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PREDICTION OF HUMAN CLEARANCE OF TWENTY-NINE DRUGS FROM HEPATIC MICROSOMAL INTRINSIC CLEARANCE DATA: AN EXAMINATION OF IN VITRO HALF-LIFE APPROACH AND NONSPECIFIC BINDING TO MICROSOMES

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ABSTRACT:

Twenty-nine drugs of disparate structures and physicochemical properties were used in an examination of the capability of human liver microsomal lability data ("in vitro $T_{1/2}$" approach) to be useful in the prediction of human clearance. Additionally, the potential importance of nonspecific binding to microsomes in the in vitro incubation milieu for the accurate prediction of human clearance was investigated. The compounds examined demonstrated a wide range of microsomal metabolic labilities with scaled intrinsic clearance values ranging from less than 0.5 ml/min/kg to 189 ml/min/kg. Microsomal binding was determined at microsomal protein concentrations used in the lability incubations. For the 29 compounds studied, unbound fractions in microsomes ranged from 0.11 to 1.0. Generally, basic compounds demonstrated the greatest extent of binding and neutral and acidic compounds the least extent of binding. In the projection of human clearance values, basic and neutral compounds were well predicted when all binding considerations (blood and microsome) were disregarded, however, including both binding considerations also yielded reasonable predictions. Including only blood binding yielded very poor projections of human clearance for these two types of compounds. However, for acidic compounds, disregarding all binding considerations yielded poor predictions of human clearance. It was generally most difficult to accurately predict clearance for this class of compounds; however the accuracy was best when all binding considerations were included. Overall, inclusion of both blood and microsome binding values gave the best agreement between in vivo clearance values and clearance values projected from in vitro intrinsic clearance data.

The use of in vitro drug metabolism data in the understanding of in vivo pharmacokinetic data has recently become an area of scientific interest (Houston, 1994; Houston and Carlile, 1997; Iwatsubo et al., 1997). This has partially stemmed from a trend in the pharmaceutical industry to use in vitro drug metabolism data, using human-derived reagents, as a criterion to select compounds for further development (Rodrigues, 1997). Thus, in vitro metabolism data is used in a prospective manner to choose those compounds for further development that are expected to possess commercially acceptable pharmacokinetic properties (e.g., half-life permitting once-per-day administration regimens, low oral clearance to reduce dose, etc.). Several investigators have recently described methods whereby preclinical drug metabolism and pharmacokinetic data can be used to predict human pharmacokinetic parameters (Obach et al., 1997; Lave et al., 1997a,b; Mahmood, 1998a,b).

The first demonstration of the correlation between in vivo clearance values and clearance values calculated from liver microsomal metabolism intrinsic clearance data was made by Rane et al. (1977) for the rat. Intrinsic clearance data were obtained by determination of the enzyme kinetic parameters (V_{max} and K_M). In our work, we described two related methods whereby human clearance could be predicted from in vitro metabolism data (Obach et al., 1997). In one method, the enzyme kinetic parameters V_{max} and K_M were determined and converted to intrinsic clearance (CL'_{int})[1], which is similar to that described by Rane et al. (1977). In the other method, referred to as the "in vitro $T_{1/2}$ method", CL'_{int} was determined by measuring the first-order rate constant for consumption of the substrate at a low concentration. Interestingly, for both of these methods, a better correlation was observed between the actual and predicted clearance values if the free fraction in blood was disregarded in the well-stirred or parallel-tube equations describing hepatic extraction.

One possible reason for the observation that a better prediction of human clearance was made when disregarding plasma protein binding was that the substrates were bound in the microsomal incubations, and that the extent of this binding could be great enough so as to almost cancel out the plasma protein binding term in the well-stirred and parallel-tube equations (Obach, 1996). This possibility was further substantiated in an examination of probe substrates propranolol, imipramine, and warfarin (Obach, 1997). In this report, it was demonstrated that the lipophilic amines propranolol and imipramine were bound to microsomes, and that incorporation of this binding term aided in the accurate prediction of human clearance from in vitro intrinsic clearance data. The acidic drug, warfarin, exhibited this phenomenon to a much lesser extent. However, for all three drugs overall, incorporation of both plasma protein and microsome binding

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[1] Abbreviations used are: CL'_{int}, intrinsic clearance; $f_{u(mic)}$, unbound fraction in microsomal incubation mixtures; $f_{u(blood)}$, unbound fraction in blood; Q, hepatic blood flow; ISTD, internal standard.

