrin epoxidation were 12-20- and 2.4-fold, respectively. The metabolism of debrisoquine was not correlated with cytochrome P-450 content \((r = 0.26)\), whereas both the metabolism of bufuralol \((r = 0.45; r^2 = 0.20)\) and the epoxidation of aldrin \((r = 0.72; r^2 = 0.52)\) were correlated. Rates of debrisoquine and bufuralol metabolism were significantly correlated \((r = 0.73)\), whereas only weak correlations existed between debrisoquine:aldrin \((r = 0.49)\) and bufuralol:aldrin \((r = 0.51)\). Because biphase kinetics have been observed in human liver microsomes for the 7- and 5-hydroxylation of AAF, two concentrations of this substrate were used. The disappearance of AAF at either 0.37 or 50 \(\mu M\) was not correlated with debrisoquine, bufuralol, or aldrin metabolism. Similarly, at 0.37 \(\mu M\) AAF, no correlation existed between the formation of N-, 1-, 3-, 5-, 7-, and 9-hydroxylation products of AAF and debrisoquine, bufuralol, or aldrin metabolism. At 50 \(\mu M\) AAF, only the 7-hydroxylation of this substrate correlated with bufuralol metabolism \((r = 0.47)\). This lack of, or weak correlation between pathways leading to metabolic activation (N-hydroxylation) or detoxication (C-hydroxylation) of the carcinogen AAF and debrisoquine, bufuralol, and aldrin metabolism strongly suggests that different forms of cytochrome P-450 are involved in these pathways. In contrast, exceptionally high correlations \((r > 0.94)\) existed between N-OH-AAF:1-OH-AAF, N-OH-AAF:7-OH-AAF, and 7-OH-AAF:1-OH-AAF at the low concentration of AAF, and imply that similar forms of cytochrome P-450 produce these metabolites. However, at 50 \(\mu M\) AAF, these correlations are considerably weaker and explain less than 35% of the variance in the data. It is concluded, based on these multiple cross-correlations, that common cytochrome P-450 isoforms are involved in the formation of AAF metabolites; while the metabolism of debrisoquine, bufuralol, and aldrin is unrelated to the metabolism of this carcinogen in human liver microsomes.

INTRODUCTION

Since most chemical carcinogens are stable, nonreactive compounds (also called procarcinogens), initial metabolic activation of these compounds is required to form highly reactive electrophilic derivatives which are then capable of reacting with nucleophilic sites on cellular macromolecules \((12, 33, 41)\). The cytochrome P-450-dependent monooxygenase system catalyzes the initial metabolic activation of most procarcinogens and, consequently, attempts have been made to correlate both content and enzymatic activity of this enzyme with the incidence of cancer \((1, 11, 14-16, 24-26, 28, 34, 38, 40)\).

In both laboratory animals \((17, 20, 27)\) and humans \((3)\), the cytochrome P-450 system comprises a family of isoforms which differ in both substrate and positional specificity for the metabolism of exogenous and endogenous substrates \((8, 13, 15, 21, 31, 44)\). Recent studies in humans have demonstrated genetic variability in cytochrome P-450-mediated drug oxidations, particularly in the 4-hydroxylation of the antihypertensive agent debrisoquine \((10, 19)\). Two distinct phenotypes have been observed for the 4-hydroxylation of debrisoquine, designated extensive and poor metabolizers, and a number of other drugs have been shown to be under the same monogenic control \((10, 19)\). The frequency of the poor metabolizer phenotype varies between 2 and 9% in Caucasian populations \((10, 19)\). Poor metabolizers of debrisoquine and related drugs metabolize other substrates such as antipyrine, tolbutamide, acetanilide, and theophylline at normal rates \((6, 10)\). The oxidative defect in debrisoquine metabolism has been linked to a deficiency or absence of a specific form of hepatic cytochrome P-450 \((4)\).

