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The effect of cigarette smoking on 7-ethoxyresorufin *O*-deethylase and other monooxygenase activities in human liver: analyses with monoclonal antibodies

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1 Four cytochrome P-450 enzyme activities, 7-ethoxyresorufin *O*-deethylase (ERDE), coumarin 7-hydroxylase (CH), 7-ethoxycoumarin *O*- deethylase (ECDE) and aryl hydrocarbon hydroxylase (AHH) were measured in human liver needle biopsy samples from smokers and non-smokers. Cigarette smoking was verified and quantitated by measuring plasma cotinine levels.

3 Enzyme inhibitory monoclonal antibodies (MAb) to a 3-methylcholanthrene-induced (MAb 1-7-1) and phenobarbitone-induced (MAb 2-66-3) rat hepatic cytochrome P-450 were used to measure the contribution of MAb-defined, epitope-specific cytochromes P-450 to the total reaction measured for each of the above activities.

3 ERDE activity was significantly elevated in the livers of cigarette smokers, whereas AHH, CH or ECDE activities were not affected by cigarette smoking. No correlation was observed between plasma cotinine concentration and ERDE activity.

4 MAb 1-7-1 inhibited hepatic ERDE activity to a variable extent (from 0 to 65%), but had very little or no effect on AHH, CH or ECDE activities. The inhibitory effect of MAb 1-7-1 on ERDE activity was greater than 50% in the non-smokers. MAb 2-66-3 had no inhibitory effect on any of the enzyme activities studied.

5 In contrast to liver both ERDE and AHH on human placental microsomes from cigarette smokers were inhibited by MAb 1-7-1. The MAb 2-66-3 was without effect.

6 Cigarette smoking induces a form of P-450 in human liver, responsible for ERDE activity, that contains an epitope recognized by MAb 1-7-1. This form of cytochrome P-450 is insensitive to MAb 2-66-3 and is not contributing to AHH, CH or ECDE activities of human liver.

Keywords cigarette smoking liver drug metabolism cytochrome P-450 monoclonal antibodies

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Introduction

Cigarette smoking increases the elimination of several drugs (see Jusko, 1978) and increases the metabolism of polycyclic aromatic hydrocarbons and some other model substances in certain human tissues and cultured cells (see e.g. Pelkonen, 1976; Pelkonen *et al.*, 1984). It is thought that the increase in the plasma elimination of a number of drugs is due to the induction of hepatic drug-metabolizing enzymes, but unequivocal proof in man has not been provided. Although Pelkonen *et al.* (1975) and Boobis *et al.* (1980) reported small increases in some drug-metabolizing enzymes in liver biopsy samples from cigarette smokers, other studies (Pelkonen *et al.*, 1980) have provided evidence that does not support the role of the liver in the cigarette smoke-induced increase in the clearance of drugs. Vähäkangas *et al.* (1983) studied antipyrine elimination and hepatic drug metabolism *in vitro* in smokers and non-smokers and found, that antipyrine elimination was clearly enhanced in smokers, but the level of hepatic cytochrome P-450-dependent aryl hydrocarbon hydroxylase (AHH) did not differ between smokers and non-smokers. It is possible that AHH is not a good indicator of cytochrome P-450 changes in human liver induced by polycyclic aromatic hydrocarbons. 7-ethyoxyresorufin *O*-deethylase activity has been suggested to reflect very specifically the cytochrome P-450 form induced by PAHs and cigarette smoking (Prough *et al.*, 1978; Phillipson *et al.*, 1984). In the present study we investigated the levels of 7-ethoxyresorufin *O*-deethylase (ERDE) as well as cytochrome P-450-dependent AHH, coumarin 7-hydroxylase (CH) and 7-ethoxycoumarin *O*-deethylase (ECDE) in liver biopsy samples from smokers and non-smokers, in whom the smoking status was verified by plasma cotinine measurements. In addition we examined the effect of monoclonal antibodies (MAb) specific for and inhibitory to methycholanthrene (MC)- and phenobarbitone (PB)-induced cytochromes P-450 to characterize the epitope specific cytochromes P-450 that contribute to the total tissue activities for each of the enzymes measured. The extraordinary utility of monoclonal antibodies for cytochrome P-450 analysis, purification and tissue and reaction phenotyping has recently been reviewed (Gelboin & Friedman, 1985).