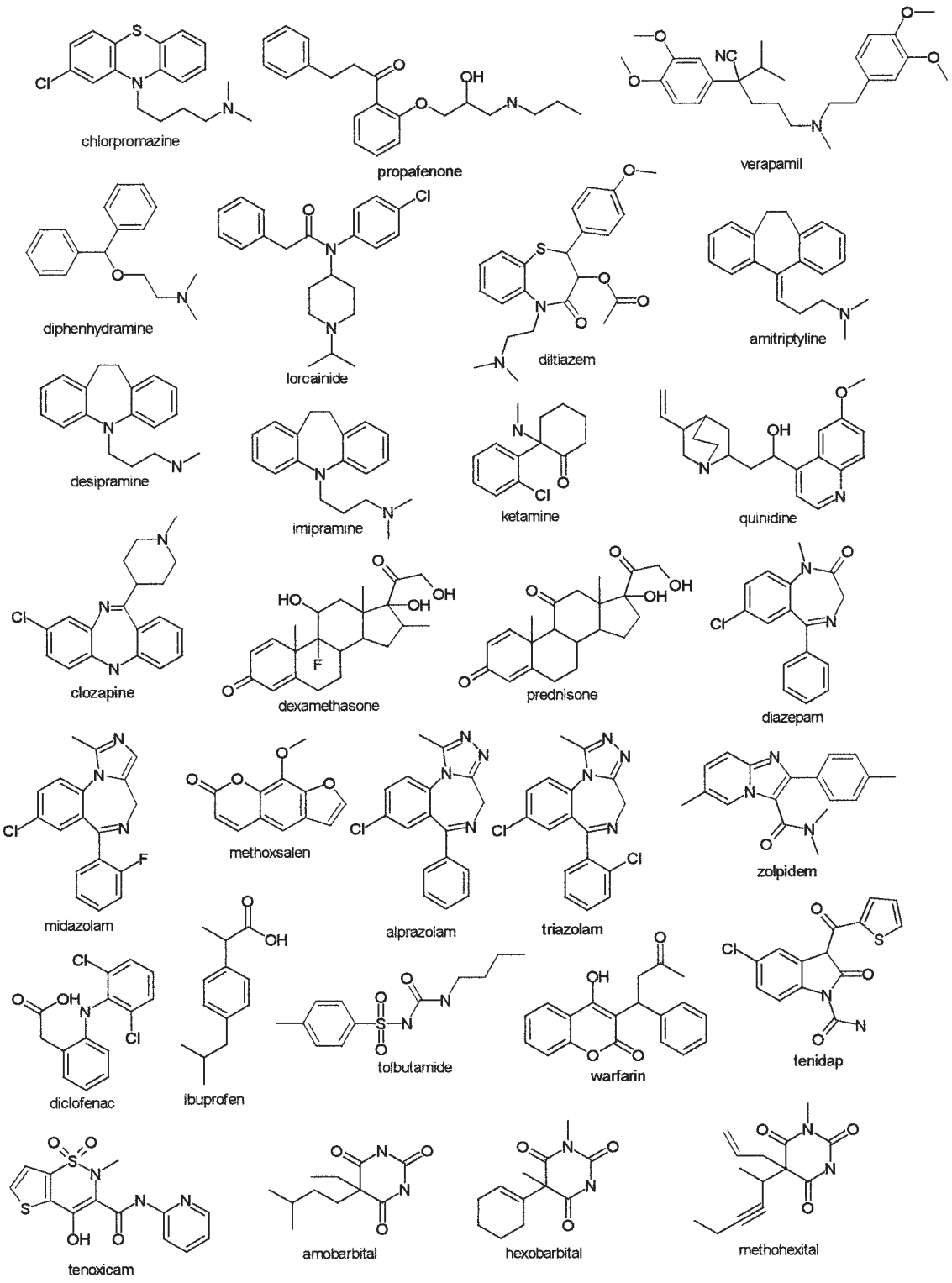

FIG. 1. *Chemical structures of the 29 drugs examined in this study.*

terms generally yielded more accurate predictions of human clearance.