The poor metabolizer phenotype has now been associated with increased pharmacodynamic and toxicological effects of some drugs in humans \((10)\). In contrast, Idle et al. \((18)\) have recently shown that a group of cancer patients contain a disproportionate number of extensive metabolizers of debrisoquine as compared to controls. These authors infer that the extensive metabolizer phenotype would be more likely to develop tumors, due to their enhanced ability to activate chemical carcinogens. To test this hypothesis, we have assessed the capacity of human liver microsomes from 28 individuals to metabolize debrisoquine and bufuralol, 2 drugs that show genetic variability in cytochrome P-450-dependent metabolism in humans characterized by extensive and poor metabolizer phenotypes, as well as the hepatocarcinogen 2-acetylaminofluorene. In addition, we have determined aldrin epoxidation as an indicator of the activity of another, unrelated, isoform of cytochrome P-450 \((43)\) and measured the cytochrome P-450 content of each microsomal preparation. Multiple cross-correlations of these parameters revealed possible common cytochrome P-450 isoforms involved in the formation of 2-acetylaminofluorene metabolites and indicated that debrisoquine, bufuralol, and aldrin metabolism are unrelated to the metabolism of this carcinogen.
MATERIALS AND METHODS

Chemicals. Randomly labeled [3H]AAF2 (18 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA) and was purified to greater than 98% purity by high-pressure liquid chromatography (30). Unlabeled AAF and 2-aminofluorene were obtained from Eastman Organic Chemicals (Rochester, NY). Authentic N-OH-AAF and 1-OH-AAF were generously donated by Dr. Elizabeth Weisburger, National Cancer Institute, and 3-OH-AAF, 5-OH-AAF, 7-OH-AAF, 9-OH-AAF, and 2-acetylaminofluorene-9-one were prepared as described previously (35). Desferrioxamine mesylate was purchased from Ciba Pharmaceuticals (Summit, NJ) and NADPH from Sigma Chemical Co. (St. Louis, MO). Debrisoquine hemisulfate, 4-hydroxydebrisoquine hemisulfate, bufuralol hydrochloride, and 1-hydroxybufuralol were generously provided by Roche Products, Ltd. (Welwyn Garden City, United Kingdom); [3H]-4-hydroxy-debrisoquine was prepared biologically as described previously (22). Aldrin and the epoxide of aldrin, dieldrin, were a gift from the Shell Toxicology Laboratory (Sittingbourne, United Kingdom).

Human Tissue Samples. Microsomal fractions of human liver were obtained as described previously (4), either from wedge biopsy samples taken at laparotomy or from samples of liver of renal transplant donors maintained on life-support systems until the kidneys could be removed. The use of such tissue in these studies had local Research Ethics Committee permission and, where appropriate, coroner’s permission. Samples were stored at -80° until required, during which time there was no loss of activity. Liver samples from renal transplant donors had activities very similar to that of wedge biopsy samples with normal histology (22), and therefore no further distinction has been made between the 2 groups of samples in this study.

Xenobiotic Assays. AAF metabolism was measured essentially as described previously, except sodium fluoride was omitted from the incubation mixture (30). Concentrations of AAF used were 0.37 and 50 μM, and microsomal protein contents per ml of incubation mixture were 25 and 100 μg, respectively, at these concentrations. Debrisoquine 4-hydroxylase and bufuralol 1-hydroxylase activities were measured according to the methods of Kahn et al. (22) and Boobis and Davies (3), respectively. Aldrin epoxidation was measured according to the method of Wolff et al. (39). Incubation conditions used gave reaction rates that were zero order with respect to cofactor and linear with respect to protein concentration and time. Multiple cross-correlations were calculated as described previously (37).

RESULTS

The cytochrome P-450-mediated metabolism of AAF involves oxidation at both nitrogen and carbon atoms; the former leads to metabolic activation, while hydroxylation at positions 1, 3, 5, 7, and 9 on the fluorene ring is considered a detoxication pathway (36, 42). Recently in this laboratory, it was shown that the relative quantity of each metabolite of AAF produced by human liver microsomes and therefore the balance between metabolic activation (N-hydroxylation) and detoxication (C-hydroxylation), varied markedly with substrate concentration (29, 30). Thus, in order to assess the capacity of liver microsomes from 28 human subjects to metabolize AAF, 2 concentrations of substrate were used. A concentration of 0.37 μM AAF was chosen to detect those forms of cytochrome P-450 that have a high affinity for AAF, while a 50 μM concentration was used to detect lower affinity forms. The rate of AAF deacetylation was slow, and did not affect the rate of N- and ring-hydroxylations (data not shown).

