In the urine sample from the Indian subject 19% of total HD was in the form of the (*R*)-enantiomer (Fig. 1). For comparison a chromatogram from the analysis of a Caucasian PM urine sample is also shown (Fig. 1).

Discussion

All of the black Nigerians in this study were similar to Caucasian EMs [7, 8] in their ability to 4-hydroxylate D with a high degree of stereoselectivity. In the majority of subjects only (*S*)-HD was detectable, although it is possible that small amounts of the (*R*)-enantiomer were formed. Nevertheless, in the samples where (*R*)-HD could be measured, stereoselectivity remained high. A greater ability to eliminate D by 4-hydroxylation is associated with increased stereoselectivity. The considerably lower stereoselectivity observed in the Indian subject was characteristic of that observed in Caucasian PMs [7, 8] and this, together with the high D/HD ratio of 60, is compatible with this subject also being of the PM phenotype. The enantiomer data from the present study confirm previous findings using the D/HD ratio [6] in providing no evidence that D metabolism is under polymorphic control in Nigerians. The possible reasons for this have been discussed elsewhere [6].

In summary, a high degree of stereoselectivity was observed for the 4-hydroxylation of D in a black Nigerian population, the (*S*)-metabolite enantiomer being the predominant product. This characteristic is shared with Caucasians of the EM phenotype for debrisoquine.

Acknowledgements—We thank Dr C. O. Meese for valuable comments and Frau P. Thalheimer for technical assistance. Part of this work was funded by a grant from the University of Sheffield Medical Research Fund. M.S.L. is a Wellcome Trust Lecturer.

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Biochemical Pharmacology, Vol. 37, No. 1, pp. 98–102, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Journals Ltd.

DNA recombinant and monoclonal antibody directed methods for determining cytochrome P-450 specificity

We have introduced two new approaches to the analysis of individual cytochrome P-450 function. These new technologies are highly applicable to understanding cytochrome P-450 specificity in respect to both substrate and product formation. These methods engage modern immunochemical technologies and DNA recombinant methods and although we have used them largely to study the product specificity of individual P-450s, they can easily be applied to the study of stereospecific aspects of cytochrome P-450 function and therefore are germane and appropriate to the subject of chirality and cytochrome P-450.

The cytochromes P-450 are a large family of hemoproteins capable of metabolizing xenobiotics, such as drugs, carcinogens and environmental pollutants [1]. It does not exaggerate the situation to suggest that the vast majority of drugs of therapeutic use are substrates for the cytochrome P-450 class of enzymes. In addition, the P-450s metabolize endobiotics, such as steroids, fatty acids and prostaglandins. This group of cytochrome-P-450 enzymes carries out beneficial metabolic activities by detoxification of xenobiotics as well as harmful metabolic conversion of xenobiotics to toxic, mutagenic and carcinogenic forms [1]. There is a multiplicity of cytochrome P-450 forms numbering at least 20–30 and perhaps many more.

The multiplicity and the common properties of the cytochrome P-450 render difficult the separation of different forms of cytochrome P-450, especially the minor forms. This has prevented a full understanding of the role of individual forms of cytochromes P-450 in metabolism, detoxification, and activation of xenobiotic and endobiotic substrates. The complexities and uncertainties of purification have also prevented the defining of individual cyto-

chrome P-450 specificity and assigning the contribution of each P-450 to the total metabolism of an individual drug in a tissue.

We have used monoclonal antibodies (MAbs) (1) to analyze the contribution of individual P-450s to the total reaction in a tissue and vaccinia virus expression vectors containing cDNA for a single P-450 and (4) to analyze individual cytochrome P-450 specificity. We have constructed a library of panels of monoclonal antibodies to different forms of cytochrome P-450. In some cases the panels contain some hybridomas that produce MAbs that inhibit completely the enzymatic activity of the P-450 to which the MAb binds. The inhibiting MAbs are especially useful for what we have termed "reaction phenotyping".

