A comparative study of the formation of chemically reactive drug metabolites by human liver microsomes

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1 The metabolism of amodiaquine (A), ethinyloestradiol (E), mianserin (M), phenytoin (Ph), sulphanimide (S) and paracetamol (Pa) to both stable and chemically reactive, i.e. irreversibly protein bound, metabolites was investigated using microsomes prepared from histologically normal human liver obtained from eight kidney donors.

2 All drugs, except amodiaquine, were metabolized by NADPH-dependent microsomal enzymes to chemically reactive metabolites. The degree of NADPH-dependent binding varied between drugs (E, $11.5 \pm 5.8\%$ incubated drug; M, $3.0 \pm 1.9\%$; Ph, $0.10 \pm 0.09\%$; S, $0.57 \pm 0.38\%$; Pa, $1.2 \pm 1.2\%$; mean of eight livers $\pm$ s.d.).

3 Inclusion of glutathione (1 mM) or ascorbic acid (1 mM) in the incubation reduced the NADPH-dependent binding for all substrates, indicating the involvement of electrophilic oxidation products.

4 Binding of M and Pa correlated with each other (Spearman's $r = 0.86$) and with total cytochrome P-450 content ($r = 0.76$ and 0.78 respectively). E binding also correlated with the binding of M ($r = 0.79$) and Pa ($r = 0.81$) but not with cytochrome P-450. Binding of Ph and S did not correlate with any of the other measured metabolic parameters.

Keywords liver microsomes reactive metabolites

Introduction

The metabolism of chemicals, including drugs, to reactive metabolites which can bind covalently to proteins is now a generally accepted mechanism of chemical toxicity (Parke, 1987). The role of reactive metabolites in chemical carcinogenesis and non-carcinogenic organ-directed toxicity has been established by parallel *in vitro* and *in vivo* studies of the metabolism of model compounds such as benzo-(a)-pyrene (Miller, 1970), ipomeanol (Boyd, 1977) and paracetamol (Mitchell & Jollow, 1975).

In contrast, the role of chemically reactive metabolites in immunotoxic reactions is poorly understood; partly because of the low prevalence and the lack of dose dependency associated with

this type of adverse reaction. According to the hapten hypothesis of drug hypersensitivity, drugs may become immunogenic once conjugated to a macromolecular carrier (Park *et al.*, 1987). This hypothesis was evolved largely from studies with penicillin antibiotics, which contain a β -lactam nucleus that has inherent chemical reactivity towards nucleophilic groups on proteins (Levine *et al.*, 1966). For a number of other drugs associated with hypersensitivity reactions in man it has been assumed that drug haptens are formed from chemically reactive metabolites, rather than parent drug but there is little or no experimental evidence for such a mechanism in most cases (Park *et al.*, 1987).

Quantitatively, the most important source of reactive drug metabolites is the hepatic microsomal monooxygenase system of enzymes. However, direct measurement of reactive metabolites is difficult *in vitro* and has not been achieved *in vivo* in man. The purpose of this study was to investigate the formation of reactive metabolites from drugs, using irreversible binding to protein as an indirect means of quantifying their formation, under standardized conditions in a microsomal preparation derived from human liver. In particular, drugs which are either known or thought to elicit occasional immunotoxic adverse reactions were selected for this study since the formation of reactive intermediates may be an obligatory event leading to their toxicity. The ability of these drugs to yield reactive metabolites was compared with that of paracetamol, which produces hepatic necrosis associated with the formation of a quinone imine intermediate (Dahlin & Nelson, 1982) but which has a low reported incidence of hypersensitivity reactions. The generation of potentially toxic metabolites was compared with other metabolic parameters, such as cytochrome P-450 content of the liver samples, alkoxy coumarin-*O*-dealkylase activity and total metabolic turnover of the individual drugs.