Chart 1C shows the frequency distribution of cytochrome P-450 in human liver microsomes from 28 subjects. The mean cytochrome P-450 content was 0.37 ± 0.11 nmol per mg liver microsomal protein (mean ± S.D.), and a plot of the data approximated a normal distribution. No correlation existed between AAF disappearance at either the low (r = -0.21) or high (r = 0.027) substrate concentration used and cytochrome P-450 content. Chart 1, A and B shows the frequency distribution of AAF disappearance at 0.37 and 50 μM, respectively. A 5.7-fold variation in AAF disappearance was observed between subjects at 0.37 μM AAF, whereas at 50 μM, the interindividual variation was 2.6-fold. Within the constraints of the number of microsomal fractions tested, no evidence for polymorphism in AAF disappearance was apparent.

The frequency distribution of the rate of formation of different metabolites of AAF at 0.37 μM are shown in Chart 2. A 50- and 55-fold variation was observed in the rate of formation of both N-OH-AAF (Chart 2A) and 1-OH-AAF (Chart 2B), respectively. While this marked difference in metabolism between subjects was skewed by a few individuals, the differences in rates observed at this concentration for all metabolites were generally greater than the variability in AAF disappearance. Except for the subject exhibiting the high rate of 3-OH-AAF formation (Chart 2C), efficient producers of one metabolite were...
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Chart 2. Frequency distributions for the formation of 2-acetylaminofluorene metabolites in human liver microsomes. AAF concentration was 0.37 μM. A, N-OH-AAF; B, 1-OH-AAF; C, 3-OH-AAF; D, 5-OH-AAF; E, 7-OH-AAF; F, 9-OH-AAF.

Table 1

Correlation coefficients for AAF metabolites formed with human liver microsomes
For 26 d.f., r > 0.37; p < 0.05. The incubation mixture contained 0.37 μM AAF.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>9-OH-AAF</th>
<th>5-OH-AAF</th>
<th>3-OH-AAF</th>
<th>1-OH-AAF</th>
<th>N-OH-AAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-OH</td>
<td>0.84</td>
<td>0.79</td>
<td>0.37</td>
<td>0.97</td>
<td>0.94</td>
</tr>
<tr>
<td>9-OH</td>
<td>0.75</td>
<td>0.24</td>
<td>0.77</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>5-OH</td>
<td>0.30</td>
<td>0.80</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-OH</td>
<td>0.27</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.98</td>
</tr>
</tbody>
</table>

was determined by the sample:blank ratio. At >5 μM concentrations of AAF, the specific activity of the substrate AAF was insufficient to accurately determine the quantity of these metabolites formed. Intersubject variation in the rate of metabolism was similar to the variability in substrate disappearance at 50 μM AAF (Chart 1B). For example, the rates of formation of N-OH-AAF and 1-OH-AAF varied 6.8- and 10.6-fold, respectively. The data indicate that 2 populations of cytochrome P-450 may exist for the formation of several AAF metabolites, in particular N-OH-AAF and 7-OH-AAF. Correlations between rates of metabolite formation at 50 μM AAF were considerably weaker than those at the low AAF concentration, suggesting different populations of cytochrome P-450 were involved in the formation of these metabolites at the higher substrate concentration (Tables 1 and 2). Except for a weak correlation between 9-OH-AAF:AAF (r = 0.56), no relationship was apparent between AAF disappearance and the formation of any individual metabolite at this concentration. A significant correlation was observed between 7-OH-AAF:cytochrome P-450 (r = 0.74), whereas similar correlations for other metabolites were considerably weaker.

The rates of formation of the different metabolites of AAF plotted against debrisoquine 4-hydroxylation, bufuralol 1-hydroxylation, and aldrin epoxidation are depicted in Charts 4 and 5. Interindividual differences ranged from 12- and 20-fold for debrisoquine and bufuralol metabolism, respectively, to 2.4-fold for aldrin epoxidation. The 4-hydroxylation of debrisoquine was not correlated with cytochrome P-450 content (r = 0.26), whereas both the 1-hydroxylation of bufuralol (r = 0.45) and the epoxi-
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Chart 4. Comparison of 2-acetylaminofluorene metabolism at 0.37 µM with debrisoquine 4-hydroxylation (A to F), bufuralol 1-hydroxylation (G to L), and aldrin epoxidation (M to R) in human liver microsomes. A, G, and M, N-OH-AAF; B, H, and N, 1-OH-AAF; C, I, and O, 3-OH-AAF; D, J, and P, 5-OH-AAF; E, K, and Q, 7-OH-AAF; F, L, and R, 9-OH-AAF.

