Utility of *in vitro* clearance in primary hepatocyte model for prediction of *in vivo* hepatic clearance of psychopharmacons

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Highlights

- Primary hepatocytes offer a simple *in vitro* model for pharmacokinetic studies.
- The utility of hepatocytes for prediction of *in vivo* clearance of drugs or novel chemical entities may have some limitations.
- *In vitro* pharmacokinetic behaviour of psychopharmacons in rat hepatocytes correlated with *in vitro* human pharmacokinetics.
- Neither the elimination rates nor the intrinsic clearance in dog or rabbit cells resembled those parameters in human cells.
- For prediction of *in vivo* hepatic clearance, rat hepatocyte model appeared to be the most relevant to human.
- Human bioavailability values predicted from *in vitro* clearance were in good agreement with clinical bioavailability data.

Abstract

Primary hepatocytes offer a simple *in vitro* model for studying biotransformation of drugs or novel chemical entities. The utility of hepatocytes for the prediction of *in vivo* clearance was investigated by using 16 psychopharmacons of disparate structures (aripiprazole, biperiden, carbamazepine, citalopram, clonazepam, clozapine, duloxetine, fluoxetine, haloperidol, mianserin, mirtazapine, olanzapine, paroxetine, quetiapine, risperidone, venlafaxine). *In vitro* pharmacokinetic parameters ($t_{1/2}$ elimination half-life, Cl_{int} intrinsic clearance) were determined in human, rat, dog and rabbit hepatocytes by liquid chromatography tandem mass spectrometry, and hepatic clearance values (Cl_H), hepatic extraction ratios as well as bioavailability values were predicted.

In human hepatocytes, the most stable compounds were carbamazepine, citalopram, clonazepam, fluoxetine, mirtazapine and paroxetine, displaying *in vitro* $t_{1/2}$ longer than the 5-hour incubation period, whereas quetiapine appeared to be the most labile drug. The fastest elimination rates were observed in rabbit hepatocytes, with approximately one or two magnitude orders faster than in human liver cells. *In vitro* pharmacokinetic parameters obtained from rat hepatocytes displayed strong correlation with *in vitro* human values ($r^2>0.85$); whereas neither the elimination rates nor Cl_{int} of the drugs in dog or rabbit hepatocytes resembled those parameters in human cells ($r^2<0.2$). Similarly, there were significant interspecies differences in hepatic clearance predicted from *in vitro* elimination half-lives. Namely significant correlation was observed in predicted Cl_H values between human and rat, and no correlation was found between human and dog or rabbit. The human hepatic extraction ratios of the psychopharmacons ranged widely from the lowest values for carbamazepine and clonazepam (<0.1) to the highest for quetiapine (0.7). The human bioavailability values predicted from *in vitro* pharmacokinetic data were in good agreement with clinical bioavailability data.

In conclusion, the predicted bioavailability obtained from human hepatocytes showed an excellent rank order with *in vivo* findings. Furthermore, rat was considered to be the most relevant animal model to human subjects.

Keywords: pharmacokinetic model, primary hepatocytes, *in vitro – in vivo* extrapolation, psychopharmacons, clearance, bioavailability

Abbreviations: CYP cytochrome P450, LC-MS/MS liquid chromatography tandem mass spectrometry

1. Introduction

The early knowledge of the pharmacokinetic behaviour and the routes by which a novel drug-candidate is metabolized is important for the interpretation of toxicological data obtained in safety studies and for prediction of *in vivo* systemic clearance in human [1, 2]. The hepatic metabolic clearance is considered to be the major component of the total clearance [3]; thus, in the early phase of drug development, hepatic stability screening is a widely used method to assess the metabolic stability and to predict *in vivo* hepatic clearance of a drug candidate [1, 4]. For *in vivo* clearance prediction, theoretical aspects of *in vitro – in vivo* scaling have been developed and successfully established in laboratory animals or in human [5-9]. The first attempt by Rane et al [10] calculated *in vivo* clearance from intrinsic clearance of a drug which was obtained by determination of the enzyme kinetic constants (v_{max}: maximal velocity of enzyme activity, K_M: Michaelis constant) in hepatic microsomal metabolism. The *in vitro* $t_{1/2}$ method described by Obach [6] is based on determination of the first-order rate constant for consumption of the drug-substrate for the estimation of intrinsic clearance.

The potential advantages of *in vitro* pharmacokinetic approach include the rapid distribution of the test compound, rapid sampling for kinetic studies, relatively high purity of the biological samples and the easy procedure of the determination of depletion of the parent compounds. An appropriate *in vitro* model should resemble the *in vivo* metabolism; thus for successful prediction of *in vivo* clearance, the selection of *in vitro* models must consider the qualitative information on enzyme(s) responsible for the metabolism of a drug [1, 6, 7, 11-15]. Several *in vitro* models have been developed, such as supersomes (expressed enzymes), subcellular fractions (microsomes, cytosol or S9 fraction), primary hepatocytes (freshly isolated or cryopreserved) or intact perfused liver, and some of these models are acceptable as supportive test systems by the regulatory authorities. Hepatic microsomes are simple, affordable and can be a useful *in vitro* model for evaluating pharmacokinetics of drugs metabolized by cytochrome