Our data permit us to conclude that a specific cytochrome P-450 sensitive to MAb 1-7-1 is responsible for a significant part of total ERDE activity and that this cytochrome P-450 does not contribute to human liver AHH, CH and ECDE.

Methods

Chemicals

7-Ethoxyresorufin was obtained from Pierce Chemical Co (Rockford, IL, USA) and resorufin from Eastman Kodak Co (Rochester, NY, USA). 7-Hydroxycoumarin and coumarin were obtained from Aldrich-Europe (Beerse, Belgium). Benzo(a)pyrene was obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of the highest commercial grade available.

Antibodies

The monoclonal antibodies (MAb) were prepared by the hydridoma technique described by Köhler & Milstein (1974). The MAb towards MC-induced form of rat liver cytochrome P-450 was from the clone 1-7-1 (hereafter referred to as 'MAb 1-7-1') (Park *et al.*, 1982). The MAb to PB-induced form of rat liver cytochrome P-450 is also described previously (Park *et al.*, 1984) and the clone used in this study was 2-66-3 ('MAb 2-66-3'). As a control, an ascites fluid (NBS 1-48-5) containing non-specific IgG (Park *et al.*, 1982) was used. The preparation, characterization and properties of the monoclonal antibodies have been previously described (Park *et al.*, 1982; 1984; Fujino *et al.*, 1982; 1984). The MAb 1-7-1 inhibits the AHH and ECDE activities of the purified MC-induced cytochrome P-450 by more than 90%, while not inhibiting the same activities of the purified PB-induced cytochrome P-450 (Park *et al.*, 1982). The MAb 2-66-3 inhibits the AHH and ECDE activities of the purified PB-induced P-450, but does not inhibit MC-induced P-450 (Park *et al.*, 1984). The MAb 1-7-1 used as an immunosorbent detects two species of cytochrome P-450 (56K and 57K) in Sprague-Dawley rats, and C57BL/6 mice, one form from DBA/2 mice (56K) and one from guinea pig (53K). All of the latter are MC-induced forms of cytochrome P-450 (Cheng *et al.*, 1984a). All of the latter polypeptides have been *N*-terminal amino acid sequenced (Cheng *et al.*, 1984b) and the 56 and 57K forms detected correspond to cytochrome P-450c (MC-induced) and cytochrome P-450d (isosafrole-induced). Using a radioimmunoassay MAb 1-7-1 detects the epitope containing cytochromes P-450 in the livers of untreated rats at a level of about one hundredth of that detected in the livers of MC-treated rats. Thus there are MAb epitope specific P-450 present in control rat liver at a very low level (Song *et al.*, 1984). In cross reactivity studies, the MAb 1-7-1 reacts with the major MC-inducible form of P-450 and

the isosafrole-inducible form. It does not cross react with three purified P-450s induced by PB and two forms induced by pregnenolone 16-carbonitrile or a highly purified constitutive form of cytochrome P-450.

The MAb PB-2-66-3 immunopurifies cytochromes P-450 from the livers of PB-treated rats that develop a diffuse band when exposed to gel electrophoresis suggesting microheterogeneity. *N*-terminal amino acid sequencing (Friedman *et al.*, 1985) suggest that MAb 2-66-3 interacts with three forms of highly homologous PB-inducible P-450's designated as PB-1, PB-4 and PB-5 (Waxman & Walsh, 1982; 1983). The MAb 2-66-3 cross reacts with the rabbit PB-induced P-450 LM2 and does not cross react with any MC-, isosafrole- or PCN-induced cytochromes P-450 (unpublished data).

Tissue preparations

Liver biopsies Needle biopsies were obtained from 37 patients who were admitted to the Department of Medicine for diagnostic purposes. The obtainment of surplus tissue from samples intended primarily for diagnostic purposes has been approved by the Ethics Committee of the Medical Faculty University of Oulu. Indications for biopsy were elevations in serum aminotransferases, alkaline phosphatase or bilirubin, suspected liver disease, or liver involvement in extrahepatic diseases. Percutaneous biopsy using a Thrucut needle was performed and, if the sample was judged to be large enough it was immediately divided into two parts. One part was fixed in formalin and used for histological examination at the Department of Pathology. The remaining part was immersed in 0.1 M sodium/potassium phosphate buffer, pH 7.4, frozen within 10 min at −70° C, and used for enzymic determinations. In the histological examination of liver samples mostly fatty degeneration, mild inflammation and/or mild hepatitis ('slight changes') were seen.

