

METABOLIC CHARACTERIZATION OF HUMAN LIVER MICROSOMAL CYTOCHROMES P-450 INVOLVED IN THE OXIDATION OF DEBRISOQUINE, BUFURALOL AND THE CARCINOGEN 2-ACETYLAMINOFLUORENE

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Introduction

Over the last two 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 monooxygenases, has been addressed (1-4). This enzyme system is composed of numerous cytochrome P-450 isoenzymes that differ with respect to both substrate specificities and in their responses to enzyme inducers and inhibitors (1-6). In the case of aromatic amines and amides the first and obligatory step in their metabolic activation involves N-hydroxylation, and for the model hepatocarcinogen, 2-acetylaminofluorene (AAF), we have shown that a distinct isoenzyme of cytochrome P-450 in rabbit liver is involved in this process (7). This high degree of regio-selectivity of cytochrome P-450 isoenzymes in the oxidation of model carcinogens such as AAF (7) and benzo(a)pyrene (3) has provided the basis for the notion that the relative composition of cytochrome P-450 isoenzymes may be a major determinant in individual susceptibility to cancer as well as in target organ selection upon exposure to chemical carcinogens.

In man, numerous studies have documented large inter-individual differences both *in vivo* and *in vitro* in activities associated with the cytochrome P-450 system (5). This variability in expression and/or intrinsic activity of cytochrome P-450 has in part been attributed to environmental factors, age, disease and drug therapy. Recent studies, however, have also demonstrated genetic variability in cytochrome P-450 mediated drug oxidation, particularly in the 4-hydroxylation of the antihypertensive agent debrisoquine (8). 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 (8,9). The frequency of the poor metabolizer phenotype, depending on ethnic origin, has been shown to vary between 1-30070 of the population (8,9). This oxidative defect in debrisoquine metabolism has been linked to a deficiency or absence of a specific form of hepatic cytochrome P-450 (9,10). Further, those subjects of the poor metabolizer phenotype have been shown to exhibit increased pharmacodynamic and toxicity effects when administered drugs associated with this pharmacogenetic defect (8). In contrast to those side effects associated with the poor metabolizer defect, two recent studies by Idle and co-workers (11,12) have demonstrated disproportionate numbers of extensive metabolizers of debrisoquine in cancer groups 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 assessed the capability of human liver microsomes from 28 individuals to metabolize debrisoquine and bufuralol, 2 drugs that show genetic variability in cytochrome P-450 dependent metabolisms in humans characterised by extensive and poor metabolizer phenotypes, as well as the hepatocarcinogen 2-acetylaminofluorene. 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 (13), rabbit (7) and rat (14) 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 detoxification pathways (15). Therefore, the use of this substrate enables us to correlate pathways leading to metabolic activation and detoxification of a carcinogen, with debrisoquine 4-hydroxylation and bufuralol 1-hydroxylation. In addition, we determined aldrin epoxidation as an indicator of the activity of another unrelated isoenzyme of cytochrome P-450 (16) and measured cytochrome P-450 content of each microsomal preparation.

Materials and Methods

Chemicals

Randomly labelled [3H]AAF (18 Ci/mmol) was purchased from Moravsek Biochemicals (Brea, CA) and was purified to greater than 98% purity by high-pressure liquid chromatography (14). Unlabelled AAF, 2-aminofluorene, N-hydroxy-AAF (N-OH-AAF), 1-hydroxy-AAF (1-OH-AAF), 3-hydroxy-AAF (3-OH-AAF), 5-hydroxy-AAF (5-OH-AAF), 7-hydroxy-AAF (7-OH-AAF), 9-hydroxy-AAF (9-OH-AAF), and 2-acetylaminofluorene-9-one were obtained as described previously (14). Desferrioxamine mesylate was purchased from Ciba Pharmaceuticals (Summit, N J) and NADPH from Sigma Chemical Co. (St. Louis, MO). Debrisoquine hemisulfate, bufuralol hydrochloride, and 1-hydroxybufuralol were generously provided by Roche Products, Ltd. (Welwyn Garden City, United Kingdom); [2H9]-4-hydroxy-debrisoquine was prepared biologically as described previously (16). Aldrin and the epoxide of aldrin, dieldrin, were a gift from the Shell Toxicology Laboratory (Sittingbourne, U.K.).