P450 (CYP) enzymes [5, 6]; however, this model has some drawback in studying those compounds that are metabolized by non-microsomal enzymes or by microsomal UDP-glucuronyltransferases. Conjugation activities in microsomal preparations generally underpredict *in vivo* metabolism because of the lack of conjugation enzymes (non-microsomal localisation) or insufficient activation of UDP-glucuronyltransferase activities [16]. Several authors [5; 12] successfully applied primary hepatocytes for *in vitro* studies and demonstrated better predictability of *in vivo* clearance. The advantages of hepatocytes over subcellular fractions have been documented; namely the cells retain most of the metabolic capabilities of the intact liver, possess the full complement of drug-metabolizing enzymes and contain the cofactors at physiological concentrations [5, 7, 12, 13]. Additional advantages of hepatocytes are the possibility of determination of metabolic profiles or even of metabolite identification. Hepatocytes in suspension are appropriate for the investigation of pharmacons with short elimination half-lives (<5 hr), whereas liver cells attached on collagene surface are generally applied for drugs with long half-lives (>5 hr). In high-throughput studies, the multi-drug-in-one-cocktail approach can be an option; however, the metabolism-based drug-drug interactions must be excluded for appropriate evaluation. *In vitro* pharmacokinetic data obtained from human hepatocytes can be used for prediction of *in vivo* hepatic clearance in human, whereas *in vitro* clearance data from hepatocytes isolated from rat, mouse, dog or rabbit can be useful to identify the laboratory animal(s) with similar pharmacokinetics to human beings. Furthermore, information on species differences in the rates and pathways of metabolism is of great interest and can help to select the laboratory animal model most relevant to human and most suitable to study the toxic properties of drugs.

In the present study, we investigated *in vitro* pharmacokinetics of sixteen psychopharmacons with disparate chemical structures (aripiprazole, biperiden, carbamazepine, citalopram, clonazepam, clozapine, duloxetine, fluoxetine, haloperidol, mianserin, mirtazapine, olanzapine,

paroxetine, quetiapine, risperidone, venlafaxine, Fig. 1) in primary hepatocytes of rat, rabbit, dog and human using liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis. These antipsychotics, antidepressants, mood stabilizers and anticonvulsive agents are most frequently used in treatment of psychiatric disorders (e.g. schizophrenia, schizoaffective disorder, bipolar disorder, depressive disorder). *In vivo* pharmacokinetic parameters (hepatic clearance, hepatic extraction ratio, bioavailability) were calculated from *in vitro* clearance data using *in vitro* $t_{1/2}$ method. *In vivo* bioavailability of the psychopharmacons predicted from human hepatocytes was compared to *in vivo* clinical data.

2. Materials and methods

2.1 Chemicals

Biperiden, carbamazepine, citalopram, clozapine, fluoxetine, mianserin, mirtazapine, paroxetine, risperidone and venlafaxine were purchased from Sigma-Aldrich Co. (Deisenhofen, Germany). Aripiprazole and duloxetine were obtained from International Laboratory USA (San Francisco, CA). Olanzapine and quetiapine was from Santa Cruz Biotechnology Inc. (Heidelberg, Germany). Clonazepam was obtained from Roche Magyarország Ltd. (Budaörs, Hungary) and haloperidol was from Gedeon Richter Plc. (Budapest, Hungary). All the other chemicals for the hepatocyte isolation and for the LC-MS/MS analysis were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemie.

2.2 Hepatocyte isolation

Experiments were carried out by using pooled hepatocytes prepared from male Wistar rats, male Beagle dogs or male New-Zealand rabbits (Toxi-Coop Toxicological Research Center, Budapest, Hungary). Human liver tissues were obtained from organ-transplant donors at the Department of Transplantation and Surgery, Semmelweis University (Budapest, Hungary).

The liver cells were isolated using collagenase perfusion method of Bayliss and Skett [17]. Briefly: The liver tissues were perfused through a derivation of the portal vein with Ca^{2+}-free medium (Earle's balanced salt solution) containing EGTA (0.5 mM) and then with the same medium without EGTA, finally with the perfusate containing collagenase (Type IV, 0.25 mg/ml) and Ca^{2+} at physiological concentration (2 mM). The perfusion was carried out at pH 7.4 and at 37°C. Hepatocytes having a viability of better than 90%, as determined by trypan blue exclusion [18], were used in the experiments.

2.3 *In vitro* pharmacokinetics in primary hepatocytes

Time courses of the unchanged psychopharmacons in hepatocytes pooled from three subjects were obtained. Each compound was incubated in cell suspension (generally at the concentration of 2×10^6 cells/ml; and at the concentration of 0.5 $\times10^6$ cells/ml or 4×10^6 cells/ml with the drugs intensively or slowly metabolized, respectively) at 37°C in a humid atmosphere containing 5% CO_2. The parent compounds were added directly to the medium (Williams' medium E : Ham's nutrient mixture F12 =1:1) at the final concentration of 1 μM. Except for clonazepam and haloperidol that were dissolved in medium, the stock solutions of the psychopharmacons (1 mM) were prepared in acetonitrile; thus, the final concentration of the organic solvent was 0.1% of the incubation mixture. At various time points (0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 240, 300 min), the incubation mixtures were sampled (aliquots: 0.25 ml) and terminated by the addition of 0.17 ml ice-cold acetonitrile containing the internal standard, carbamazepine (0.13 μM) or clozapine (0.09 μM). The cell debris was separated by centrifugation and the supernatant was analysed by LC-MS/MS for quantitation of the parent compound. All measurements were performed in duplicate with <5% interday and intraday precision.

