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Original article

First-principle, structure-based prediction of hepatic metabolic clearance values in human

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ABSTRACT

The first-principle, quantitative structure–hepatic clearance relationship for 50 drugs was constructed based on selected molecular descriptors calculated by TSAR software. The R^2 of the predicted and observed hepatic clearance for the training set ($n = 36$) and test set ($n = 13$) were 0.85 and 0.73, respectively. The average fold error (AFE) of the *in silico* model was 1.28 ($n = 50$). The prediction accuracy of *in silico* model was superior to *in vitro* hepatocytes' model in literature ($n = 50$, AFE = 2.55). It is attractive to predict human hepatic clearance based on molecular descriptors merely. The structure-based model can be used as an efficient tool in the rapid identification of hepatic clearance of new drug candidates in drug discovery.

1. Introduction

The increasing numbers of new chemical entities via combinatorial chemistry and computer-aided drug design urgently call for rapid and effective approaches of identification of successful drug candidates. The promising candidates must exhibit appropriate clinical efficacy, safety and pharmacokinetic performances [1]. Therefore, the field of ADMET (absorption, distribution, metabolism, elimination, and toxicity) prediction has gained considerable attention in the drug discovery stage.

Hepatic metabolism of drugs is a key parameter to the drug ADMET performances. The precise prediction of hepatic metabolism is of great importance both for the evaluation of bioavailability [2], optimization of potential candidate drugs and for the estimation of early human trial doses and exposures in the clinic. Several reviews have focused on the prediction of drug metabolisms [3–8]. Recent works in drug metabolism prediction can be categorized into several levels, such as prediction of hepatic metabolic clearance (CL_h), potential enzyme subgroup involved, induction, inhibition or competition for relevant enzymes (drug–drug interaction), and ultimately prediction of metabolites to deduce the whole metabolic process [9].

Up to now, many attempts have been made to predict human CL_h, including simple interspecies extrapolation [10], allometric scaling with and without correction for brain weight and maximum life-span potential (MLP) [11], *in vitro* to *in vivo* correlation [12], physiologically based *in vitro* to *in vivo* (PB-IVIV) prediction using human liver microsomes [13–15] and hepatocytes [16–19], and *in silico* methods. Among these, PB-IVIV method coupled with human unbound fraction in blood (fu_b) and hepatocyte intrinsic clearance (CL_{int}) data appears to be the most accurate method (maximum ~2-fold errors) [8]. Some researchers have employed statistical methods in predicting CL_h, as summarized in Table 1. However, most of these models are based on animal (rat/dog) *in vivo–in vitro* and human *in vitro* data [16,20], or these data combined with molecular descriptors [21], both of which are limited by time, cost and resource. Few studies have been undertaken on the first-principle, structure-based CL_h *in silico* prediction. To the best of our knowledge, it is very valuable to predict human CL_h merely using molecular descriptors to accelerate the screening of new chemical entities in the drug discovery stage.

In this study, structure-based CL_h prediction model was constructed just based on molecular descriptors for a data set of 50 drugs covering a range of molecular properties. All of the molecular descriptors were calculated using TSAR™ software version 3.3 (Accelrys Inc.) [22]. Then, the feature selection was performed by Weka version 3.5.6, a free open-source data-mining software [23]. The principal objectives of the study were (i) to develop the quantitative relationship between the molecular descriptors and observed human CL_h; (ii) to estimate the predictability of our *in silico* model by comparing with the *in vitro* hepatocytes' model in literature and (iii) to identify the important molecular descriptors influencing CL_h.