Materials

[Quinoline-2-¹⁴C] amodiaquine dihydrochloride monohydrate (3.2 mCi mmol⁻¹), 5,5-diphenyl [4-¹⁴C]hydantoin (phenytoin) (58 mCi mmol⁻¹) and [U-¹⁴C]-sulphanilamide (11.4 mCi mmol⁻¹) were supplied by Amersham International plc. [6,7-³H]17 α -ethinyloestradiol (42 Ci mmol⁻¹) and *p*-[³H (G)] hydroxyacetanilide (paracetamol) (4.8 Ci mmol⁻¹) were supplied by New England Nuclear Corporation. [13-³H]-mianserin (20.4 Ci mmol⁻¹) was a gift from Organon International B.V. [¹⁴C]-amodiaquine was repurified by t.l.c. according to the method of Maggs *et al.* (1988). All other compounds were shown to be $\geq 97\%$ pure by h.p.l.c. or were repurified before use by h.p.l.c. under conditions (Table 2) known to separate the parent drug from all known metabolites.

Human liver samples

Whole or part livers were obtained from kidney donors, who died from cerebrovascular or other accidents, immediately following cessation of life support. Ethical approval was granted by the Mersey Regional Ethics Committee and consent to removal of the liver samples was obtained from the donors' relatives. Samples were trans-

ferred, on ice, to the laboratory within 1 h where they were sectioned into 10–20 g portions, placed in 20 ml plastic scintillation vials and immediately frozen in liquid nitrogen. The vials were stored at -80°C until required. Approximately 1 g of liver was transferred directly into buffered formalin for histological examination by the Department of Pathology, University of Liverpool. Apart from livers II and VIII, which demonstrated normal/mild inflammation of portal tracts and mild fatty change, respectively, all of the organs were histologically normal. Donor details, where obtained, are given in Table 1.

Table 1 Details of donors of human liver samples

Liver	Donor's age (years)	Sex	Drugs
I	4	M	unknown
II	28	M	unknown
III	35	M	unknown
IV	66	F	warfarin
V	60	M	penicillin, augmentin
VI	21	M	unknown
VII	59	F	mannitol, heparin, cefaloxin, solumedin, frusemide
VIII	53	F	unknown

Preparation of liver microsomes

Washed microsomes were prepared from human liver samples as previously described (Purba *et al.*, 1987). Microsomes were either used immediately or stored for a maximum of 18 h as an intact pellet at -70°C . Cytochrome P-450 content was measured by the method of Omura & Sato (1964) and microsomal protein was determined by the method of Lowry *et al.* (1951).

7-Alkoxy coumarin *O*-dealkylase activity

7-Ethoxycoumarin *O*-deethylase activity and 7-methoxycoumarin *O*-demethylase activity were determined for each liver sample (1–8 triplicate determinations per liver) under conditions previously shown to be linear with respect to time and protein concentration; NADPH concentration was non-limiting. Incubations of either 7-ethoxycoumarin or 7-methoxycoumarin (10 μM or 100 μM) were carried out in round bottom 20

ml glass tubes containing 1 ml sodium potassium phosphate buffer (pH 7.4; 0.067M), 150 µg microsomal protein and reduced nicotinamide adenine dinucleotide phosphate (NADPH, 1 mM). Reactions were performed in a shaking water bath at 37° C for 15 min and were initiated by addition of NADPH. Control incubations contained no NADPH. Extraction and quantification of the product, 7-hydroxycoumarin, was performed as reported by Greenlee & Poland (1978).