Chart 5. Comparison of 2-acetylaminofluorene metabolism at 50 µM with debrisoquine 4-hydroxylation (A to D), bufuralol 1-hydroxylation (E to H), and aldrin epoxidation (I to L) in human liver microsomes. A, E, and I, N-OH-AAF; B, F, and J, 1-OH-AAF; C, G, and K, 7-OH-AAF; D, H, and L, 9-OH-AAF.

The disappearance of AAF at either the low or high substrate concentration used was not correlated with debrisoquine, bufuralol, or aldrin metabolism. Similarly, at 0.37 µM AAF, no correlation existed between the rate of formation of any AAF metabolite and the latter 3 parameters (Chart 4). At 50 µM AAF, only the 7-hydroxylation of AAF correlated with bufuralol metabolism (r = 0.47) (Chart 5). The correlation coefficients for the N-hydroxylation of AAF at 0.37 and 50 µM AAF with debrisoquine 4-hydroxylase were -0.09 and -0.21, respectively, with bufuralol 1-hydroxylase at 0.15 and 0.06, respectively, and with aldrin epoxidation at -0.21 and -0.11, respectively.

DISCUSSION

Over the last 2 decades, considerable attention has been focused on the relationship between carcinogen metabolism and cancer risk in humans. In particular, the association between carcinogenesis and the enzyme system responsible for the metabolism of most lipophilic chemicals, the cytochrome P-450-dependent monooxygenases, has been addressed (1, 11, 14-16, 24-26, 34, 38). Numerous studies have documented large interindividual differences both in vivo and in vitro in activities associated with this enzyme system (1, 3, 5, 10, 23, 38). These differences are regulated by both genetic and environmental factors (3, 10, 19, 38). Recent studies in humans have demonstrated a genetic polymorphism in oxidative metabolism, and 2 phenotypes, the extensive and poor metabolizers, have generally been observed (10, 19). Speculation has arisen as to whether extensive metabolizers of drugs such as debrisoquine would also be extensive metabolizers of carcinogens and consequently be more prone to chemically induced cancer (18).

In the present study, we have screened 28 human liver microsomal samples for their capacity to metabolize debrisoquine and bufuralol, 2 substrates that are oxidized polymorphically in humans (7, 19). The metabolism of AAF, a model carcinogen, was also determined in these same samples. AAF was chosen as a substrate, since recent studies in this laboratory have shown it to be a useful probe for determining multiple forms of cytochrome P-450 in human (30), rabbit (21), and rat (29) liver microsomes.
The cytochrome P-450-mediated metabolism of AAF involves oxidation at both nitrogen and carbon atoms. The former leads to metabolic activation, while hydroxylation at positions 1, 3, 5, 7, and 9 on the fluorene ring are considered detoxication pathways (36, 41). Therefore, the use of this substrate has enabled us to correlate pathways leading to metabolic activation and detoxication of a carcinogen, with debrisoquine 4-hydroxylation and bufuralol 1-hydroxylation.

N-Hydroxylation is considered an obligatory step in the bioactivation of AAF and other aromatic amines and amides to their ultimate carcinogenic or mutagenic forms (32, 33, 42). We have reported previously a $K_m$ of 1.63 $\mu$M and a $V_{max}$ of 61 pmol/mg/min for this reaction in human liver microsomes (30). While a marked variation in the rate of N-hydroxylation of AAF (55-fold) is observed between subjects at the low substrate concentration (0.37 $\mu$M), only a 7-fold difference is apparent at 50 $\mu$M, a concentration which would saturate the metabolic pathway. The mean AAF N-hydroxylation activity of human liver microsomes in this study (405 ± 245 pmol/min/nmol P-450; $n$ = 28) compares favorably with previously reported values (2, 9, 30). Neither debrisoquine 4-hydroxylation nor bufuralol 1-hydroxylation was correlated with AAF N-hydroxylation (Chart 4). This lack of correlation strongly suggests that different forms of cytochrome P-450 are involved in the N-hydroxylation of AAF compared to those oxidizing debrisoquine and bufuralol. The good correlation ($r = 0.73$) between debrisoquine 4-hydroxylation and bufuralol 1-hydroxylation supports the suggestion that a similar form or population of cytochrome P-450 catalyzes these reactions. Further, the N-hydroxylation of AAF was not correlated with aldrin metabolism ($r = -0.11$) which has previously (43) and in this present study ($r = 0.72$) been shown to correlate with total cytochrome P-450 content.