2.4 Determination of unchanged drug concentrations

An Inertsil ODS-4 column (75×2.1 mm, 3 μm, GL Sciences Inc., Tokyo, Japan) with gradient elution was applied with MilliQ water containing 0.1% of formic acid as the mobile phase A and with acetonitrile containing 0.1% of formic acid as the mobile phase B. The column was eluted at a rate of 0.3 ml/min at 25°C using an Agilent 1100 HPLC-system (Agilent Technologies Inc., Waldbronn, Germany). The running time of chromatographic separation was 10 min, and the effluent was analysed by mass spectrometry. MS/MS measurements were performed on a SCIEX API 2000 mass spectrometer (Applied Biosystems, Foster City, Canada) using the Analyst 1.4.2 software. The ionisation mode was electrospray in positive mode. The instrument was used in multiple reaction monitoring (MRM) mode for quantitation of the psychopharmacons. The source conditions were: curtain gas: 30 units, spray voltage: 5500 V, source temperature: 400°C, nebulising gas: 50 units, drying gas: 50 units. The MRM transitions and collision energy applied for monitoring the parent compounds are in Table 1. The primary stock solutions of psychopharmacons for the preparation of calibrator and quality control samples were prepared in acetonitrile or in water by separate weighing. The working solutions used for spiking the calibrator and quality control samples were diluted separately in acetonitrile by spiking an appropriate volume of the stock solutions to achieve the concentrations ranged between 1 and 0.05 mM. The working solutions were 1000 times diluted in cell culture medium to achieve the final concentrations (1-0.05 μM), and after adding 0.17 ml acetonitrile containing the internal standard (0.13 μM carbamazepine or 0.09 μM clozapine), the calibrators were analysed by LC-MS/MS.

2.5 Estimation of pharmacokinetic parameters

The intrinsic clearance for hepatocytes (Cl_{int}) [ml/(min×2x10^6cells)] was calculated from the decrease in the concentration of the psychopharmacon as follows [6]:

$$Cl_{\text{int}} = \beta = \frac{\ln 2}{t_{1/2}}$$

For scaling up the Cl_{int} value to obtain $Cl_{int\ per\ whole\ liver\ (g)/bw\ (kg)}$, the cell concentration in liver, the average liver weight and average body weight parameters were used (Table 2) [19]. The value for hepatic clearance (Cl_H) was calculated as follows [1, 5, 8]:

$$Cl_H = \frac{Cl_{\text{int}\,liver/bw} * fu * Q_{plasma}}{(Cl_{\text{int}\,liver/bw} * fu) + Q_{plasma}}$$

where the flow rate of $Q_{plasma}=Q_H*plasma/blood\ ratio$. To calculate Cl_H, the hepatic flow rate (Table 2), plasma/blood ratio=0.57 and fu=1 values were used [20]. (Q_H is the hepatic blood flow, while *fu* is the unbound fraction of the compound.) The hepatic extraction ratio was defined as [5, 12]:

$$E=Cl_H*Q_H$$

and the bioavailability (%) was [3]

$$F=(1-E)*100.$$

2.6 Data analysis

The intrinsic clearance and predicted hepatic clearance of each psychopharmacon were calculated from *in vitro* pharmacokinetics in hepatocytes of human and laboratory animals. The correlation between the clearance values in human and in laboratory animals was estimated. The correlation among human and laboratory animal clearance was quantified, and the correlation coefficients (r^2) were calculated using the Pearson approach (GraphPad InStat version 3.05, San Diego, CA). A strong correlation between the human and the animal clearance values was considered if the probability value (P) was under 0.05.