We compared smokers and non-smokers with respect to age, sex, degree of liver damage and use of drugs, which might have affected drug-metabolizing enzymes, and did not find any significant differences.

Other liver samples Larger liver samples suitable for the isolation of microsomes were obtained from 12 individuals. About 0.5–10 g wedge biopsy samples in connection with abdominal surgery were obtained from four patients with hepatoma (patients no. 1, 2, 6 and 7 in Table 3), four with cholelithiasis (no. 4, 8, 9, and 12) and two with liver cirrhosis (no. 10 and 11). One larger liver sample (about 100 g) was obtained from a renal transplant donor (no. 3). The whole liver was obtained from a forensic autopsy case (no. 5) about 8 h after the death. Other pertinent characteristics, cigarette smoking, use of inducing drug and histological picture of the liver, are mentioned in Table 3. In two cases (no. 6 and 7) it is certain that the piece used for enzymic studies contained tumour tissue, because the histology on the adjoining tissue revealed the presence of infiltrating tumour tissue. In all other cases the tissue used appeared entirely parenchymal with various degrees of histological changes. The samples were stored at −70° C before the preparation of microsomes.

Preparation of microsomes Liver samples were homogenized in 0.1 M sodium/potassium phosphate buffer, pH 7.4, with a Potter-Elvehjem teflon-glass homogenizer (about 20 strokes at 600 rev min^{-1}). Needle biopsy homogenate contained 10 mg of liver tissue ml^{-1} and larger samples 100 mg ml^{-1}. Needle biopsy homogenates were used as such for enzymic assays. The microsomes from larger liver samples were isolated by the standard ultracentrifugation schedule (Pelkonen *et al.*, 1974).

Placental preparations Placentas were obtained after normal delivery at term from women who had a history of cigarette smoking (smoking was verified by plasma cotinine assays). Microsomes were prepared by the standard ultracentrifugation technique and stored at −70° C until assays.

Enzymatic assays

In all enzyme assays the concentration of MAb used was related to the microsomal protein content. The ratio of ascites protein/microsomal protein was 1:5, except in those cases where antibody concentration curves were examined. The enzyme preparations were incubated with the MAb for 3–5 min at room temperature prior to the beginning of the enzyme assay.

The 7-ethoxyresorufin *O*-deethylase (ERDE) activity measurement was carried out according to an end-point fluorometric method of Burke *et al.* (1978). Incubation mixture (1 ml) contained the standard cofactors ($MgCl_2$ 2.5 mM, KCl 50 mM, glucose-6-phosphate 1.5 mM, NADP 62.5 μM and glucose-6-phosphate dehydrogenase 1 IU) and the substrate 100 μM 7-ethoxyresorufin. Reaction was started by the addition of the substrate and after a 5 min incubation at +37° C reaction was stopped by the addition of 2.5 ml methanol. Precipitated protein was separated by

centrifuging and the fluorescence in the supernatant was measured by an Aminco-Bowman spectrophotofluorometer with excitation and emission wavelengths of 530 and 585 nm, respectively. Resorufin (10 μM) was used as an external standard in every series of measurements.

The AHH activity was measured by the fluorometric method of Nebert & Gelboin (1968). The ECDE and CH activities were assayed by the method of Greenlee & Poland (1978). The final concentrations of the substrates were 75 μM (benzo(a)pyrene, 500 μM (7-ethoxycoumarin) and 1 mM (coumarin).

Plasma cotinine assay

The method for measuring plasma cotinine was a modification of an assay for urinary nicotine and cotinine by Wilcox *et al.* (1979). A 1 ml plasma sample was diluted and made alkaline with 3 ml of 4 N NaOH and extracted with chloroform. The organic phase was evaporated to dryness, the residue dissolved in a small volume of ethanol and cotinine was measured gas chromatographically using the OV-17 column and nitrogen detector. Column temperature was 180° C and detector and injector temperatures 250° C. External standardization was used. The coefficient of variation (at the 20 ng ml^{-1} level) was 15% ($n = 9$). The limit of the sensitivity of the method was about 1–2 ng ml^{-1} plasma.