Out of 7 highly purified forms of rabbit liver cytochrome P-450 tested, only Form 4 is capable of N-hydroxylating AAF (21, 31). The data from this study and earlier work from this laboratory (30) suggest that, as in rabbit liver, a specific form of cytochrome P-450 may be involved in the N-hydroxylation of AAF in human liver microsomes. Further, the exceptionally high correlations ($r > 0.94$) between the formation of N-OH-AAF:1-OH-AAF, N-OH-AAF:7-OH-AAF, and 7-OH-AAF:1-OH-AAF at the low concentration of AAF, imply that the same or very similar forms of cytochrome P-450 produce these metabolites (Table 1). However, at 50 $\mu$M AAF, the correlations between 7-OH-AAF:7-OH-AAF and N-OH-AAF:1-OH-AAF are considerably weaker and only explain less than 35% of the variance in the data (Table 2). The weaker correlations between 7-OH-AAF:N-OH-AAF and 7-OH-AAF:1-OH-AAF formation at this higher concentration can be explained by the fact that a 2-enzyme system best describes the 7-hydroxylation of AAF in human liver microsomes, whereas only a single enzyme system was apparent for the N-hydroxylation of this substrate (30).

The poor correlations between cytochrome P-450:debrisoquine 4-hydroxylation, cytochrome P-450:bufuralol 1-hydroxylation, aldrin:debrisoquine 4-hydroxylation, and aldrin:bufuralol 1-hydroxylation further support the hypothesis that a specific form of cytochrome P-450 is involved in both debrisoquine and bufuralol metabolism (3, 4). Except for the 7-hydroxylation of AAF at 50 $\mu$M (Chart 5), neither AAF disappearance nor the formation of AAF metabolites was correlated with the oxidation of debrisoquine or bufuralol. Although the formation of 7-OH-AAF was statistically correlated with these activities, it nonetheless accounted for less than 23% of the variance. It is of interest that 6 of the 7 purified forms of rabbit liver cytochrome P-450 can hydroxylate AAF at position 7 (31). These results demonstrate that, as with metabolic activation, pathways leading to detoxication of AAF are either not correlated or are only poorly correlated with debrisoquine and bufuralol metabolism.

The lack of correlation obtained in this study between 2 drugs that exhibit oxidative polymorphism and the oxidative metabolism of a carcinogen casts doubt on the use of prototype drugs, such as debrisoquine, to type populations for chemically induced cancer risk. Based on animal data, such an approach appears to be fraught with danger, as modulation of cytochrome P-450 activity has been associated with both an increased and decreased incidence of cancer (38, 42). For example, phenobarbital pretreatment reduces AAF carcinogenicity in rats by decreasing the percentage of the dose metabolized via N-hydroxylation and increasing the percentage of 7-OH-AAF formed, the major detoxication pathway (29, 42). By contrast, the incidence of fibrosarcomas following 3-methylcholanthrene administration was highly correlated with arylhydrocarbon hydroxylase activity (38). The multiplicity of isoenzymes of cytochrome P-450 and the overlapping substrate specificity of the different forms complicates the task of relating carcinogenicity to specific cytochrome P-450 activities. Indeed, debrisoquine 4-hydroxylation appears to be catalyzed by a specific form(s) of cytochrome P-450 (4), since deficient metabolizers of this drug show no decrease in metabolism of many other substrates for the cytochrome P-450 system. Therefore, the multiplicity of the cytochrome P-450 system, taken together with the fact that carcinogenesis is a multistage process, probably accounts for the uncertainty in the association of this enzyme activity and chemically induced cancer.

REFERENCES

3. Boobis, A. R., and Davies, D. S. Human cytochromes P-450 and the metabolism of many other substrates for the cytochrome P-450 system, taken together with the fact that carcinogenesis is a multistage process, probably accounts for the uncertainty in the association of this enzyme activity and chemically induced cancer.

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Relationship between Oxidative Metabolism of 2-Acetylaminofluorene, Debrisoquine, Bufuralol, and Aldrin in Human Liver Microsomes

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