The objective of the experiments described herein is to more exhaustively test the hypothesis that microsomal binding is an important phenomenon in the prediction of in vivo pharmacokinetics from in vitro drug metabolism data. To this end, human hepatic microsomal metabolism data were gathered for 29 drugs using the in vitro $T_{1/2}$ approach. Additionally, the extent of nonspecific binding to microsomes in the in vitro matrix was measured for each drug. The drugs used in these experiments span a broad range of structural types (Fig. 1) and include basic compounds (positively charged at pH 7.5), acidic compounds (negatively charged at pH 7.5), and neutral compounds (no charge at pH 7.5). The data set was used to project human clearance from the in vitro intrinsic clearance data to determine

TABLE 1

Sample processing and HPLC-MS conditions for 29 drugs used in this analysis

Drug	Internal Standard	Incubation Termination	Mobile Phase System	CH_3CN	MS Polarity	*m/z*	R_t
				%			min
Basic compounds							
Chlorpromazine	Amitriptyline	NaOH	1	36.5	+	318.8	1.2
Propafenone	Verapamil	NaOH	1	32.0	+	341.9	1.4
Verapamil	Propafenone	NaOH	1	32.0	+	455.1	1.6
Diphenhydramine	Propafenone	NaOH	1	32.0	+	256.0	0.8
Lorcainide	Propafenone	NaOH	1	32.0	+	371.0	1.4
Diltiazem	Propafenone	NaOH	1	32.0	+	415.0	1.0
Amitriptyline	Imipramine	NaOH	1	36.5	+	278.0	1.0
Desipramine	Amitriptyline	NaOH	1	36.5	+	266.5	0.8
Imipramine	Amitriptyline	NaOH	1	36.5	+	281.0	1.0
Ketamine	Metoprolol	NaOH	1	18.5	+	237.8	0.8
Quinidine	Ondansetron	NaOH	1	18.5	+	325.0	1.5
Clozapine	Diltiazem	NaOH	1	27.5	+	326.9	1.2
Neutral compounds							
Dexamethasone	Prednisone	NaOH	1	32.0	+	393.1	1.8
Prednisone	Dexamethasone	NaOH	1	32.0	+	359.1	1.1
Diazepam	Midazolam	NaOH	1	50.0	+	284.9	1.4
Midazolam	Diazepam	NaOH	1	50.0	+	325.8	0.8
Methoxsalen	Diazepam	CH_3CN	1	50.0	+	217.0	1.0
Alprazolam	Triazolam	NaOH	1	41.0	+	309.0	0.9
Triazolam	Alprazolam	NaOH	1	41.0	+	342.9	1.0
Zolpidem	Quinine	NaOH	1	23.0	+	308.0	1.5
Acidic compounds							
Diclofenac	Ibuprofen	HCl	2	32.0	−	294.0	1.1
Ibuprofen	Diclofenac	HCl	2	32.0	−	205.1	1.3
Tolbutamide	Warfarin	HCl	2	27.5	−	269.0	1.2
Warfarin	Tolbutamide	HCl	2	27.5	−	307.3	1.2
Tenidap	Warfarin	HCl	2	32.0	−	319.1	1.3
Tenoxicam	Piroxicam	HCl	2	27.5	−	336.1	0.8
Amobarbital	Methohexital	HCl	2	45.5	−	225.2	0.8
Hexobarbital	Methohexital	HCl	2	45.5	−	235.1	0.8
Methohexital	Amobarbital	HCl	2	45.5	−	261.1	1.5

whether the most accurate projections are made by disregarding all binding data, including only blood binding values, or including both blood and microsomal binding values.