Monoclonal antibody based reaction phenotyping

The cytochromes P-450 collectively catalyze the oxidation of literally thousands of xenobiotic and endobiotic substrates including a large variety of different classes of chemicals. One of the essential questions concerning cytochrome P-450 function relates to our ability to measure the contribution of unique forms or classes of cytochromes P-450 to the total metabolism of an individual substrate in different tissues and individuals. Available methods do not permit such a measurement. Current understanding of the specificity of unique forms of P-450 is derived from purification and isolation of the unique P-450. The purified P-450 is reconstituted with cofactors and enzymes and its substrate and product specificity is measured. The latter methods have yielded information on P-450 specificity but do not add much to understanding the contribution of the particular cytochrome P-450 to total tissue metabolism. Furthermore, the reconstituted system may not adequately reflect the *in situ* activity of the cytochromes P-450 located in the endoplasmic reticulum. Monoclonal antibodies that completely inhibit the enzyme activity of the epitope containing cytochrome P-450 to which they bind are extraordinarily useful for "reaction phenotyping". Thus, "reaction phenotyping" is the identification and quantification of the contribution of individual or epitope-defined classes of cytochrome P-450 to the total metabolism of a specific cytochrome P-450 substrate in a tissue. The inhibitory MAb is added to the tissue preparation at saturating levels, and the amount of inhibition of the reaction is a measure of the minimum contribution of the MAb-sensitive cytochrome P-450 to the total reaction in the tissue. This reflects only the minimum contribution of the sensitive cytochromes P-450 and not always the maximum. In some instances the MAb-sensitive epitope in the cytochrome P-450 may be bound or inserted in the membrane structure and unavailable to the MAb. If this is the case, a solubilization of the microsomes is required prior to MAb-inhibition measurements. After solubilization, the inhibition by the MAb-sensitive cytochrome P-450 measures its maximum contribution to the total reaction. "Reaction phenotyping" of cytochromes P-450 can be applied to analysis of a variety of reactions which may be the direct or indirect results of cytochrome P-450 activity. These include: (1) substrate disappearance, (2) substrate and product specificity including stereospecific reactions, and (3) indirect action, i.e. phenomena which are indirectly a result of cytochrome P-450 catalyzed formation of reactive metabolites. The formation of these reactive metabolites may result in (a) metabolite binding to macromolecules such as protein and DNA, (b) cell toxicity, (c) mutagenicity, and (d) carcinogenicity. The addition of an inhibitory MAb to any of the above assay systems will in each case qualitatively and quantitatively define the contribution of the MAb epitope specific cytochrome P-450 to the total activity measured.

Reaction phenotyping for substrate and product specificity

This technique has been used to reaction phenotype the contribution of MAb specific cytochromes P-450 [23]. Table 1 is an example of reaction phenotyping of the liver of different species for two cytochrome P-450 activities. The distribution of cytochromes P-450 that catalyze AHH and 7-ethoxyucoumarin *O*-deethylase (ECD) were studied with MAB 1-7-1, which completely inhibits these activities of a purified 3-methylcholanthrene (MC) induced rat liver cytochrome P-450. The degree of inhibition by MAb 1-7-1 quantitatively assesses the contribution of antigenically defined cytochromes P-450 in the liver from untreated, MC- and phenobarbital (PB)-treated rats, mice, guinea pigs

Table 1. MAb 1-7-1 Inhibition of hepatic AHH and ECD of different species and strains

		AHH (pmol/min/mg)			ECD (nmole/min/mg)		
Species	Induction	Control	1-7-1	Inhibition (%)	Control	1-7-1	Inhibition (%)
Rat							
Sprague–Dawley	none	367	384	0	0.82	0.95	0
	PB	600	615	0	1.27	1.16	8
	MC	11704	2872	75	5.28	1.56	71
Mouse							
C-57BL/6	none	88	87	1	6.4	5.6	12
	PB	128	125	2	7.0	5.8	17
	MC	5716	699	88	21.9	13.2	39
Mouse							
DBA/2	none	70	70	0	4.0	3.5	11
	PB	51	94	0	4.7	3.6	25
	MC	501	486	3	16.9	18.9	0
Guinea pig							
NIH	none	282	209	25	2.0	2.1	0
Outbred	PB	703	722	0	2.9	3.0	0
	MC	1642	804	47	5.3	6.2	0
Hamster							
Golden	none	100	136	0	8.7	9.1	0
Syrian	PB	192	219	0	18.0	17.0	5
	MC	387	204	51	22.1	23.7	0

and hamsters. Enzyme sensitivity to MAb 1-7-1 inhibition defines two types of cytochrome P-450 contributing to AHH and ECD. The MAb 1-7-1 sensitive cytochrome P-450 is a major contributor to AHH in liver, of MC-treated rats, $C_{57}B1/6$ mice, guinea pigs and hamsters. This form, however, makes little or no contribution to liver aryl hydrocarbon hydroxylase of control or PB-treated animals. ECD is also a function of both the MAb 1-7-1 sensitive and insensitive classes of cytochrome P-450. The ratio of the classes contributing to AHH and ECD differs in the various tissues and species and after inducer treatment. Liver AHH from MC-induced rats and $C_{57}B1/6$ mice was inhibited by 75% and 88%, respectively, indicating that MAb 1-7-1 sensitive cytochromes P-450 contribute 75% and 88% of the total tissue activity. With ECD, however, the results were different. In the same tissues the inhibition of ECD was 75% and 40% respectively. Thus, in C57 mice, 60% of the ECD activity is contributed by cytochromes P-450 other than the MAb 1-7-1 sensitive type. In related experiments we found that, in the livers of control and PB-treated rats and $C_{57}B1/6$ mice, AHH was unaffected by MAB 1-7-1 while ECD was inhibited by only 10%–15%. This indicated that in these tissues virtually all of the AHH and 85%–90% of the ECD were contributed by an MAb 1-7-1 insensitive form of P-450. Furthermore, in hamster and guinea pig none of the ECD was sensitive to MAb 1-7-1. In DBA/2 mice, although the AHH was induced 5-fold by MC, it was insensitive to MAb 1-7-1, indicating that this induced activity of AHH active cytochrome P-450 is different than the highly induced AHH active cytochrome P-450 in MC-treated $C_{57}B1/6$ mice.