Human Tissue Samples

Microsomal fractions of human liver were obtained as described previously (16), 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 (16), 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 that sodium fluoride was omitted from the incubation mixture (14). Concentrations of AAF used were 0.37 and 50 μ M, and microsomal protein contents per ml of incubation mixture were 25 and 100 μ g, respectively, at these concentrations. Two concentrations of AAF were used since we have shown previously that the relative quantity of each metabolite of AAF produced by human and rat liver microsomes and therefore the balance between metabolic activation (N-hydroxylation) and detoxification (C-hydroxylation) varied markedly with substrate concentration (12,13). Debrisoquine 4-hydroxylase and bufuralol 1-hydroxylase activities were measured according to the methods of Kahn *et al.* (17) and Boobis and Davies (3), respectively. Aldrin epoxidation was measured according to the method of Wolff *et al.* (18). Incubation conditions used gave reaction rates that were zero order with respect to cofactor and linear with respect to protein concentration and time. Multiple crosscorrelations were calculated as described previously (16).

TABLE 1. Correlation Coefficients for AAF Metabolites formed with Human Liver Microsomes.

	9-OH-AAF	5-OH-AAF	3-OH-AAF	1-OH-AAF	N-OH-AAF
7-OH	0.64	0.79	0.37	0.97	0.94
9-OH		0.75	0.24	0.77	0.85
5-OH			0.30	0.80	0.82
3-OH				0.27	0.37
1-OH					0.98

For 26 d.f., $r > 0.37$; $p < 0.05$. The incubation mixture contained 0.37 μ M AAF.

Results and Discussion

The mean cytochrome P-450 content of the 28 human liver microsomes tested was 0.37 ± 0.11 nmol per mg protein (mean \pm S.D.) and at the low concentration of AAF (0.37 μ M) there were statistically significant correlations between cytochrome P-450 content and the rates of formation of some of the metabolites of AAF. Correlation coefficients ranged from 0.59 for 7-OH-AAF and cytochrome P-450, to 0.32 for 9-OH-AAF and cytochrome P-450. At 50 μ M AAF only a significant correlation was observed between 7-OH-AAF and cytochrome P-450 ($r = 0.74$). Table 1 shows that except for the 3-hydroxylation of AAF, exceptionally high correlations ($r > 0.94$) existed between the rates of formation of individual metabolites of AAF at 0.37 μ M ($p < 0.05$), and imply that similar forms of cytochrome P-450 produce these metabolites. However, at 50 μ M AAF correlations between rates of metabolite formation 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 this concentration (16).

The rates of formation of the different metabolites of AAF at 0.37 μ M and 50 μ M plotted against debrisoquine 4-hydroxylation, bufuralol 1-hydroxylation, and aldrin epoxidation are depicted in Figs. 1 and 2. Marked interindividual differences were observed in the rates of formation of different AAF metabolites at 0.37 μ M with these being most marked for the N-hydroxylation (50-fold) and 1-hydroxylation (55-fold). Interindividual differences in debrisoquine, bufuralol and aldrin metabolism were 12-, 20- and 2.4-fold, respectively. The 4-hydroxylation of debrisoquine was not correlated with cytochrome P-450 ($r = 0.26$) whereas both the 1-hydroxylation of bufuralol ($r = 0.45$) and the epoxidation of aldrin ($r = 0.72$) were correlated with this parameter. While bufuralol 1-hydroxylation was correlated with cytochrome P-450 content, the correlation only explained 20% of the total variance. Rates of debrisoquine and bufuralol metabolism were significantly correlated ($r = 0.73$) which supports the suggestion that a similar form of cytochrome P-450 catalyzes these reactions (9).

N-hydroxylation is considered the first and obligatory step in the bioactivation of AAF and other aromatic amines and amides to their ultimate carcinogenic or mutagenic forms (14). At both concentrations of AAF used neither debrisoquine 4-hydroxylation nor bufuralol 1-hydroxylation was correlated with AAF N-hydroxylation. Further, except for a weak correlation between 7-OH-AAF and bufuralol ($r = 0.47$) at 50 μ M, no significant correlations were observed between debrisoquine and bufuralol metabolism and any of the C-hydroxylation pathways at the two substrate concentrations employed. This lack of correlation strongly suggests that different forms of cytochrome P-450 are involved in the metabolic activation and detoxification of this carcinogen compared to those oxidizing debrisoquine and bufuralol.