Experimental Procedures

Materials. The 29 drugs examined in these experiments were obtained from Sigma Chemical Co. (St. Louis, MO) with the exception of lorcainide (obtained from ICN, Aurora, OH), methoxsalen (obtained from Aldrich Chemical, Milwaukee, WI), zolpidem (obtained from Research Biochemicals International, Natick, MA), and methohexital (obtained from Radian Inc., Dallas, TX). NADPH was obtained from Sigma. Solvents and other reagents were from common sources and were of HPLC grade or better. Human liver microsomes were from an in-house bank of liver microsomes maintained at Pfizer Central Research (Groton, CT). A pool was prepared from six liver microsomal preparations from six individual donors that were selected on the basis of having average activities for five of the major drug metabolizing cytochrome P-450 (CYP) enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A) normalized per microsomal protein content. Microsomes from putative CYP2D6 and CYP2C19 poor metabolizers were excluded. The P-450 content of this pool, as determined by spectral means (Omura and Sato, 1964) was 0.26 nmol/mg microsomal protein. CYP isoform specific marker substrate activities were as follows: CYP1A2, phenacetin *O*-deethylase of 0.147 nmol/min/mg protein (at 50 μM phenacetin); CYP2C9, tolbutamide 4-hydroxylase of 0.23 nmol/min/mg protein (at 1.0 mM tolbutamide); CYP2C19, *S*-mephenytoin 4′-hydroxylase of 0.093 nmol/min/mg protein (at 1.0 mM *S*-mephenytoin); CYP2D6, bufuralol 1′-hydroxylase of 0.075 nmol/min/mg protein (at 10 μM bufuralol); and CYP3A4, testosterone 6β-hydroxylase of 2.7 nmol/min/mg protein (at 250 μM testosterone). All glassware was subjected to gas phase silylation before use.

Metabolic Incubations. Human liver microsomal incubations were conducted in triplicate. General conditions are described as follows with details specific to each drug listed in Table 1. Incubation mixtures consisted of liver microsomes (0.3–10 mg microsomal protein/ml), substrates (1.0 μM), $MgCl_2$ (3.3 mM), and NADPH (1.3 mM) in a total volume of 0.5 ml potassium phosphate buffer (25 mM, pH 7.5). Reactions were commenced with the addition of NADPH and shaken in a water bath open to the air at 37°C. At $T = 0$ and at five time points ranging to 40 min, aliquots (50 μl) were removed and added to termination mixtures containing internal standards as listed in Table 1. The samples were processed by extraction into methy *t*-butyl ether (3 ml), the aqueous layer was frozen in a dry ice-acetone bath, the organic solvent was decanted and evaporated under N_2 at 30°C. The residue was reconstituted in 50 μl HPLC mobile phase A (see below). For methoxsalen samples, the work-up procedure consisted of precipitation of protein with CH_3CN (100 μl), removal of precipitated materials by centrifugation, and analysis of the supernatant by HPLC-mass spectrometry (MS).

Equilibrium Dialysis. Drugs (1.0 μM) were mixed with human liver microsomes (at protein concentrations used for the respective metabolic incubations), $MgCl_2$ (3.3 mM) and potassium phosphate buffer (25 mM; pH 7.5). The mixtures were subjected to equilibrium dialysis versus buffer/$MgCl_2$ at 37°C using a Spectrum apparatus (Spectrum Industries, Los Angeles, CA) as per instructions of the manufacturer. Spectra-Por no. 4 membranes, with molecular mass cutoff of 12 to 14 kDa, were used and the cells were rotated at 20 rpm for 5 h. (These dialysis conditions had been previously shown to give equilibrium for this dialysis apparatus; Obach, 1997). Dialysis experiments were done in triplicate. On completion of the dialysis period, the microsome and buffer samples were removed, processed as described above, and analyzed by HPLC-MS. Microsome samples (50 μl) were mixed with control buffer (100 μl), and buffer samples (100 μl) were mixed with control microsomes (50 μl) to yield an identical matrix before sample work-up. Drug recovery through the dialysis procedure was determined by analyzing samples of the mixtures that were not subjected to dialysis, and recovery values were 86% or greater.

HPLC-MS Analysis. The HPLC-MS system consisted of a Hewlett-Packard 1100 quaternary gradient HPLC pump with membrane degasser (Hewlett-Packard, Palo Alto, CA), a CTC PAL autoinjector (Leap Technologies, Carrboro, NC), and a PE-Sciex API 100 single quadrupole mass spec-

TABLE 2

Values for systemic clearance, fraction unbound in plasma, and blood-to-plasma ratio for 29 drugs examined in this analysis