3. Results and Discussion

3.1 *In vitro* pharmacokinetics of psychopharmacons in primary hepatocytes

In vitro Cl_{int} was estimated for sixteen psychopharmacons with diverse chemical structures in primary hepatocytes of rat, rabbit, dog and human (Fig. 1). The time courses of unchanged parent compounds were determined, and the elimination half-life of each compound was calculated (an example of paroxetine in Fig. 2). The unchanged drug profiles at the concentration of 1 μM displayed linear log concentration declines; thus, the biotransformation follows a first-order reaction kinetics under these conditions. The metabolic stability of the set of psychopharmacons varied in a wide range, and large interspecies differences were observed in the pharmacokinetic behaviour of these drugs. In human hepatocytes, the most stable compounds were carbamazepine, citalopram, clonazepam, fluoxetine, mirtazapine and paroxetine, displaying *in vitro* elimination half-lives longer than the 5-hour incubation period. Similarly to human, slow elimination rates of these drugs were also observed in rat hepatocytes (Fig. 3a); however, the elimination half-lives were about one order of magnitude lower in the rat cells than in human hepatocytes. Besides carbamazepine and clonazepam, haloperidol was found to be the most stable drug in dog hepatocytes, whereas mirtazapine and paroxetine belonged to the most labile compounds in dog cells. In contrast to human, rat and dog, risperidone displayed the longest elimination half-life (*in vitro* $t_{1/2}$ longer than the 5-hour incubation period) and the lowest Cl_{int} in rabbit liver cells. Quetiapine appeared to be the most labile compound in all species except for dog. Mianserin was found to be one of the most labile drugs in hepatocytes isolated from laboratory animals. Interestingly, mirtazapine and paroxetine, which were the most stable psychopharmacons in human and rat, appeared to be fast-elimination drugs in dog and rabbit liver cells. In general, the rabbit hepatocytes were found to have the most active metabolic

capability displaying elimination half-life shorter than 15 min for 12 of the set of psychopharmacons investigated.

Species comparison of the *in vitro* pharmacokinetic behaviour of the set of psychopharmacons demonstrated that the metabolic capabilities of human and rat hepatocytes closely resembled each other, displaying strong correlation of *in vitro* $t_{1/2}$ or Cl_{int} (r^2=0.8878 and 0.9516, respectively) (Fig. 3). However, in human hepatocytes, the elimination half-lives were approximately 5 times longer, whereas the Cl_{int} values were about 27 times lower than in rat cells. On the other hand, neither the elimination rates nor Cl_{int} of the drugs in human liver cells correlated with those parameters in dog or rabbit hepatocytes (r^2<0.2).

3.2 Prediction of human clearance and bioavailability of psychopharmacons from *in vitro* pharmacokinetic data

The *in vitro* Cl_{int} values were expressed per kg of body weight taking physiological parameters, such as cell number in the liver, liver weight and body weight, into consideration (Table 2) (see 2.5). The calculation of hepatic clearance (Cl_H) additionally takes hepatic flow rate into account. In human, low hepatic clearance was predicted for aripiprazole, carbamazepine, citalopram, clonazepam, fluoxetine, mirtazapine and paroxetine, intermediate Cl_H was estimated for biperiden, clozapine, duloxetine, haloperidol, mianserin, olanzapine, risperidone and venlafaxine, whereas the hepatic clearance of quetiapine was predicted to be high (Fig. 4a). Similarly to the Cl_{int} (3.1), there were significant interspecies differences in hepatic clearance predicted from *in vitro* elimination half-lives. The predicted hepatic clearance for the psychopharmacons displayed significant correlation between human and rat, whereas neither dog nor the rabbit hepatic clearance resembled human Cl_H values (Fig. 4). This means that in safety studies, the rat is the most relevant laboratory animal to human, since the metabolic properties of the rat is similar to those of human. During drug

development, the experimental approaches are generally based on animal models; however, the drug metabolizing systems in the laboratory animals can differ from each other or from human [21-24]. For example in rabbit, the enzymes of CYP2C subfamily are the most important in drug metabolism, contrary to human where CYP3A enzymes are involved in the metabolism of most of the drugs (approximately 50%) in the market [22, 25]. Additionally, CYP2D orthologues in rabbit have a marginal role in biotransformation, whereas CYP2D6 in human catalyses the metabolism of 30% of drugs despite the fact that the concentration of CYP2D6 protein is about 2% of the total hepatic CYP enzymes [25]. Because of the substantial metabolic differences between rabbit and man, the predictive value of the rabbit model can be considered to be poor [22]. The properties of the dog CYP2D15 mostly resembles the human CYP2D6; thus, dog model may be recommended for investigation of the drugs that are known to be metabolized by CYP2D6 in human [21, 22]. Although plenty of the psychopharmacons included in the present study are known to be metabolized by CYP2D6 (aripiprazole, citalopram, duloxetine, fluoxetine, haloperidol, mianserin, mirtazapine, olanzapine, paroxetine, quetiapine, risperidone, venlafaxine) [26], no correlation of the clearance properties was observed between the dog and the human hepatocytes. It can be explained by the facts 1) that some human CYP2D6 substrates are not metabolized or poorly metabolized by the canine CYP2D15 [24] or 2) that the dog CYP2D15 or the human CYP2D6 are not the exclusive enzymes in the metabolism of most of the drugs.

Hepatic clearance predicted from *in vitro* pharmacokinetic parameters allows the classification of drugs into low, intermediate and high hepatic extraction ratio (E) groups. Houston [5] suggested the cut-off values between the hepatic extraction ratio groups: under 0.3 for low extraction drugs, between 0.3 and 0.7 for intermediate, and above 0.7 for high extraction drugs. Considering these cut-off values, the psychopharmacons displaying low hepatic clearance in human hepatocytes were classified as low extraction drugs, those having

intermediate Cl_H were intermediate extraction compounds, whereas quetiapine with high hepatic clearance was a high extraction drug (Fig. 4 and Table 3). Significant interspecies differences in hepatic extraction properties of the psychopharmacons were observed (Table 3), anticipating different systemic exposure of the laboratory animals and human at the same dose level. In rabbit, three fourth of the psychopharmacons were classified as high extraction drugs, and only one, risperidone displayed low hepatic extraction (E=0.08). Risperidone was found to be an intermediate or high extraction compound in the species other than rabbit. In rat and dog, 56% of the drugs were high extraction compounds, and only carbamazepine was classified as a low extraction drug in dog (E=0.27).