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Table 1
Summary of human hepatic metabolic clearance (CL_h) prediction researches based on statistical models

Year	Authors	Statistical method	Training/test set	Software	Model performance
1999	Schneider et al. [16]	BPN, PLS, and MLR	20/2	–	MLR (all variables): $R^2 = 0.84$, $Q^2 = 0.74$ MLR (rat + human *in vitro*): $R^2 = 0.84$, $Q^2 = 0.79$ PCA + MLR: $R^2 = 0.85$, $Q^2 = 0.79$ PLS: $R^2 = 0.83$, $Q^2 = 0.79$ ANN linear: $R^2 = 0.86$, $Q^2 = 0.79$ ANN sigmoidal: $R^2 = 0.88$, $Q^2 = 0.77$
2001	Zuegge et al. [20]	BPN Physiologically based method Empirical *in vitro–in vivo* correlation	20/2	–	BPN: $R^2 = 0.78$ Physiologically based methods: $R^2 = 0.77$ Empirical *in vitro–in vivo* correlation: $R^2 = 0.84$
2007	Lee and Kim [21]	MLR	Data set 1: $n = 19$ Data set 2: $n = 30$ No test set	PreADMET	Hepatocyte data: $R^2 = 0.921$, RMSE = 2.13 Microsomal data: $R^2 = 0.883$, RMSE = 2.41

BPN, back propagation neural; PLS, partial least square; MLR, multiple linear regression; PCA, principal component analysis.

2. Materials and methods

2.1. Data collection

The observed human CL_h ($CL_{h,observed}$) and human CL_h predicted by hepatocyte ($CL_{h,predicted,hepatocyte}$) data of 50 drugs were obtained from the literature [24]. Among 56 compounds of the original data set in literature, six compounds were excluded in our data set. Three compounds, FK 079, FK 1052 and FK 480, were not open to the public. The molecular weight of cyclosporin A is 1202.63, which is too large for model construction. Antipyrine and cimetidine could not generate molecular descriptors successfully in TSAR. The CL_h value of each drug was log-transformed ($\log CL_{h,observed}$) to normalize the data and to reduce unequal error variances. Data for human $\log CL_{h,observed}$ of 50 drugs are listed in Table 2.

2.2. Calculation of descriptors

The 2D structures of 50 drugs were searched in SciFinder Scholar 2007 [25] and the saved *mol* files were imported into TSAR 3.3. The 3D structures and the partial charges of drugs were derived using CORINA 3D and Charge-2 packages in TSAR 3.3, respectively. The geometries of the molecules were optimized using the Cosmic module of TSAR. The calculations were terminated if the energy difference or the energy gradient were smaller than 1e−005 and 1e−010 kcal/mol, respectively.

Then, a set of 132 molecular descriptors was calculated with TSAR 3.3, including molecular surface area and volume, moments of inertia, ellipsoidal volume, dipole moments, lipole moments, molecular mass, Wiener index, molecular connectivity indices, molecular shape indices, electrotopological state indices, log *P*, rings (aromatic and aliphatic), and groups (methyl, hydroxyl, etc.). Vamp, a semi-empirical molecular orbital package in TSAR 3.3, was used to calculate the electrostatic properties like total energy, electronic energy, nuclear repulsion energy, accessible surface area, atomic charge, mean polarizability, heat of formation, HOMO and LUMO, ionization potential, total dipole, and dipole components and perform structural optimizations *in vacuo* with default parameters using Hamiltonian method AM1 and formalism type of restricted Hartree–Fock (RHF).

2.3. Feature selection

Descriptors with the same values for all drugs were discarded. Then, correlation matrixes of the descriptors were built and the number of descriptors was filtered to 70 according to a correlation threshold of 0.85. The easily interpretable descriptor was retained. The algorithms implemented in Weka (version 3.5.6) were used to automatically select subset of variables that was highly correlated with $\log CL_{h,observed}$. WrapperSubsetEval (B weka/classifiers/functions/LinearRegression-F 50 -T 0.01-R 1– -S 1-R 1.0E-8) was chosen as attribute evaluator, combining with either the BestFirst or GreedyStepwise search method.