Antipyrine determination

The antipyrine test, with oral dose of 20 mg kg^{-1} body weight was performed on patients, from whom also needle biopsy sample was available. The protocol, antipyrine assay in plasma samples and calculation of clearance, half-time and volume of distribution were as previously described in detail (Sotaniemi *et al.*, 1977; Pelkonen *et al.*, 1985).

Results

Properties of ERDE in the human liver

Various conditions for the measurement of the ERDE activity in human liver homogenate and microsomes were determined. The reaction displayed the usual cofactor requirements, i.e. NADPH or a NADPH-regenerating system. The addition of NADH did not enhance the reaction, although it supported, in the absence of NADPH, a low level (< 10%) of activity. The Michaelis-Menten constant was about 7 μM, as measured with two microsomal preparations. With liver homogenates, the reaction was linear up to 4 mg of homogenate protein and during 10 min of incubation. Dicoumarol (10 μM) was routinely included in the incubation mixture, to inhibit possible resorufin reduction, although preliminary experiments with larger liver samples (three individual livers) indicated that there were no consistent differences between incubations with or without dicoumarol. With liver microsomes, the reaction proceeded linearly until up to 200 μg of microsomal protein and during incubation for 5 to 10 min, depending on the individual liver.

ERDE, AHH, ECDE and CH of liver biopsy samples from smokers, ex-smokers and non-smokers

ERDE, AHH, CH and ECDE were measured in liver biopsy homogenates from patients whose smoking status was verified by measuring plasma cotinine concentration (Table 1). Of the four cytochrome P-450-dependent activities examined the ERDE was the only activity that was statistically significantly higher in verified smokers. The CH and ECDE activities were similar in smokers and non-smokers. The AHH activity was measured subsequently after the samples had been frozen and thawed twice. Consequently, the AHH activities of these samples were much lower than those reported in the previous study (Vähäkangas *et al.*, 1983). Nevertheless, there was no significant observable difference between smokers and non-smokers (data not illustrated). There was no significant difference in any of the enzyme activities of livers from individuals who never smoked and who were ex-smokers.

ERDE activities were plotted against plasma cotinine concentrations, and no significant correlation was found between these two parameters (Figure 1).

The elimination of antipyrine was also measured in the same patients. In agreement with the previous study (Vähäkangas *et al.*, 1983), antipyrine clearance was significantly increased. The half-life of antipyrine was shortened in the smokers, although the difference was not statistically significant (Table 2).

The effect of cytochrome P-450 inhibitory MAbs on enzyme activities in human liver microsomes

MAbs to specific epitope-containing cytochromes P-450 were used to characterize the nature of monooxygenases in human liver, that contribute to the studied enzyme activities. The MAb 1-7-1 moderately to strongly inhibited in a concentra-

Table 1 Cytochrome P-450 catalyzed monooxygenase activities in human liver biopsy homogenates from smokers, ex-smokers, and non-smokers. Classification with respect to smoking status was based on the measurement of cotinine in plasma samples. Ex-smokers had stopped smoking at least two months before the biopsy date

Group	*Enzyme activity (nmol g^{-1} liver min)**		
	ERDE	*CH*	*ECDE*
Smokers	1.045 ± 0.980 (17) (0.098–3.10)	14.4 ± 6.11 (15) (4.39–28.0)	4.35 ± 1.43 (14) (1.91–7.12)
All non-smokers	0.318 ± 0.174 (20) (0.061–0.650)	16.2 ± 9.31 (18) (4.40–43.2)	4.16 ± 2.05 (17) (1.82–10.8)
—Never-smokers	0.288 ± 0.167 (13)	16.0 ± 7.26 (12)	4.12 ± 2.42 (11)
—Ex-smokers	0.371 ± 0.187 (7)	16.8 ± 13.3 (6)	4.22 ± 1.26 (6)
Statistical difference between smokers and non-smokers			
t	3.26	0.65	0.31
P	< 0.01	NS	NS

*Mean ± s.d. (number of samples and range in parentheses).