In vitro pharmacokinetic behaviour of a drug provides useful information for the prediction of bioavailability. The bioavailability of the psychopharmacons was predicted from *in vitro* pharmacokinetic parameters obtained in human hepatocytes, and the predicted values were compared with *in vivo* oral bioavailability determined in clinical studies (Table 4) [26-32]. The human bioavailability predicted from *in vitro* pharmacokinetic studies appeared to be in good agreement with *in vivo* clinical data, which confirmed the utility of the primary hepatocyte model for prediction of *in vivo* pharmacokinetic parameters. This also means that the precision of *in vivo* prediction from *in vitro* data was found to be excellent, and that the *in vitro* approaches can be recommended for assaying drug-candidates in a prospective manner.

4. Conclusions

The rate of metabolism of a drug influences both its pharmacodynamic effect and its toxic potential; thus, pharmacokinetic studies are essential for understanding the results of safety studies on a drug-candidate. In the early phase of drug development, there is an urgent need to predict the *in vivo* pharmacokinetic behaviour of a molecule in human. Therefore, substantial efforts have been made in development of metabolically competent *in vitro* models

appropriate for the extrapolation to *in vivo* with acceptable degree of confidence. Primary hepatocytes offer a simple *in vitro* model because the cells provide an opportunity to study *in vitro* biotransformation of novel chemical entities and to predict *in vivo* pharmacokinetic parameters. The present study with sixteen psychopharmacons (antipsychotics, antidepressants, mood stabilizers) demonstrated the utility of *in vitro* pharmacokinetic data obtained from primary liver cells isolated from human in prediction of *in vivo* hepatic clearance and bioavailability. The predicted human bioavailability showed an excellent rank order with *in vivo* clinical data. The comparison of *in vitro* Cl_{int} or predicted hepatic clearance values in human with those obtained from hepatocytes of laboratory animals indicated that the metabolism mediated species differences should be taken into account in *in vitro-in vivo* extrapolation. Rat hepatocytes were considered to be the most relevant *in vitro* animal model to human, whereas the knowledge of the pharmacokinetic differences between human and dog or rabbit facilitates the interpretation of the results of safety studies in these animal models.

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Table 1 The MRM transitions and collision energy applied for monitoring of psychopharmacons

Psychopharmacon	**Q1 (amu)**	**Q3 (amu)**	**Collision energy (eV)**
Aripiprazole	449.2	286.1	39
Biperiden	312.2	98.1	35
Carbamazepine	237.1	194.1	30
Citalopram	325.0	108.9	37
Clonazepam	316.0	269.9	37
Clozapine	327.1	270.1	33
Duloxetine	298.1	154.0	9
Fluoxetine	309.9	44.0	32
Haloperidol	376.1	164.9	34
Mianserin	265.2	208.0	30
Mirtazapine	266.2	195.0	35
Olanzapine	313.3	256.1	31
Paroxetine	330.2	192.1	30
Quetiapine	384.1	253.0	32
Risperidone	411.1	191.1	40
Venlafaxine	278.2	58.0	40

Table 2 Physiological parameters for calculation of clearance values

Parameter*	**Rat**	**Rabbit**	**Dog**	**Human**
Number of hepatocytes in liver ($\times 10^6$ cells /g liver)	117	114	215	139
Liver weight (g)	10	77	320	1.660
Body weight (kg)	0.25	2.5	10	70
Liver blood flow (ml/min/kg)	55.2	70.8	30.9	19

*[19, 20]

Table 3 Extraction ratio values of psychopharmacons predicted in hepatocytes from human, rat, dog and rabbit

Psychopharmacon	Extraction ratio			
	Human	Rat	Dog	Rabbit
Aripiprazole	0.24	0.51	0.94	0.32
Biperiden	0.34	0.90	0.66	0.96
Carbamazepine	<0.14	0.46	0.27	0.34
Citalopram	0.19	0.49	0.53	0.79
Clonazepam	<0.14	0.44	0.36	0.33
Clozapine	0.38	0.85	0.91	0.73
Duloxetine	0.45	0.83	0.98	0.92
Fluoxetine	0.16	0.43	0.45	0.85
Haloperidol	0.30	0.77	0.35	0.77
Mianserin	0.36	0.84	0.99	0.93
Mirtazapine	0.18	0.38	0.94	0.89
Olanzapine	0.43	0.91	0.75	0.87
Paroxetine	0.19	0.42	0.98	0.75
Quetiapine	0.70	0.96	0.85	0.92
Risperidone	0.37	0.80	0.71	0.08
Venlafaxine	0.31	0.75	0.56	0.84

Table 4 Comparison of bioavailability of psychopharmacons predicted in human hepatocytes with *in vivo* bioavailability data