Analysis of metabolites and determination of irreversible binding

[¹⁴C]-amodiaquine (10 µM, 0.13 µCi), [³H]-ethinyloestradiol (10 µM, 1.0 µCi) [³H]-mianserin (10 µM, 0.5 µCi), [¹⁴C]-phenytoin (10 µM, 2.3 µCi), [¹⁴C]-sulphanilamide (10 µM, 0.3 µCi) or [³H]-paracetamol (10 µM, 0.25 µCi) was incubated with 2 mg ml⁻¹ microsomal protein and NADPH (1 mM) in 4 ml (2.5 ml for sulphanilamide) sodium potassium phosphate buffer (0.067 mM; pH 7.4) at 37° C for 30 min in 25 ml conical flasks. Drug substrates were added in a maximum volume of 20 µl absolute ethanol, which was shown to have no effect on enzyme activities. The reaction was initiated by addition of the NADPH, and terminated by cooling in ice and prompt addition of organic solvents for either protein precipitation or drug extraction. NADPH was omitted from control incubations. In some incubations either ascorbic acid (1 mM) or glutathione (1 mM) was added as a potential inhibitor of irreversible binding. Unchanged parent compound and metabolites were either extracted into organic solvents, concentrated to dryness and redissolved in mobile phase before h.p.l.c. analysis (recoveries of incubated radioactivity in absence of NADPH: A, 80–90%, E, 75–85%, and Ph, 85–95%) or were analysed directly in the supernatant following precipitation of protein (see Table 2 for conditions used for each individual drug). After separation by h.p.l.c., parent compound and metabolites were quantified by measuring radioactivity, in 0.5-min eluate fractions, in 4 ml scintillant (Aqua Luma Plus, Lumac/3M BV, Schaesberg, Netherlands); the counting efficiencies of ³H and ¹⁴C in the various eluates were 32%–42% and 91%–92%, respectively.

Irreversible binding of radiolabelled compounds to microsomal protein was quantified following exhaustive solvent extraction of the precipitated protein. Protein was precipitated overnight at 4° C with either trichloroacetic acid (TCA) or organic solvent and was subsequently washed with solvents of increasing polarity until no further radioactivity was extractable (the pro-

cedure used for each drug is shown in Table 2). The protein was dissolved in 3 ml sodium hydroxide (0.5 M) at 60° C for 3–6 h. Aliquots (1 ml) of the solubilized protein were neutralized with glacial acetic acid (160 µl) and radioactivity was quantified in 12 ml scintillant.

Analysis of results

Irreversible binding of radiolabelled material was taken as a measure of reactive metabolite formation and was expressed as percentage of the initial radioactivity bound to the incubated microsomal protein. Stable metabolites were expressed as percentage of initial radioactivity and the sum of reactive and stable metabolite formation was taken as total metabolic turnover. All values given are mean ± s.d. and values were compared using Wilcoxon's signed rank test for paired data. Correlations were performed using Spearman's rank correlation test.

Results

Values for total cytochrome P-450 varied between 0.23 and 0.67 nmol mg⁻¹ microsomal protein (Table 3) and were comparable with those reported in other studies with human liver microsomes (Boobis *et al.*, 1980). Similarly, 7-ethoxy- and 7-methoxycoumarin *O*-dealkylase activities varied widely between samples but 7-methoxycoumarin was invariably 2–3-fold more rapidly dealkylated than 7-ethoxycoumarin (Table 3).

The presence of NADPH in incubations of all drugs, with the exception of amodiaquine, increased the irreversible binding of radiolabelled material to microsomal protein (Table 4). Inclusion of glutathione in the incubations reduced the binding of all drugs (Table 5), presumably through reacting with electrophilic metabolites to form glutathione adducts. This inhibition supports the concept that the irreversible binding observed represents covalently bound drug metabolites and not non-extractable metabolites. The antioxidant ascorbic acid was also a potent inhibitor of binding for some of the drugs (Table 5).