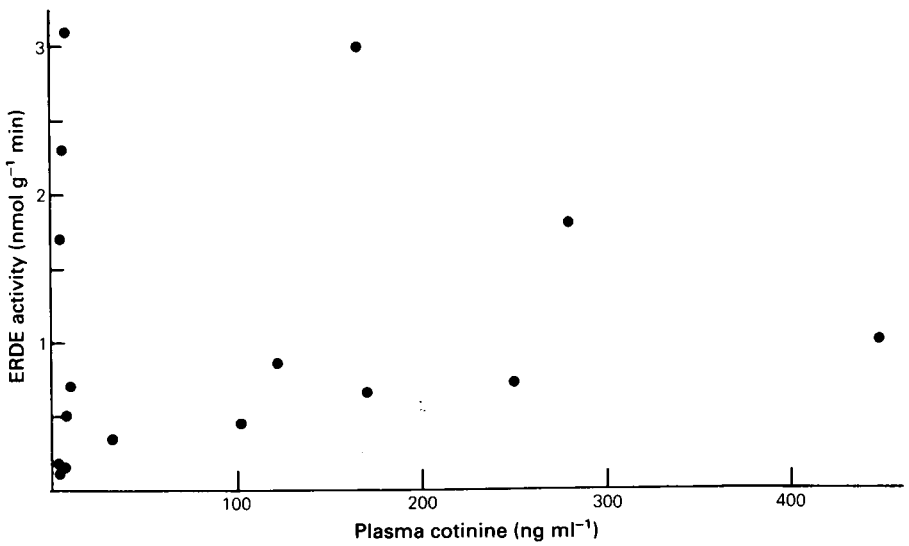

Figure 1 Relationship between plasma cotinine levels and 7-ethoxyresorufin *O*-deethylase activities in human liver biopsy homogenates; $r = 0.12$, $n = 17$.

tion-dependent manner the ERDE activity in 10 of 12 human liver samples examined. The MAb 1-7-1 however had no significant effect on AHH, CH, or ECDE activities in any of the samples except for a single sample where AHH was inhibited by 35% (Figure 2 and Table 3). The MAb 2-66-3 and the ascites fluid containing control MAbs had very little effect on any of the enzyme activities measured (Figure 2 and Table 3). The slight enhancement of AHH activity by the MAbs was observed in two cases (no. 2 and 6 in Table 3). The interindividual variation in the inhibitory effect of MAb 1-7-1 on ERDE was relatively large. The amount of inhibition of the ERDE by MAb 1-7-1 in 12 liver samples studied varied from 0 to 65% and seemed to be generally greater in smokers as compared with non-smokers (Table 3). Thus, in four samples from smokers the MAb 1-7-1 inhibited ERDE by 51 to 66%. In two of eight samples from non-

Table 2 Antipyrine elimination in smokers and non-smokers

Group	*Clearance** *(ml min^{-1})*	*Half-time** *(h)*	*Distribution volume** *(l kg^{-1})*
Smokers (15)	57.1 ± 24.4 (26.0–117.7)	9.88 ± 3.47 (4.5–17.3)	0.536 ± 0.085 (0.391–0.707)
Non-smokers (16)	39.1 ± 10.9 (22.8–65.1)	11.96 ± 3.69 (7.1–21.2)	0.509 ± 0.071 (0.395–0.672)
Statistical difference between smokers and non-smokers			
t	2.67	1.61	0.96
P	< 0.05	NS	NS

*Mean ± s.d. (range in parentheses)