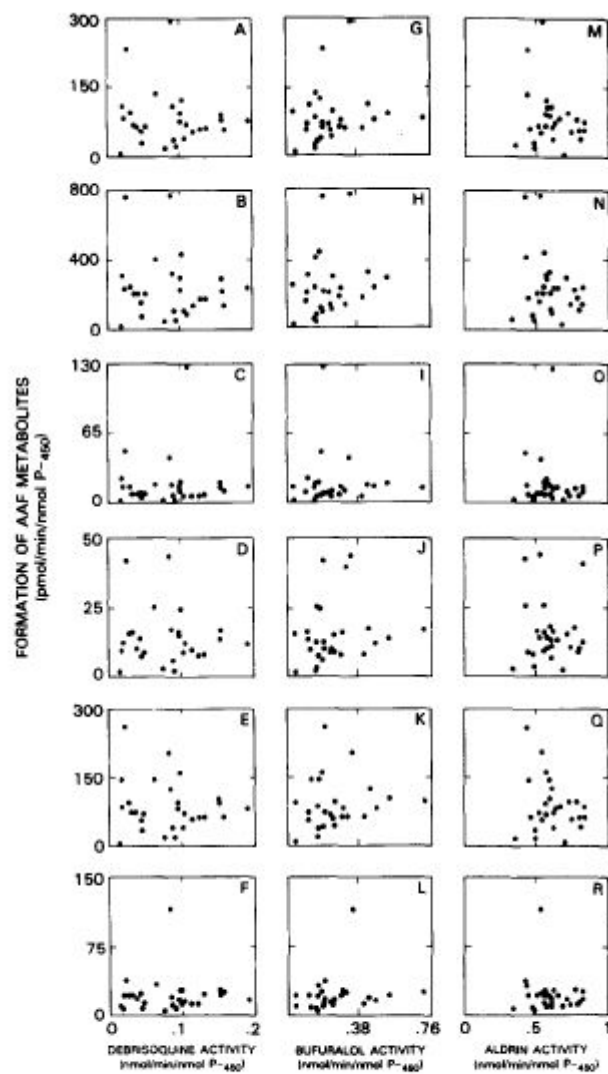


FIG. 1. Comparison of 2-acetylaminofluorene metabolism at $0.37 \mu\text{M}$ with debrisoquine 4-hydroxylation (A to F), bufuralol 1-hydroxylation (G to L), and aldrin epoxidation (M to R) in human liver microsomes, A, G and M, N-OH-AAF; B, H and N, 1-OH-AAF; C, I and O, 3-OH-AAF; D, J and P, 5-OH-AAF; E, K and Q, 7-OH-AAF; F, L and R, 9-OH-AAF.

Probit plots of debrisoquine 4-hydroxylase and bufuralol 1-hydroxylase activities (data not shown) as well as cytochrome P-450 content (Fig. 3) of the 28 human liver samples tested were linear indicating that values were normally distributed about their respective means. However, similar analysis of AAF metabolism at $0.37 \mu\text{M}$, a realistic environmental exposure level, showed distinct biphasic probit plots for both metabolic activation and detoxification pathways of this carcinogen (Fig. 3). Biphasic probit plots are indicative of two phenotypes for these activities being present in our population sample. For the N hydroxylation of AAF (metabolic activation) 79% of the sample were slow metabolizers and their values ranged from 2 to 31 pmol N-OH-AAF formed/min/mg protein. Values for the fast or extensive metabolizers ranged from 42 to 133 pmol/min/mg protein. The 1- and 7-hydroxylations of AAF exhibited identical probit plots to N-hydroxy 2-acetylaminofluorene (Fig. 4) (19). However, the slow metabolizers for the formation of 9- and 3-hydroxy-acetylaminofluorene represented 89/70 of the population (Fig. 4).