Amodiaquine, ethinyloestradiol, mianserin and phenytoin were appreciably metabolized to stable metabolites (Table 6), most of which were identified as known metabolites (Claesen *et al.*, 1982; De Jongh *et al.*, 1981; Maggs *et al.*, 1983b; Winstanley *et al.*, 1987) by co-chromatography with authentic standards. Desethylamodiaquine and 5-(4-hydroxyphenyl)-5-phenylhydantoin were the sole metabolites of amodiaquine and phenytoin, respectively, whilst 2-hydroxyethinyloestradiol

Table 2 Procedures for extraction of radiolabelled drugs from incubations of human liver microsomes and h.p.l.c. analytical conditions

Drug	Extraction procedure		H.p.l.c. analytical conditions				Detection λ (nm)
	Organic extraction	Protein precipitant	Exhaustive extraction	Solvent A	Solvent B	Gradient flow (%A) (ml min ⁻¹)	Column
[¹⁴ C]-amodiaquine	Ether (2 × 5 ml)	Acetone (5 ml)	acetone (2 × 5 ml) methanol (2 × 5 ml)	methanol	triethylamine 1.25% in H ₂ O (pH 2.8)	20% isocratic	μBondapak Phenyl (Rad pak)
[³ H]-ethinyl- oestradiol	Ether (2 × 5 ml)	Acetone (5 ml)	70% methanol (1 × 5 ml) 0.1M sodium phosphate pH 7.4 (1 × 5 ml) 70% acetone (2 × 5 ml)	methanol	water	50–65% 2%/min	Spherisorb C ₁₈ , 10 μm
[³ H]-mianserin	*	Methanol (5 ml)		aceto- nitrile	NH ₄ H ₂ PO ₄ (0.1M; pH 4.6) +0.5M C ₇ sulphonate	38% isocratic	μBondapak C ₁₈ , 10 μm
[¹⁴ C]-phenytoin	Ethyl acetate (2 × 5 ml)	Acetone (5 ml)	methanol (2 × 5 ml)	methanol	water	35–55% 1%/min	Rad pak C ₈ , 10 μm
[¹⁴ C]-sulphamit- amide	*	Methanol (5 ml)	70% methanol (2 × 5 ml)	methanol	NH ₄ H ₂ PO ₄ 0.1M; (pH 2.3.)	0.5–30% 2%/min	Partisil C ₁₈ , 10 μm
[³ H]-paracetamol	*	Methanol (5 ml)	0.6M TCA (2 × 5 ml) methanol (2 × 5 ml) 70% methanol (2 × 5 ml)	methanol/ sodium acetate (1.5%)	sodium acetate (pH 3.5, 0.05M)	50% isocratic	μBondapak C ₁₈ , 10 μm (Rad pak)

* Metabolites in the supernatants obtained following protein precipitation were analysed directly

Table 3 Microsomal cytochrome P-450 content and alkoxycoumarin *O*-dealkylase activity in human liver samples

Liver	Cytochrome P-450 (nmol mg ⁻¹ protein)*	n	Alkoxycoumarin O-dealkylase activity (pmol mg ⁻¹ min ⁻¹)**					
			7-ethoxycoumarin		7-methoxycoumarin			
			10 μM	100 μM	10 μM	100 μM	10 μM	100 μM
I	0.60 ± 0.13	9	57	223	199	542		8
II	0.23 ± 0.02	4	59	193	193	539		1
III	0.20 ± 0.08	8	75	195	245	541		2
IV	0.67 ± 0.15	10	129	505	359	1088		3
V	0.29 ± 0.10	3	92	291	310	756		3
VI	0.26 ± 0.04	7	41	208	62	269		2
VII	0.37 ± 0.20	6	180	468	613	1522		3
VIII	0.23 ± 0.03	3	128	293	176	467		1
Mean ± s.d.	0.36 ± 0.18		95 ± 47	297 ± 134	169 ± 165	716 ± 403		

* Mean ± s.d. (*n* = 3–10 determinations).** Mean of triplicate determinations (*n* = triplicate determinations).**Table 4** Irreversible binding of radiolabelled material to protein following incubation of various drugs with human liver microsomes. Values are expressed as percentage of incubated radioactivity bound in the presence of NADPH (1 mM) (NADPH-dependent binding in parentheses) for each individual liver