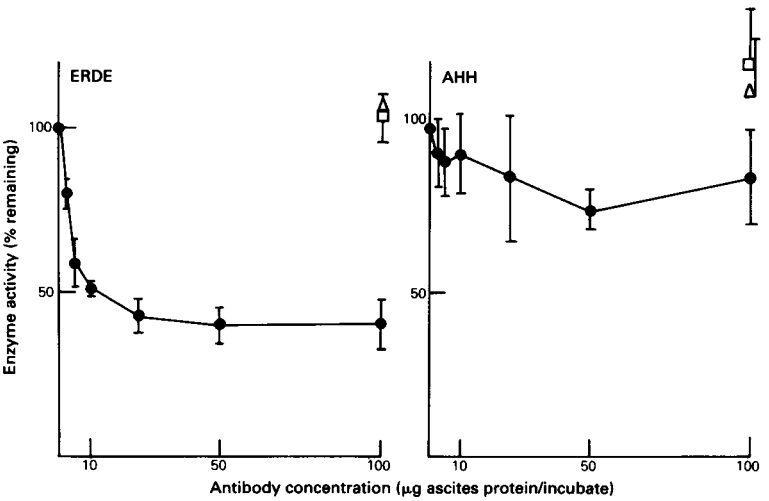

Figure 2 The effect of monoclonal antibodies (MAb) 1-7-1 (MC) and MAb 2-66-3 (PB) on human liver microsomal 7-ethoxyresorufin *O*-deethylase (ERDE) and aryl hydrocarbon hydroxylase (AHH) activities. The relative activities are shown as compared to the values without MAb. Experiments were conducted with three liver samples (nos. 1 to 3 in Table 3) with activities ranging between 20 and 30 (ERDE) and 146 and 180 (AHH) pmol mg^{-1} microsomal protein min^{-1}. Symbols: MAb 1-7-1 (●——●); MAb 2-66-3 (△); NBS (□).

smokers or questionable smokers no inhibition was observed, whereas inhibition of 30–56% was observed in the remaining six non-smokers.

The effect of MAb on enzyme activities in human placental microsomes

In contrast to liver, in human placental microsomes, the MAb 1-7-1 inhibited both AHH and ERDE activities strongly (Figure 3) as well as ECDE activity (data not shown). The MAb 2-66-3 (PB) or control NBS 1-48-5 had no effect on any of these activities. The inhibition of AHH and ERDE activities by the MAb 1-7-1 was uniformly strong, ranging around 80% inhibition, whereas the percentage inhibition of the ECDE activity between 16 and 85%, which is consistent with the results of Fujino *et al.* (1982; 1984a).

Discussion

Cigarette smoking enhances the elimination of a number of drugs and other foreign chemicals in humans, while the elimination of other substances is unaffected (Jusko, 1978). Since polycyclic aromatic hydrocarbons are a prominent component of cigarette smoke and are known inducers of hepatic xenobiotic metabolism in experimental animals a widely held view is that changes in liver enzyme levels are largely responsible for the change in drug metabolism in smokers. However, animal experiments have

Table 3 Effect of monoclonal antibodies (MAb) 1-7-1 (MC) and MAb 2-66-3 (PB) on cytochrome P-450-dependent ERDE, AHH, CH and ECDE of individual human liver microsomes. Diagnoses of the patients (histological pictures of the biopsies in parentheses) were the following: 1 and 2: hepatoma (only tumour-free tissue was used for studies); 3: renal transport donor (normal histology); 4: cholelithiasis, (portal inflammation); 5: not known; 6 and 7: hepatoma (samples probably contained tumour tissue); 8: cholelithiasis (proliferation of bile canaliculi, otherwise normal parenchyma); 9: cholelithiasis (bile stasis, parenchyma mostly normal); 10 and 11: hepatic cirrhosis (cirrhotic parenchyma); 12: cholelithiasis (slight fatty degeneration)

Case number (smoking/ inducers)‡	*Antibody added*‡	*Enzyme activity* (pmol mg^{-1} microsomal protein min^{-1})*			
		ERDE	*AHH*	*CH*	*ECDE*
1 (+/−)	C	20.3	180	not tested	nt
	MAb-MC	39	96	(nt)	
	MAb-PB	100	98		
	NBS	100	108		
2 (+/−)	C	21.0	146	nt	nt
	MAb-MC	49	92		
	MAb-PB	112	123		
	NBS	108	132		
3 (+/−)	C	30.8	156	nt	nt
	MAb-MC	34	69		
	MAb-PB	105	96		
	NBS	98	90		
4 (+/−)	C	74.3	nt	nt	nt
	MAb-MC	44			
	MAb-PB	92			
	NBS	84			
5 (−/−)	C	224	407	330	213
	MAb-MC	56	90	101	97
	MAb-PB	100	117	99	100
	NBS	91	107	92	109
6 (?/?)	C	55.9	723	244	77.5
	MAb-MC	98	120	108	102
	MAb-PB	98	118	106	105
	NBS	101	120	101	97
7 (−/?)	C	16.9	2060	200	48.2
	MAb-MC	71	108	110	104
	MAb-PB	86	104	111	94
	NBS	93	93	113	96
8 (−/−)	C	48.1	2454	182	35.3
	MAb-MC	65	113	102	119
	MAb-PB	90	112	111	122
	NBS	93	116	111	107
9(−/−)	C	10.1	nt	146	nt
	MAb-MC	96		99	
	MAb-PB	107		95	
	NBS	103		94	
10 (−/−)	C	11.8	nt	180.5	nt
	MAb-MC	68		99	
	MAb-PB	94		102	
	NBS	103		100	
11 (−/−)	C 26.4		nt	124	nt
	MAb-MC	53		105	
	MAb-PB	101		97	
	NBS	101		99	
12 (?/?)	C	47.9	nt	160	nt
	MAb-MC	55		96	
	MAb-PB	103		96	
	NBS	99		104	