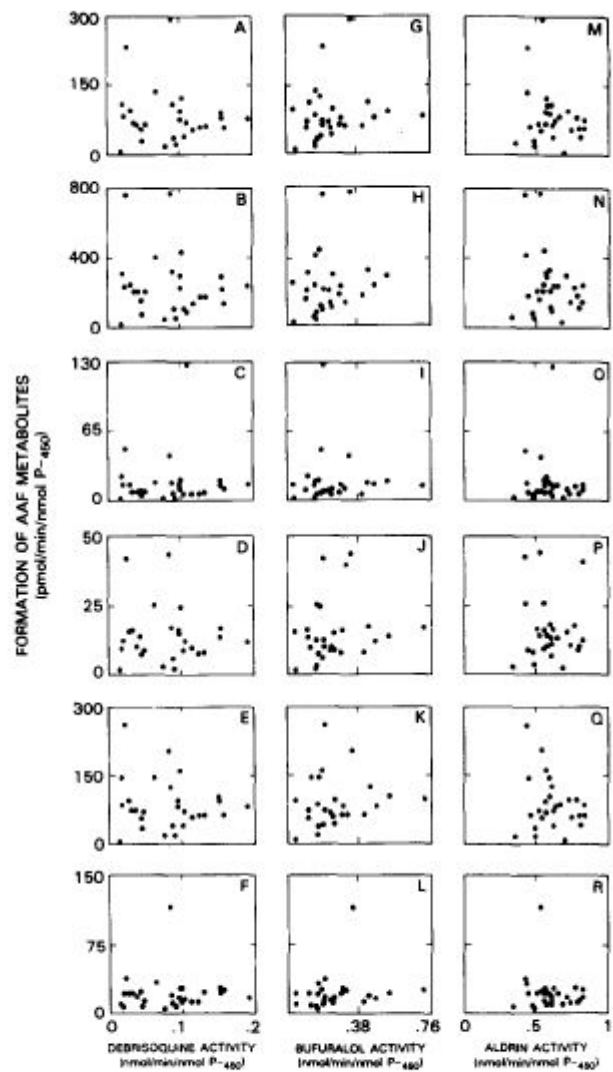


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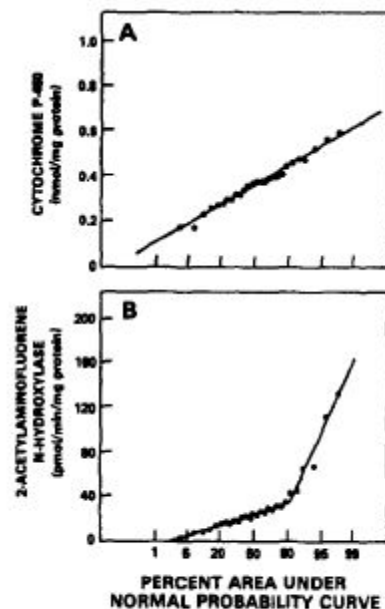


FIG. 3. Probit plots for cytochrome P-450 content and 2-acetylaminofluorene *N*-hydroxylase activity in 28 samples of human liver microsomes.

Despite the lack of correlation between either debrisoquine and bufuralol metabolism and AAF bioactivation or detoxification, these data nonetheless clearly demonstrate a metabolic polymorphism in human liver samples for AAF metabolism. The determinants of each phenotype for the metabolism of the carcinogen appear to be controlled by factors independent of those regulating debrisoquine 4-hydroxylation and bufuralol 1-hydroxylation. Further the ultimate consequence of polymorphic bioactivation of chemical carcinogens in man is complicated by the possible existence of a metabolic detoxification polymorphism, as has been demonstrated in the present study with the model hepatocarcinogen 2-acetylaminofluorene. In general, we observed that individuals who were fast activators of the carcinogen were also fast detoxifiers of the chemical. However, the different phenotype patterns for *N*-hydroxylation and oxidation in the 3- and 9- positions (relatively minor metabolites) suggest that fast activators of a toxin need not also be phenotyped as fast deactivators (19). Studies with other classes of carcinogens are required to further understand whether certain individuals may be more susceptible to chemical carcinogenesis because of their metabolic disposition.

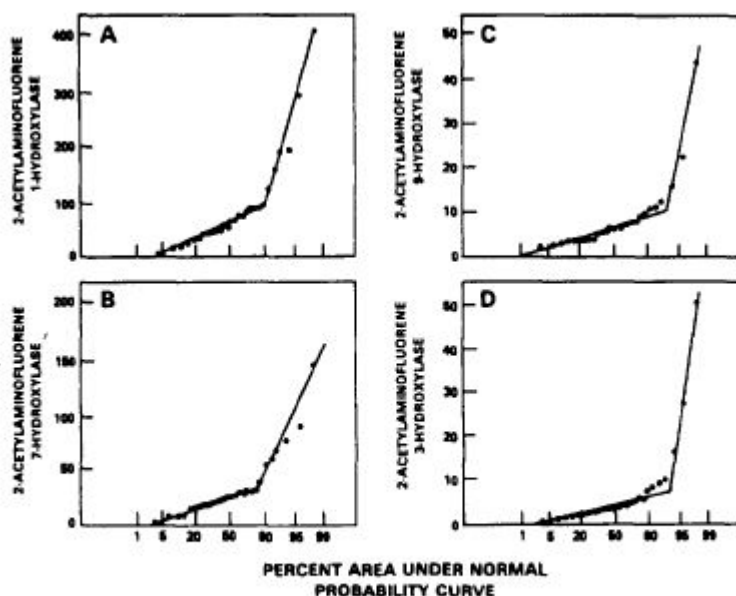


FIG. 4. Probit plots for the formation of 1-hydroxy-, 7-hydroxy-, 9-hydroxy- and 3-hydroxy-2-acetylaminofluorene in human liver microsomes.

Acknowledgements—

I wish to thank Dr. S. S. Thorgeirsson of National Cancer Institute, USA, for his encouragement and guidance throughout these studies, and the excellent cooperation of Professor D. S. Davies and Dr. A. R. Boobis of the Royal Postgraduate Medical School, London, U.K., and Dr. R. F. Minchin, Department of Pharmacology, University of Western Australia.

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