Liver	Irreversible binding (%)					
	Amodiaquine	Ethinylloestradiol	Mianserin	Phenytoin	Sulphanilamide	Paracetamol
I	2.1 (-3.6)	20.5 (20.1)	5.9 (5.1)	0.2 (0.2)	1.3 (1.1)	3.7 (3.3)
II	0.9 (-0.3)	14.7 (13.7)	2.8 (2.3)	0.1 (0.1)	1.5 (1.2)	4.2 (1.0)
III	1.9 (-2.2)	8.4 (7.9)	2.5 (2.1)	0.1 (0.0)	1.0 (0.6)	1.4 (-0.2)
IV	3.2 (-2.0)	19.6 (19.2)	6.5 (5.5)	0.1 (0.1)	0.9 (0.4)	2.9 (2.5)
V	1.5 (-0.1)	10.8 (10.2)	2.8 (2.2)	0.3 (0.3)	0.9 (0.6)	2.4 (0.7)
VI	1.3 (-1.1)	3.8 (3.4)	0.8 (0.3)	0.1 (0.0)	0.7 (0.3)	2.4 (0.0)
VII	1.4 (-0.8)	9.3 (8.8)	5.8 (4.7)	0.0 (0.0)	0.5 (0.1)	1.8 (1.2)
VIII	3.4 (-2.0)	9.5 (9.1)	2.8 (1.9)	0.2 (0.2)	0.6 (0.3)	1.2 (0.8)
Mean	2.0* ± 0.9	12.1* ± 5.8	3.7* ± 2.1	0.1* ± 0.10	0.9* ± 0.3	2.5* ± 1.1

Values are means of three (A, E, Ph and S) or four (M and Pa) determinations

*Significantly different from value obtained in absence of NADPH. *P* < 0.01 (Wilcoxon's signed rank test).

was either the sole or principal metabolite of ethinyloestradiol. 8-Hydroxymianserin, des-methylmianserin and mianserin *N*-oxide were major metabolites but some of mianserin's metabolites were not identified. Neither paracetamol nor sulphanilamide yielded detectable stable metabolites.

The correlation matrix (Table 7) shows significant correlations between cytochrome P-450 content and the irreversible binding observed with only two of the five drugs studied (mianserin and paracetamol); there was a clear lack of association with sulphanilamide and phenytoin. There was close correlation between the *O*-dealkylase activities towards 7-ethoxy and 7-

methoxycoumarin but these values did not relate to cytochrome P-450 content nor to the binding or turnover of any drug. The total metabolic turnover of ethinyloestradiol had a rank order within the eight livers that was almost identical to the order of binding of phenytoin and mianserin to microsomal protein (correlation coefficient = 0.95). Finally, although binding varied greatly between livers (e.g. ethinyloestradiol, 3.4–20.1%), the rank order of NADPH-dependent binding was very similar with each liver: ethinyloestradiol and mianserin being first and second, respectively, in every case and phenytoin the lowest except with one liver.

Table 5 Inhibition by ascorbic acid and glutathione of irreversible binding of radiolabelled material to human liver microsomal protein in incubations with various drugs

<i>Drug and liver</i>	<i>Inhibition of irreversible binding (% inhibition)*</i>	
	<i>+ ascorbate (1 mM)</i>	<i>+ glutathione (1 mM)</i>
Amodiaquine (V)	90% #	82% #
Ethinyloestradiol (I)	81%	83%
Mianserin (I)	17%	71%
Phenytoin (I)	97%	72%
Sulphanilamide (IV)	ND	79%
Paracetamol (IV)	38%	23%

Inhibition in absence of NADPH

ND not determined

* Derived from triplicate determinations of binding to one liver's microsomes in presence and absence of ascorbate/glutathione. The coefficients of variation of the irreversible binding determinations were 2%–12%.