Psychopharmacon	Bioavailability (%)	
	Predicted in human hepatocytes	In vivo bioavailability (clinical data)*
Aripiprazole	76.3	70-90
Biperiden	65.7	40-87
Carbamazepine	>86.1	89-100
Citalopram	81.5	80
Clonazepam	>86.1	80-98
Clozapine	61.6	50-60
Duloxetine	54.8	50
Fluoxetine	84.1	70-85
Haloperidol	69.6	60-70
Mianserin	64.4	30-80
Mirtazapine	81.5	50-75
Olanzapine	56.9	60-80
Paroxetine	80.9	60-100
Quetiapine	30.7	30
Risperidone	63.1	70
Venlafaxine	69.4	45-92

* [26-32]

Figure legends

Figure 1. Chemical structures of the model psychopharmacons.

Figure 2. The time course of paroxetine depletion in primary hepatocytes.

The hepatocyte concentrations were 2×10^6/ml for rat and human; and 0.5×10^6/ml for dog and rabbit. The starting concentration of paroxetine was 1 μM.

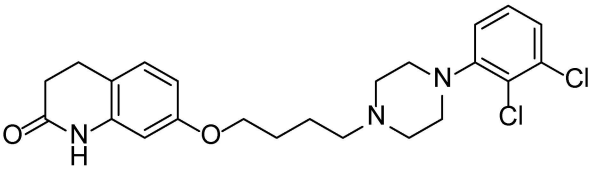
Figure 3. Correlation of the elimination half-life and Cl_{int} values of the psychopharmacons between human and rat hepatocytes.

Ari: aripirazole, Bip: biperiden, Carb: carbamazepine, Cit: citalopram, Clon: clonazepam, Clz: clozapine, Dulo: duloxetine, Fluo: fluoxetine, Hal: haloperidol, Mia: mianserin, Mirt: mirtazapine, Ola: olanzapine, Par: paroxetine, Quet: quetiapine, Risp: risperidone, Ven: venlafaxine

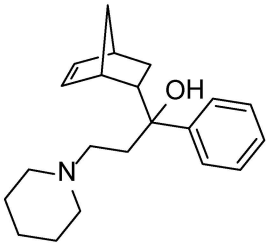
Figure 4. Correlation of the hepatic clearance values predicted from *in vitro* pharmacokinetic parameters.

(a) correlation between human and rat, (b) between human and dog, (c) between human and rabbit