Drug	Fraction Unbound in Plasma (f_u)	Blood-to-Plasma Ratio	Nonrenal Clearance[a]		References
			Plasma	Blood	
			ml/min/kg		
Basic compounds					
Chlorpromazine	0.05	0.78	8.6[b]	11	Dahl and Strandjard, 1974; Maxwell et al., 1972; Lund, 1980
Propafenone	0.04	0.70	13	19	Bryson et al., 1993
Verapamil	0.10	0.77[c]	15	19	Eichelbaum et al., 1984
Diphenhydramine	0.22	0.65[c]	6.2	9.5	Blyden et al., 1986
Lorcainide	0.15	0.77	14	18	Somani et al., 1987; Klotz et al., 1978
Diltiazem	0.22	1.0	12	12	Echizen and Eichelbaum, 1986; Smith et al., 1983
Amitriptyline	0.05	0.86	10	12	Schulz et al., 1983
Desipramine	0.18	0.96	12	12	Brosen and Gram, 1988
Imipramine	0.10	1.1	13	12	Sallee and Pollack, 1990; Abernathy et al., 1985
Ketamine	0.88	0.82[c]	16	20	White et al., 1985
Quinidine	0.13	0.92	2.5	2.7	Greenblatt et al., 1977; Rakhit et al., 1984; Hughes et al., 1975
Clozapine	0.05	0.87	2.5	2.9	Cheng et al., 1988
Neutral compounds					
Dexamethasone	0.32	0.93	3.5	3.8	Tseui et al., 1979; Peterson et al., 1983
Prednisone	0.25	0.83[c]	4.1	4.9	Schalm et al., 1977
Diazepam	0.013	0.71	0.4	0.6	Greenblatt et al., 1980; Maguire et al., 1980
Midazolam	0.05	0.53	4.6	8.7	Heizmann et al., 1983
Methoxsalen	0.09	0.67	12	18	Billard et al., 1995; Pibouin et al., 1987
Alprazolam	0.32	0.78[c]	0.59	0.76	Smith et al., 1984
Triazolam	0.10	0.62[c]	2.9	4.7	Smith et al., 1987
Zolpidem	0.08	0.76[c]	4.3	5.7	Durand et al., 1992
Acidic compounds					
Diclofenac	0.005	0.55[c]	4.2	7.6	Willis et al., 1979; Chan et al., 1987
Ibuprofen	0.01	0.55[c]	0.8	1.5	Martin et al., 1990
Tolbutamide	0.04	0.55[c]	0.2	0.36	Balant, 1981; Scott and Poffenbarger, 1979
Warfarin	0.01	0.55	0.045	0.081	O'Reilly, 1972
Tenidap	0.0007	0.56	0.058	0.10	Gardner et al., 1995
Tenoxicam	0.009	0.67	0.02	0.03	Heintz et al., 1984
Amobarbital	0.39	1.5	0.53	0.35	Bachmann, 1987; Sawada et al., 1985
Hexobarbital	0.53	1.0	3.6	3.6	Breimer et al., 1975; Sawada et al., 1985
Methohexital	0.27	0.70[c]	11	16	Breimer, 1976; Gillis et al., 1976

[a] All clearance values from the literature were from i.v. dosing. In the case of dependence of clearance on genetic polymorphism of drug-metabolizing enzymes, data from poor metabolizers was excluded. Nonrenal clearance values were calculated by: $Cl_{non\text{-}renal} = Cl_{total} \cdot (1 -$ fraction of the dose excreted unchanged in urine).

[b] Chlorpromazine clearance values from i.m. dose; assumes complete absorption from i.m. route.

[c] Denotes blood-to-plasma ratios that were unavailable in the scientific literature. Values were determined in duplicate after incubation of drug at 1.0 μg/mL in whole blood at ambient temperature for 45 min.

trometer (Sciex, Thornhill, Ontario, Canada) with a turbo ionspray interface. There were various mobile phases used for the different drugs as listed in Table 1. Mobile phase system 1 consisted of 20 mM acetic acid (adjusted to pH 4 with NH_4OH) and CH_3CN used at various percentages of organic solvent (as listed in Table 1). System 2 consisted of 5 mM NH_4OAc (pH unadjusted) and CH_3CN at various percentages as listed in Table 1. The column used was a Phenomenex Luna C18 narrow bore column (2.5 × 50 mm) with a 3-μm particle size (Phenomenex, Torrance, CA). The flow rate was 0.5 ml/min and the mobile phase composition was held isocratically for each analyte. The injection volume was 25 μl.

The effluent was split with approximately 0.25 ml/min introduced into the turbo ionspray source of the mass spectrometer. Source parameters (e.g., orifice voltage, temperature, gas flow rates, etc.) were individually optimized for each drug, and the molecular ion (either $M + H^+$ or $M - H^-$, depending on the orifice polarity) was followed for each compound and internal standard in the selected ion monitoring mode.