Table 6 Metabolism of drugs to stable and chemically reactive (irreversibly bound) metabolites in incubations with human liver microsomes and NADPH

<i>Drug</i>	<i>Total stable metabolites (% incubated radioactivity)</i>	<i>Reactive metabolites (% incubated radioactivity irreversibly bound*)</i>	<i>Total metabolic turnover (% stable + reactive metabolites)</i>
Amodiaquine	95.3 ± 8.3	–1.5 ± 1.1	95.3 ± 8.3
Ethinyloestradiol	7.6 ± 4.1	11.5 ± 5.8	19.1 ± 8.6
Mianserin	20.0 ± 10.6	3.0 ± 1.9	23.0 ± 11.6
Phenytoin	2.2 ± 1.6	0.10 ± 0.1	2.3 ± 1.6
Sulphanilamide	–	0.6 ± 0.4	0.6 ± 0.4
Paracetamol	–	1.2 ± 1.2	1.2 ± 1.2

* Control values from incubations without NADPH have been subtracted. Data were mean ± s.d. of values from eight livers

Table 7 Correlation matrix showing values of r (Spearman's rank correlation coefficient) for metabolism and binding of drugs, cytochrome P-450 content and alkoxy coumarin O-dealkylation activities in eight samples of human liver

	P-450	7-EC	7-MC	E	Irreversible binding			Metabolic turnover		
					M	Ph	S	E	M	Ph
Cytochrome P-450	-	0.28	0.52	0.59	0.76*	0.3	-0.02	0.77*	0.30	0.38
7-ethoxycoumarin (7-EC) (10 μ M)	-	-	0.74*	0.07	0.32	0.10	-0.43	0.30	0.19	0.19
7-methoxycoumarin (7-MC) (10 μ M)	-	-	-	0.23	0.61	-0.01	-0.15	0.55	0.14	0.05
Ethinylloestradiol (E)	-	-	-	-	0.79*	0.59	0.61	0.86*	0.77*	0.57
Mianserin (M)	-	-	-	-	-	0.12	0.28	0.95**	0.42	0.31
Phenytol (Ph)	-	-	-	-	-	-	0.52	0.26	0.76*	0.57
Sulphanilamide (S)	-	-	-	-	-	-	-	0.29	0.27	0.05
Paracetamol (Pa)	-	-	-	-	-	-	-	0.95**	0.41	0.64
Ethinylloestradiol	-	-	-	-	-	-	-	-	0.45	0.57
Mianserin	-	-	-	-	-	-	-	-	-	0.67

* $P < 0.05$, ** $P < 0.01$

Discussion

There is increasing acceptance of a causal relationship between the toxicity associated with certain drugs and other chemicals and their ability to form chemically reactive metabolites that can bind covalently to endogenous macromolecules. Generally, as in the case of paracetamol (Jollow *et al.*, 1973) and phencyclidine (Law, 1981) a cytotoxic effect is postulated, with the metabolite

combining with functionally important protein groups such as thiols on enzymes (Jewell *et al.*, 1982). Alternatively, a mutagenic mechanism, in which the reactive metabolite binds to nuclear DNA (Gelboin, 1983), may operate. A third possible mechanism has been suggested to explain allergic, or hypersensitivity, type reactions to drugs, whereby the reactive metabolite binds to a circulating macromolecule or to a cell surface and thereby creates an immunogen capable of eliciting a humoral immune response (Park *et al.*, 1987). Although there is little direct evidence to support this hypothesis it has recently been shown that amineptine, a tricyclic antidepressant that causes hepatitis of suspected immunological aetiology (Genève *et al.*, 1987a), is activated in human liver microsomes to a metabolite that binds to microsomal protein (Genève *et al.*, 1987b).