2.4. Model development and evaluation

Multiple linear regression (MLR) analysis was applied to develop the *in silico* model. In order to examine the predictive power and robustness of our model, the entire data set was subdivided into training set ($n = 36$) and test set ($n = 14$) by the cluster analysis of $\log CL_{h,observed}$ (as listed in Table 2). Meanwhile, leave-one-out (LOO) cross-validation and test set validation procedures were performed. Then R^2, RMSE (the root mean square error) and Q^2 resulted from LOO (Q^2_{LOO}) were calculated to evaluate the model predictability.

For CL_h predictions, another commonly employed accuracy test criterion was the fold error, as represented by Eq. (1). A prediction was usually thought to be successful if the value of fold error is less than 2 [26]. The percentage of drugs in the data set where the fold error was more than two ($E_{2\text{-fold}}$) and three ($E_{3\text{-fold}}$) was calculated, respectively, in our study.

$$\text{fold error} = \begin{cases} \dfrac{CL_{h,predicted}}{CL_{h,observed}}, & \text{if } CL_{h,predicted} > CL_{h,observed} \\ \dfrac{CL_{h,observed}}{CL_{h,predicted}}, & \text{else} \end{cases} \quad (1)$$

Furthermore, 50 drugs were divided into three classes according to their values of $CL_{h,observed}$ [16]: (i) if $CL_{h,observed} < 6$ mL/min/kg, low hepatic clearance; (ii) if 6 mL/min/kg $\leq CL_{h,observed} < 14$ mL/min/kg, medium hepatic clearance; (iii) if $CL_{h,observed} > 14$ mL/min/kg, high hepatic clearance. Then, the percentage of correctly predicted (cp%) was calculated, combined with $E_{2\text{-fold}}$ and $E_{3\text{-fold}}$ to evaluate the accuracy of the model.

The average fold error (AFE) of the prediction method was also calculated to provide a measure of bias with equal value to under- and over-predictions (Eq. (2)). Therefore, the tendency of under- or over-prediction was determined via plotting the $\log(CL_{h,predicted}/CL_{h,observed})$ against the number of drugs.

$$\text{average fold error} = 10^{\frac{\sum_{i=1}^{n} \log\left|\frac{CL_{h,predicted}}{CL_{h,observed}}\right|}{n}} \quad (2)$$

3. Results and discussion

3.1. Quantitative structure–hepatic clearance relationship

For the data set with 50 drugs and 70 molecular descriptors, 13 descriptors were chosen via the feature selection to construct the

Table 2
Data for log $CL_{h,observed}$ and selected 13 TSAR molecular descriptors for 50 drugs