Vaccinia virus expression of a cDNA for a specific cytochrome P-450

In a second approach we sought to develop a system in which a cDNA coding for a specific P-450 could be inserted into an infectious vector which we could inject into a cell in which the cDNA P-450 sequence would be expressed into fully active enzyme [4]. Two cDNA clones representing the mRNA coding sequences for mouse cytochromes P_1-450 and P_3-450 were inserted into the thymidine kinase gene of the wild-type vaccinia virus under the control of the vaccinia virus promoter (Fig. 1). Murine and human cells infected with each of the resulting infectious recom-

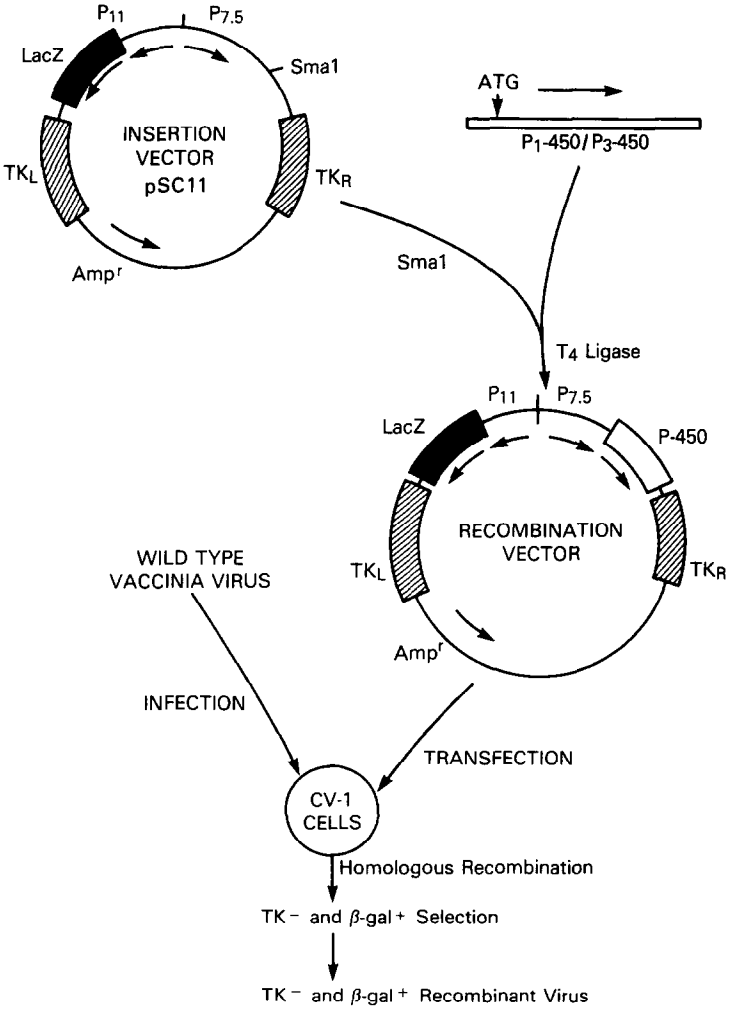

Fig. 1. The construction of vaccinia virus containing the P-450 cDNA under the control of the TK promoter.

binant viruses efficiently expressed their respective P-450 proteins. The newly synthesized protein products are translocated into the microsomes, and their characterization by immunochemical analysis indicates that the sizes of the polypeptides expressed were indistinguishable from their cytochrome P-450 counterparts found in mammalian liver microsomes. Functional analysis of each of the proteins by spectral and enzymatic analysis indicates that the expressed proteins have incorporated heme, and the holoenzymes displayed catalytic activities characteristic of their respective cytochrome P-450 enzymes (Figs. 2 and 3). Thus, this system can be used to produce properly processed and catalytically active P-450 gene products in a wide variety of cells. The remarkable fidelity of expression and processing of these enzymes suggests that the vaccinia virus recombinants can be used for a wide variety of studies, including analysis of the effects of defined mutations produced *in vitro*, and directly correlate the structure/activity relationships of the cytochrome P-450 enzymes.