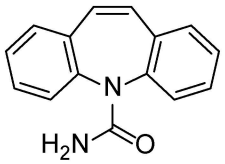
Figure 1



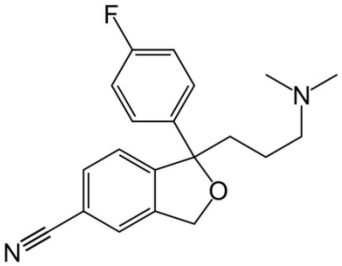
aripiprazole



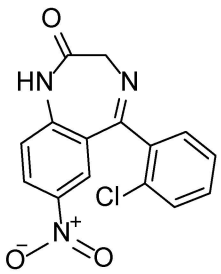
biperiden



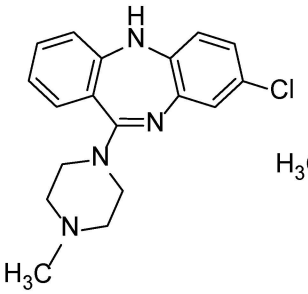
carbamazepine



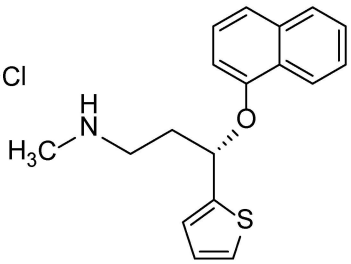
citalopram



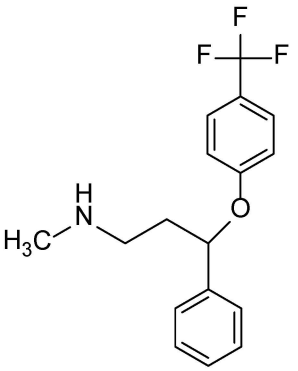
clonazepam



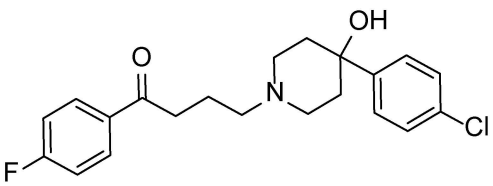
clozapine



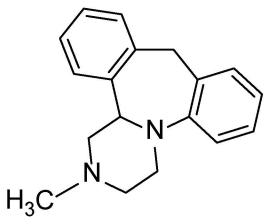
duloxetine



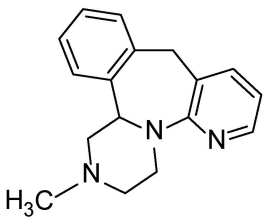
fluoxetine



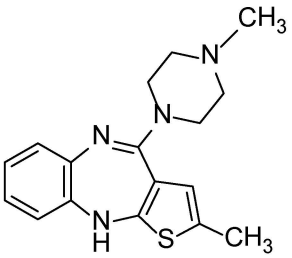
haloperidol



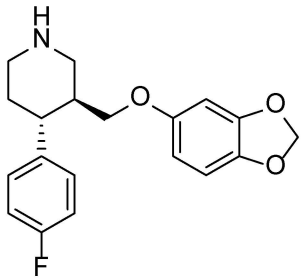
mianserin



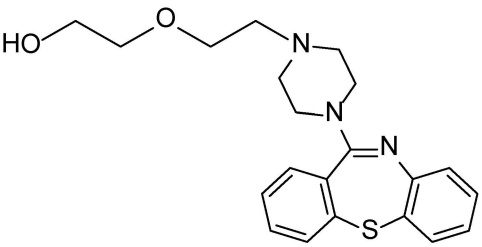
mirtazapine



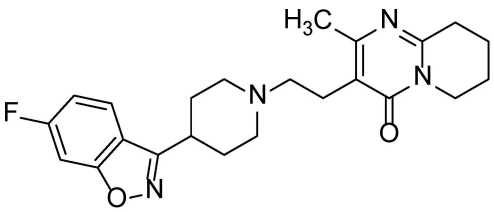
olanzapine



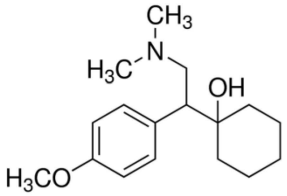
paroxetine



quetiapine



risperidone



venlafaxine

Figure 2

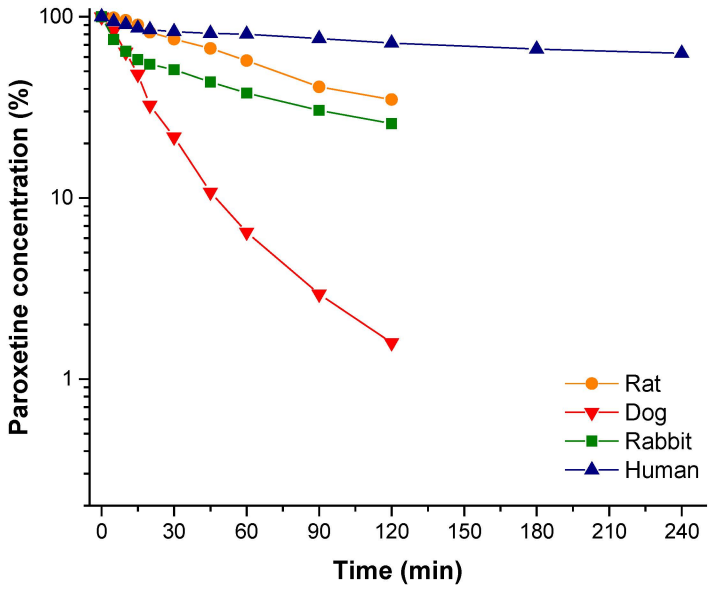

Paroxetine concentration (%)
100
10
1
0
30
60
90
120
150
180
210
240
Time (min)
Rat
Dog
Rabbit
Human