* Activities in the presence of antibodies are expressed as % of the control activities. Duplicate assays were performed and variation between tubes was always less than 10%.

† The protein/antibody ratio was constant (5:1) in all experiments. NBS is a MAb control made from unimmunized mice.

‡ Refer to cigarette smoking and known exposure to inducer drugs.

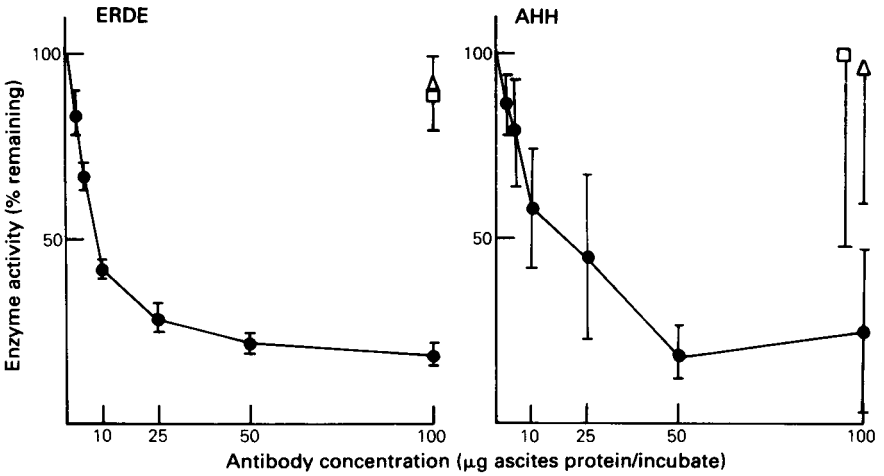

Figure 3 The effect of monoclonal antibodies (MAb) 1-7-1 (MC) and 2-66-3 (PB) on human placental 7-ethoxyresorufin *O*-deethylase (ERDE) and aryl hydrocarbon hydroxylase (AHH) activities. Experiments were conducted with three placental samples from smokers with activities ranging between 15 and 26 (ERDE) and 10 and 180 (AHH) pmol mg^{-1} microsomal protein min^{-1}. Symbols as in Figure 2.

demonstrated that the exposure to cigarette smoke does not induce hepatic xenobiotic metabolism, whereas the same treatment induces extrahepatic xenobiotic metabolism (for ref. see Pelkonen, 1976; Pelkonen *et al.*, 1984; Vähäkangas *et al.*, 1983). The few available studies with human tissues have not yielded definitive results (Boobis *et al.*, 1980; Pelkonen *et al.*, 1975; 1980; Vähäkangas *et al.*, 1983).

In the present study we found that ERDE activity was significantly higher in the liver biopsy samples from smokers. There were no differences however between smokers and non-smokers in the levels of CH, ECDE or AHH activity. Cigarette smoking status was verified by measuring plasma cotinine, which proved to be essential for the purposes of the study. On the basis of self-reporting about half of ex-smokers and 20% of non-smokers would have been wrongly classified as non-smokers, although plasma cotinine measurements showed them to be really smokers. After this correction, the classification could be confidently made and it showed that although both low and high ERDE activities were present in smokers, the levels in non-smokers were invariably low.

We did not observe any significant correlation between plasma cotinine level and hepatic ERDE activity. A possible reason for this finding may be the short half-life of cotinine, about 15 h (Darby *et al.*, 1984), when compared with the much longer of half-life of cytochrome P-450 (Omura, 1980). Admittance to the hospital probably results in some changes in the frequency and level of smoking, with consequent changes in plasma cotinine levels. Another possible reason may be that clotting does not reflect the levels of inducers absorbed from smoke.