Calculations. In the determination of the in vitro $t_{1/2}$, the analyte/ISTD peak height ratios were converted to percentage drug remaining, using the $T = 0$ peak height ratio values as 100%. The slope of the linear regression from log percentage remaining versus incubation time relationships (−k) was used in the conversion to in vitro $T_{1/2}$, values by in vitro $T_{1/2} = -0.693/k$. Conversion to in vitro CL'_{int} (in units of ml/min/kg) was done using the following formula (Obach et al., 1997):

$$CL'_{int} = \frac{0.693}{\text{in vitro } T_{1/2}} \cdot \frac{\text{ml incubation}}{\text{mg microsomes}} \cdot \frac{45 \text{ mg microsomes}}{\text{gm liver}} \cdot \frac{20 \text{ gm liver}}{\text{kg b.w.}}$$

For microsomal binding, the fraction unbound in the incubation mixture was calculated by:

$$f_{u(mic)} = \frac{\text{drug/ISTD peak height ratio in buffer sample}}{2 \cdot \text{drug/ISTD peak height ratio in microsome sample}}$$

with the factor of 2 in the denominator because the aliquot volume of buffer samples analyzed was twice that analyzed for the microsome samples (see above).

The overall accuracies of clearance prediction methods were determined by (Obach et al., 1997):

$$\text{average fold error} = 10^{\left|\frac{\Sigma \log\left(\frac{\text{predicted}}{\text{actual}}\right)}{N}\right|}$$

Literature values for i.v. clearance, plasma binding, and blood-to-plasma ratio for the 29 compounds are listed in Table 2. For those compounds in which renal excretion of unchanged drug represents a significant component of total clearance, clearance values were corrected to nonrenal clearance values by:

$$CL_{nonrenal} = CL_{total} \cdot (1 - \text{fraction of dose excreted unchanged in urine})$$

Results

The use of HPLC-atmospheric pressure ionization-MS was an important tool in the gathering of these metabolic lability and microsomal binding data. The selectivity and sensitivity of this instrumentation permitted facile quantitation of a wide variety of drug structures. Chromatographic methods were developed for each compound

TABLE 3

In vitro intrinsic clearance values and fraction unbound in the incubation conditions for 29 drugs examined

Each in vitro $T_{1/2}$ and microsomal binding value represents mean ± S.D. for triplicate determinations. Intrinsic clearance values were calculated from in vitro $T_{1/2}$ data as described in *Experimental Procedures*.

Drug	Microsomal Concentration	In Vitro $T_{1/2}$	CL'_{int}	$f_{u(mic)}$
	mg/ml	*min*	*ml/min/kg*	
Basic compounds				
Chlorpromazine	1.0	25 ± 6	25 ± 6	0.11 ± 0.02
Propafenone	0.5	8.0 ± 0.4	166 ± 8	0.26 ± 0.04
Verapamil	0.5	10 ± 0.2	122 ± 2	0.43 ± 0.10
Diphenhydramine	6.0	49 ± 24	2.1 ± 0.9	0.29 ± 0.02
Lorcainide	1.0	13 ± 2	50 ± 6	0.52 ± 0.03
Diltiazem	2.0	21 ± 3	15 ± 2	0.76 ± 0.10
Amitriptyline	0.5	92 ± 13	14 ± 2	0.15 ± 0.04
Desipramine	0.5	74 ± 24	17 ± 7	0.21 ± 0.01
Imipramine	0.5	66 ± 5	19 ± 2	0.18 ± 0.04
Ketamine	1.0	23 ± 3	27 ± 4	0.49 ± 0.02
Quinidine	5.0	37 ± 5	3.4 ± 0.5	0.32 ± 0.17
Clozapine	5.0	27 ± 5	4.6 ± 0.9	0.13 ± 0.01
Neutral compounds				
Dexamethasone	5.0	42 ± 3	3.0 ± 0.2	1.00 ± 0.07
Prednisone	5.0	47 ± 1	2.7 ± 0.0	0.20 ± 0.02
Diazepam	5.0	54 ± 19	2.3 ± 0.7	0.28 ± 0.05
Midazolam	1.0	3.9 ± 0.1	160 ± 3	0.88 ± 0.12
Methoxsalen	0.5	31 ± 3	40 ± 3	0.94 ± 0.11
Alprazolam	5.0	105 ± 66	1.6 ± 1.0	0.66 ± 0.04
Triazolam	1.0	33 ± 2	19 ± 1	0.78 ± 0.09
Zolpidem	5.0	44 ± 5	2.8 ± 0.3	0.58 ± 0.10
Acidic compounds				
Diclofenac	0.3	11 ± 3	189 ± 39	1.00 ± 0.13
Ibuprofen	2.0	36 ± 4	8.8 ± 0.9	0.84 ± 0.13
Tolbutamide	10	71 ± 12	0.90 ± 0.15	0.95 ± 0.03
Warfarin	10	>120	<0.52	0.47 ± 0.05
Tenidap	3.0	26 ± 2	8.3 ± 0.7	0.32 ± 0.01
Tenoxicam	10	38 ± 11	1.7 ± 0.4	0.78 ± 0.03
Amobarbital	10	66 ± 5	0.94 ± 0.07	0.76 ± 0.08
Hexobarbital	5.0	48 ± 6	2.3 ± 0.3	0.81 ± 0.05
Methohexital	1.0	13 ± 2	49 ± 8	0.86 ± 0.13