Figure 3

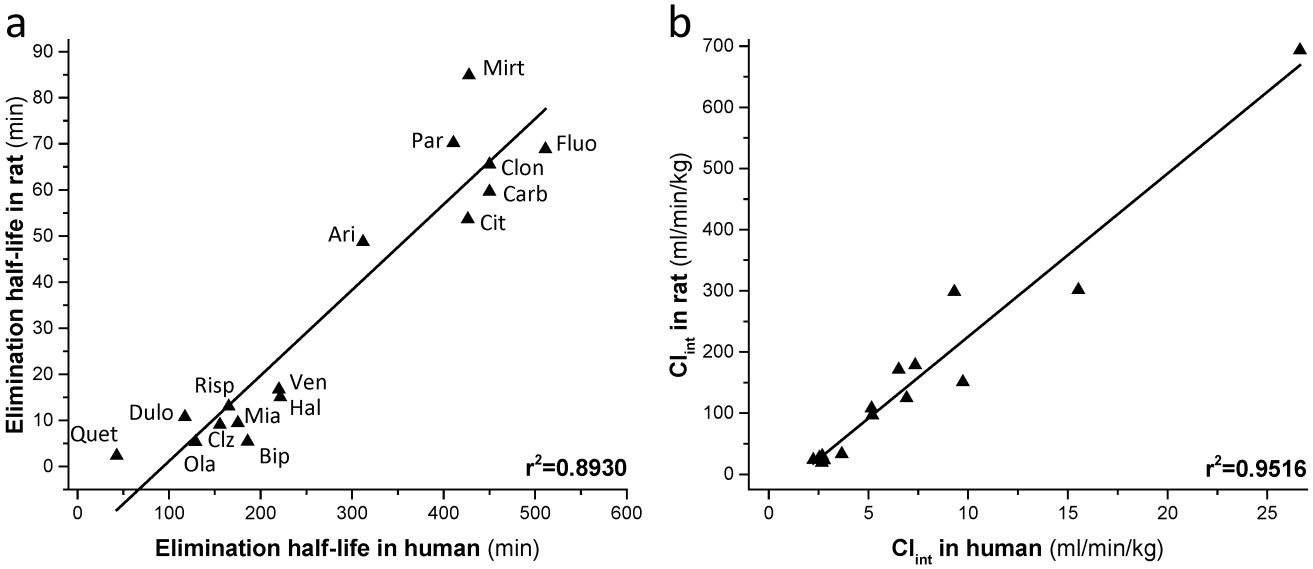
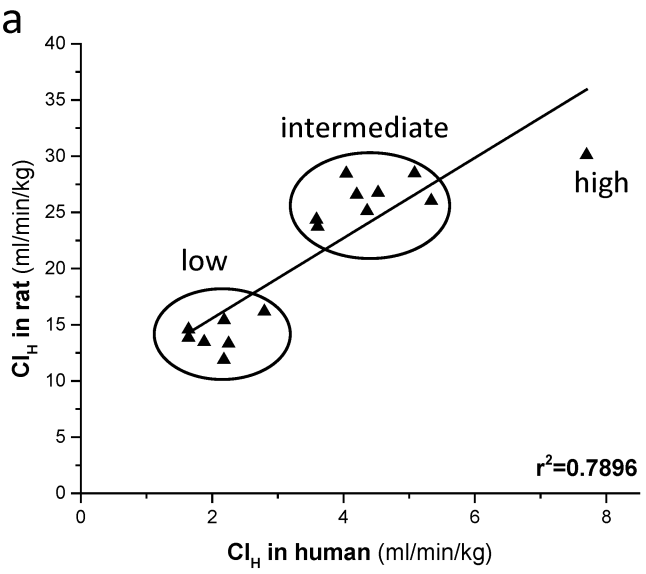
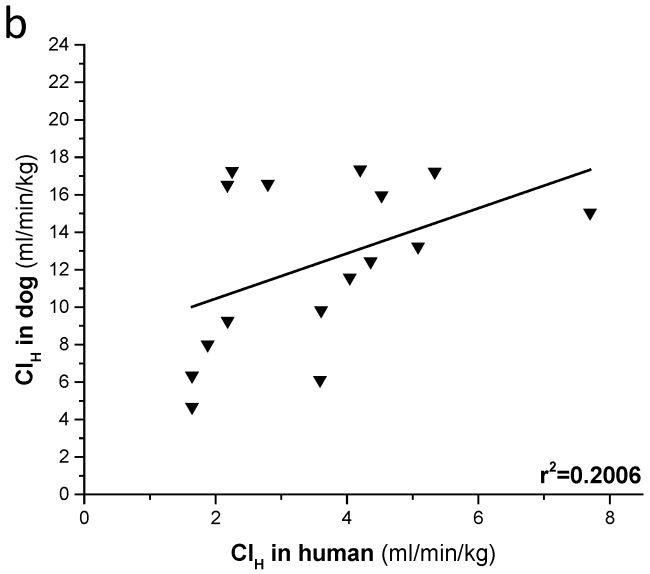
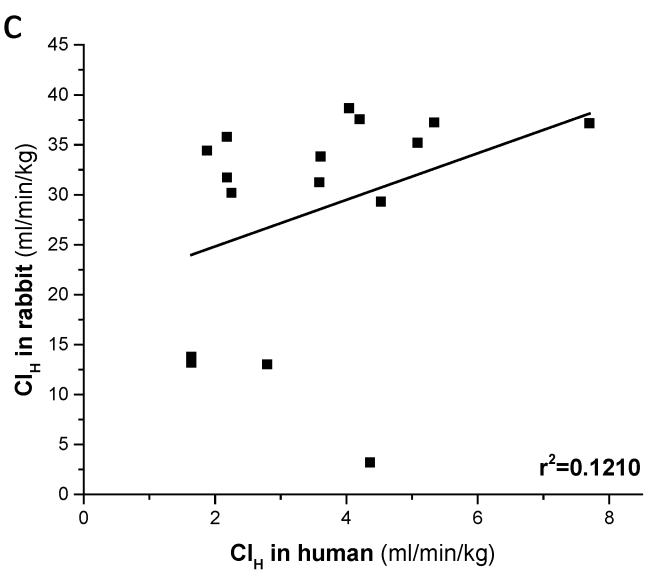

a
Elimination half-life in rat (min)
Elimination half-life in human (min)
Mirt
Par
Fluo
Clon
Carb
Cit
Ari
Risp
Ven
Hal
Dulo
Mia
Quet
Clz
Ola
Bip
r²=0.8930
b
CLint in rat (ml/min/kg)
CLint in human (ml/min/kg)
r²=0.9516

Figure 4


a
intermediate
high
low
r^2=0.7896
Cl_H in rat (ml/min/kg)
Cl_H in human (ml/min/kg)


b
r^2=0.2006
Cl_H in dog (ml/min/kg)
Cl_H in human (ml/min/kg)


c
r^2=0.1210
Cl_H in rabbit (ml/min/kg)
Cl_H in human (ml/min/kg)