Previously AHH and ECDE activity has been commonly used as measures of a cytochrome P-450 form associated with the induction by polycyclic aromatic hydrocarbons in experimental animals (Gelboin, 1980; Pelkonen & Nebert, 1982). In our studies with humans however, neither of these enzyme activities is induced by cigarette smoking in the liver, indicating that these are catalyzed primarily by P-450 forms that are not significantly induced in human liver with cigarette smoking.

It has been suggested that ERDE is an activity that is much more sensitive to polycyclic aromatic hydrocarbon-induction than either AHH or ECDE (Phillipson *et al.*, 1984). The present study indicates, that this is also true for human liver. Our study also demonstrates that the ERDE activity in human liver is inhibited by a MAb specific for an epitope present in both MC-induced P-450 forms c and d, whereas the MAb

against PB-induced liver P-450 was without effect. The lack of effect of the MAb 2-66-3 on ERDE activity is likely due to an absence of MAb 2-66-3 epitope specific P-450s in human liver that are active for ERDE activity. A less likely explanation is that the epitope specific P-450s are present but their binding by the MAb does not interfere with ERDE activity. This is less likely since in an RIA binding study of control and MC-treated rat liver, the MAb 2-66-3 bound to less than one fiftieth the binding of MAb 2-66-3 to the livers of rats induced by PB (Song *et al.*, 1984).

The MAb 1-7-1 recognizes epitopes that are common to both rat liver P-450c and P-450d (Cheng *et al.*, 1984a, b), which are analogous to rabbit P-450 LM6 and LM4, respectively. Our studies indicate that a significant amount of the ERDE of human livers from non-smokers and a still greater proportion of the ERDE from smokers contain an epitope recognized by MAb 1-7-1 and common to forms P-450c and P-450d. Nebert *et al.* (1982) have clearly demonstrated the presence in MC-treated mouse liver of three separate P-450 forms which are associated with the Ah locus. It is not known, whether the human hepatic P-450 associated with the induced ERDE activity is analogous to any one of these isozymes.

The inhibition of ERDE by the MAb 1-7-1 was rather variable in liver samples from different individuals. In some cases the activity was inhibited by 60 to 65% at the protein/antibody ratio of 5:1, whereas in another case it was not inhibited at all. Cigarette smoking seemed to increase the relative amount of ERDE inhibited, but because the number of individuals was small, we must wait for a larger study to confirm this finding. Also there is a discrepancy between the magnitude of induction of ERDE activity in needle biopsy samples and the inhibition of the activity by the antibody in the larger liver samples. The resolution of this discrepancy needs further studies. However, on the basis of the present results we can suggest that the ERDE activity in human liver is catalyzed by more than one P-450 form, one of which is clearly related to the exposure to cigarette smoking and shares an epitope with the cytochrome P-450 in rat liver, that is recognized by MAb 1-7-1.

Studies with the placental enzymes yielded similar results. The ERDE activity is induced in the placenta by cigarette smoking and it is strongly inhibited by the MAb 1-7-1. The characteristics of the ERDE activity in the liver are very similar, i.e. the induction by cigarette smoking and the inhibition by the MAb 1-7-1. An interesting contrast was provided by the experiments with AHH activity. The placental enzyme was inhibited by the MAb 1-7-1 (Fujino *et al.*, 1982; 1984; this study), whereas the hepatic activity did not respond to the antibody, a finding confirming that of Fujino *et al.* (1982). We could also confirm the earlier findings concerning the inhibition by MAbs of the placental and hepatic ECDE activities (Fujino *et al.*, 1982). The placental ECDE was partially inhibited by the MAb 1-7-1, whereas the hepatic enzyme was not inhibited by the same antibody. These findings strongly suggest that the cytochromes P-450 for hepatic AHH and ECDE are different from the cytochromes P-450 that are responsible for the induced levels of AHH and ECDE in placenta. Furthermore the MAb 1-7-1 studies indicate that the P-450 responsible for a significant part of ERDE in the liver of both smokers and non-smokers is different from the class of P-450 responsible for AHH and ECDE in human liver.

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