using the same column and only two types of mobile phases, with virtually the only customization required for each compound being determination of an optimal percentage of organic modifier (CH_3CN) to effect elution of drug and internal standard within a reasonable run time.

In vitro $T_{1/2}$ data in pooled human liver microsomes for the 29 compounds examined are listed in Table 3. Metabolic lability of this set of compounds spanned a wide range, the most stable compound being warfarin (in vitro $T_{1/2}$ was immeasurably long at a microsomal protein concentration of 10 mg/ml), and the most labile being diclofenac, propafenone, and midazolam (scaled CL'_{int} values of 160 ml/min/kg or greater). Within each general class of compounds (weak bases, weak acids, and neutral compounds), intrinsic clearance values spanned a broad range. Bases ranged from low intrinsic clearance values of 3.4 and 4.6 ml/min/kg for quinidine and clozapine, respectively, to high intrinsic clearance values of 122 and 166 ml/min/kg for verapamil and propafenone, respectively. Intrinsic clearance values for acids ranged from less than 0.52 ml/min/kg for warfarin and 0.90 and 0.94 ml/min/kg for tolbutamide and amobarbital, respectively, up to 189 ml/min/kg for diclofenac. Intrinsic clearance values for the neutral compounds ranged from 1.6 ml/min/kg for alprazolam to 160 ml/min/kg for midazolam.

The extent of microsomal binding was determined for each compound using a microsomal protein concentration equal to that used in the metabolic incubations (Table 3). Because different protein concentrations were used, the compounds cannot be rank ordered with regard to extent of binding to microsomes. The values ranged from no binding to approximately 90% bound. Furthermore, those compounds that exhibited the greatest extent of binding were not necessarily those in which the microsomal protein concentration was highest. In general, the weak bases demonstrated greater binding to microsomes, despite the fact that microsomal concentrations used for the bases were, on average, lower than those used for the neutral and acidic compounds.

A summary of human blood clearance predictions from the in vitro data is presented in Table 4 and predicted clearance values are plotted versus actual clearance values in Fig. 2. Equations for both the well-stirred and the parallel-tube models of hepatic extraction were applied under three variations: disregarding all binding values (Table 4, eqs. 1 and 4), including only blood binding (Table 4, eqs. 2 and 5), and including both blood and in vitro microsome binding (Table 4, eqs. 3 and 6). Overall accuracy values, determined as described in *Experimental Procedures*, are listed in Table 5. For all compounds examined ($n = 29$), average fold error values were just over 2-fold in the cases in which either no binding values were considered or all binding values were considered. The most accurate method was the use of the parallel-tube model with both blood and microsome binding incorporated (average fold error of 2.13). Using only the blood binding value in either model of hepatic extraction yielded very poor predictions of human clearance. When subsets of compounds were considered, some differences as to which were the most accurate methods were observed. For weak bases and neutral compounds, disregarding all binding in either model of hepatic extraction yielded the best agreement between actual human clearance values and those projected from in vitro intrinsic clearance data. However, for the acidic compounds, the most accurate clearance prediction methods

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