The vaccinia virus system used in this study to express functional proteins in heterologous cells with almost no background of enzyme activity offers a unique solution to the difficult problem of P-450 isozyme specificity and eliminates the need for enzyme purification to obtain clean enzymes in the large quantities required to study their catalytic, immunologic and structural properties. Expression of closely related P-450 forms with overlapping substrate specificities and subsequent characterization will allow analysis of the contribution of each of the enzymes to the stereospecific metabolism of carcinogens and drugs in intact cells in the absence of other P-450s.

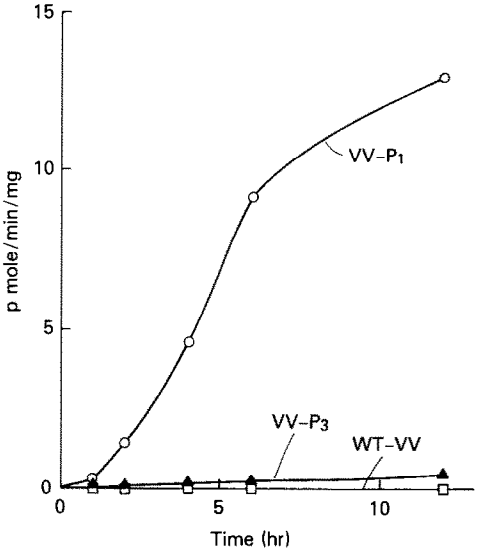

Fig. 2. The expression of aryl hydrocarbon hydroxylase activity. Infected cells were harvested at the indicated time intervals and the lysates were assayed for aryl hydrocarbon hydroxylase activity. NIH 3T3 cells were infected with VV-P_1-450, VV-P_3-450, or WT-VV. No detectable activity was found in uninfected control cells (not shown). This experiment shows the expression of fully active enzyme from the cDNA coding sequence of P-1-450 showing high AHH activity with no activity exhibited by the P_3-P-450 or the vaccinia virus only.

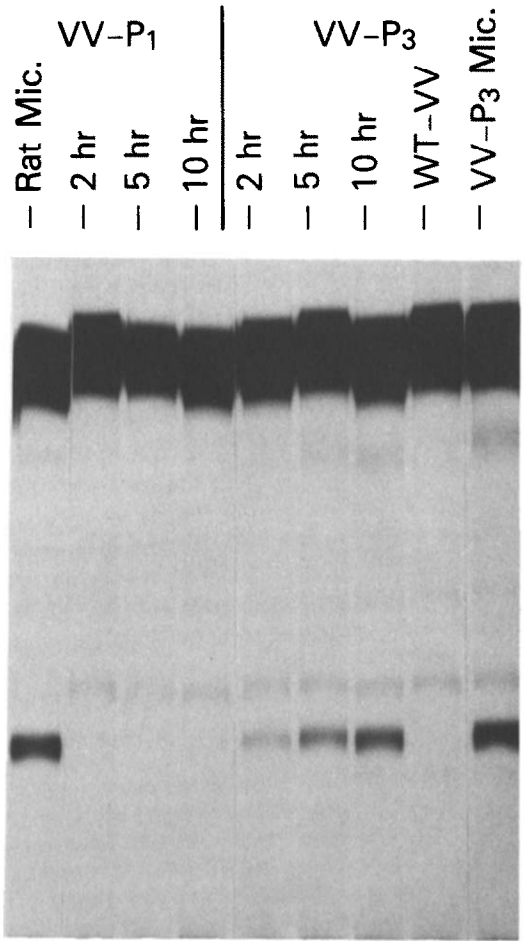

Fig. 3. The results with P_3-P450 infected cells showing acetanilide hydroxylase activity arising from infection with the P_3-P-450. The TLC analysis of acetanilide hydroxylase activity is shown. Cell lysates were assayed and the products were separated by TLC. The numbers 0.74 and 0.20 represent the R_f values of the substrate and product, respectively. Lysates of WT-VV-infected cells were prepared 10 hr after infection. Here only the P_3-P-450 injected cells showed acetanilide hydroxylase activity.

Both the monoclonal antibody and cDNA vector system should prove useful for determining the stereospecificity of cytochrome P-450 catalyzed reactions. These systems offer advantages that are unique and provide conditions that largely overcome the serious problems of previously used approaches which required P-450 purification and reconstitution and even when successful, failed to define individual P-450 contribution to total metabolism. The latter can easily be accomplished with monoclonal antibodies. The primary advantage of the vaccinia expression system is the high specificity for a single P-450 form in its natural microsomal environment and thus likely to reflect physiological specificity. These techniques are uniquely suited for determining the stereospecificity of different forms of cytochrome P-450 in the metabolism of xenobiotics and endobiotics.

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