In the present study, we have looked for irreversible binding with five drugs, amodiaquine (Larrey *et al.*, 1986), mianserin (Stricker *et al.*, 1985), ethinylloestradiol (Beaumont *et al.* 1982), phenytoin (Siegal & Berkowitz, 1961) and sulphanilamide (Yamamoto & Eyanagi, 1977), which have been reported to cause adverse reactions characteristic of an immunologically mediated toxic reaction. However, it must be emphasised that the incidence, in each case, is probably less than 1 in 2000. In addition, the model hepatotoxin paracetamol was studied.

With the exception of amodiaquine, an increase in drug binding was observed when NADPH was included in the incubation, suggesting that these drugs can be metabolized by cytochrome P-450 enzymes to a reactive species capable of forming a covalent bond with functional groups on proteins. Amodiaquine did not require metabolic activation and we have now shown that this drug can undergo autooxidation to a reactive species in neutral solutions (Maggs *et al.*, 1988); in this case NADPH presumably acts as a reducing agent and protects against covalent binding.

As we have reported previously, ethinylloestradiol undergoes remarkably extensive metabolic activation *in vitro* (Purba *et al.*, 1987), its binding being highest in all eight livers. However, it has been shown in animal experiments that covalent binding to hepatic protein *in vivo* is extremely low and that metabolic phase II processes provide an efficient defence against toxic metabolite formation (Maggs *et al.*, 1983a). Thus, although all the drugs investigated here can be oxidised to protein-reactive metabolites, and may therefore generate new antigens, whether or not such a mechanism will occur *in vivo* depends upon the balance between toxic (reactive) metabolite formation and the activity of detoxication processes.

It would be useful if a marker of enzyme activity could be linked to formation of reactive drug metabolites in order to identify those individuals who are more likely to generate such potentially toxic metabolites. Towards this end we measured both total cytochrome P-450 content and the dealkylase activities towards two model substrates, 7-ethoxycoumarin and 7-methoxycoumarin, in each liver sample, and attempted to correlate these with the metabolic and binding characteristics of the drugs. Cytochrome P-450 values related well to the binding of ethinyloestradiol, mianserin and paracetamol, but values for the correlation coefficient with phenytoin and sulphanilamide were very close to zero. *O*-Dealkylase activity towards 7-ethoxy- and 7-methoxycoumarin did not correlate with the binding or metabolism of any of the drug substrates studied. The best predictor of irreversible binding was the overall metabolism of ethinyloestradiol, which not only related to its own binding but correlated almost perfectly with the binding of mianserin and paracetamol. Correlations performed with a relatively small sample size should be interpreted with caution. Nevertheless, when ranking of the drugs for each individual liver was performed a fairly consistent pattern emerged demonstrating that, although quantitatively large interindividual variation in binding occurred, qualitatively there was little difference between the liver samples investigated.

The fact that ethinyloestradiol, which has the lowest incidence of side effects of the drugs studied, showed by far the greatest capacity to form reactive metabolites indicates that this

simple *in vitro* screening procedure cannot be used to predict the toxicological characteristics of a new drug, without additional information from parallel *in vivo* studies. This indication is reinforced by the inverse relationship displayed by sorbinil, an aldose reductase inhibitor, which does not undergo metabolic activation under the conditions employed here (Maggs & Park, 1988) but is associated with a high incidence of hypersensitivity reactions in man (Jaspan, 1986). Nevertheless, all the drugs studied here have been reported to cause immunotoxic and other adverse reactions and all were capable of producing a metabolite that binds to protein. It is thought that individuals who are deficient in defence mechanisms against reactive metabolites (or, conversely, who have an exaggerated ability to generate such metabolites) may show adverse effects to a drug which is apparently safe within the normal population (Spielberg *et al.*, 1981). However, the relationship between the production of protein-binding drug metabolites *in vitro* and a drug's toxic reactions *in vivo* is currently unpredictable (Streeter *et al.*, 1984). Thus the knowledge that a drug forms such metabolites in a human liver preparation can only alert investigators to the possibility of adverse reactions and aid the design of appropriate *in vivo* metabolism and toxicity studies.

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