Compounds	Data set	log $CL_{h,observed}$	*X*1	*X*2	*X*3	*X*4	*X*5	*X*6	*X*7	*X*8	*X*9	*X*10	*X*11	*X*12	*X*13
Acetaminophen	Training	0.6021	1.3441	2.518	−2.0282	0	2	1	1	0	0.25344	−8.4609	2.86	0.072	−1.55
Buspirone	Training	1.2833	10.468	3.4562	−0.04448	0.125	5	3	0	0	0.12892	−8.7955	3.5657	2.165	128.01
Caffeine	Test	0.1461	3.271	3.561	−0.00608	0	3	1	3	0	−0.34868	−8.9624	3.6178	0.038	0.198
Carvedilol	Test	0.9395	0.29794	4.9584	1.6425	0	5	3	1	1	−0.09646	−8.3297	1.7107	0.098	191.25
Chlorpromazine	Training	0.9345	3.4947	6.1494	1.8699	0	1	3	2	0	−0.2686	−7.5349	2.5845	0.626	90.204
Desipramine	Training	1.0128	8.8834	5.4018	1.4033	0	1	2	1	0	0.38787	−8.3964	1.5469	−0.732	−19.219
Diazepam	Training	−0.3665	9.614	5.2842	−0.82523	0	2	2	1	0	−0.63441	−9.2195	3.7078	0.878	20.978
Diclofenac	Training	0.8651	4.4497	4.2563	0.20022	0	2	2	0	0	−0.29275	−8.5699	2.2302	−1.569	37.186
Diflunisal	Training	−0.7447	2.5388	3.2248	0.9444	0	3	2	0	0	−0.78512	−9.1182	1.5815	0.888	5.147
Diltiazem	Training	1.1072	9.5879	6.0551	0.33055	0	5	2	4	0	−0.30159	−8.5331	4.7074	2.478	−5.686
Etodolac	Training	0.1173	2.5795	4.6848	−0.85189	0.051031	3	2	2	0	0.15653	−8.188	1.4814	0.761	−62.164
Fenoprofen	Test	0.2279	3.3142	3.1622	0.39928	0	3	2	1	0	0.00571	−9.0484	1.7833	1.51	−13.16
Furosemide	Training	−0.2291	1.0385	4.4151	2.0015	0.062113	5	1	0	0	−0.8868	−9.298	6.5116	3.146	32.268
Gemfibrozil	Test	0.49	1.1547	3.549	−0.02424	0.17678	3	1	4	0	0.43611	−8.7607	0.90998	0.086	−3.068
Glipizide	Training	−0.0177	3.0357	5.6115	5.547	0.053791	6	3	1	0	−1.0126	−10.142	6.2127	−0.017	24.861
Granisetron	Training	1.0414	4.3212	3.7714	−0.2307	0	3	3	2	0	−0.29832	−8.8591	3.8756	−1.576	−46.468
Ibuprofen	Training	0.1461	1.1228	2.8386	0.76682	0	2	1	3	0	0.19899	−9.4013	1.9647	0.976	−6.363
Imipramine	Training	0.9759	9.161	5.2006	0.88308	0	1	2	2	0	0.4059	−8.3782	1.5691	−0.85	−30.205
Indomethacin	Training	0.3502	6.5437	5.1473	0.26582	0	4	2	2	0	−0.66264	−8.6274	1.2963	−1.186	17.482
Irbesartan	Training	0.5855	14.695	5.452	3.5342	0.055902	5	2	1	1	−0.7841	−9.8046	4.8213	2.25	154.86
Ketoprofen	Training	0.3464	4.0533	3.7096	1.7257	0	3	2	1	0	−0.57594	−9.9137	3.5474	2.182	−11.325
Lidocaine	Test	1.1761	7.588	3.8844	−0.81341	0	2	1	4	0	0.17392	−9.2828	3.268	−0.653	−15.524
Lorazepam	Test	0.0414	9.0977	5.5398	0.033303	0	3	2	0	0	−0.76468	−9.4183	5.116	0.534	−12.782
Methylprednisolone	Test	0.8751	12.065	4.2746	−2.9807	0.22492	5	3	3	0	−0.37637	−10.069	6.4321	−4.87	−410.77
Metoprolol	Test	1.0846	0.98497	2.7725	2.3612	0	4	1	3	1	0.37591	−8.9603	3.6094	−1.48	−35.983
Midazolam	Training	0.8195	9.1269	5.3795	3.7638	0	2	2	1	0	−0.77178	−8.958	4.3611	−0.642	21.624
Montelukast	Training	0.1038	9.8982	6.4136	1.3147	0.2368	4	4	2	3	−0.92039	−8.5889	3.1907	−0.954	85.788
Naloxone	Training	1.29	7.5482	4.2241	−3.035	0.096675	5	4	0	0	0.21052	−8.8237	6.0033	2.99	52.672
Nifedipine	Training	0.8921	2.9802	6.0723	−3.0158	0	6	2	4	0	−0.70209	−8.9905	6.6171	−4.052	−62.655
Ondansetron	Training	0.7709	4.0444	3.76	−0.3048	0	2	2	2	0	−0.38113	−8.6588	5.5747	−2.147	13.173
Oxaprozin	Test	−1.1549	6.2432	4.87	1.4943	0	4	2	0	0	−0.67121	−8.681	2.6724	−0.892	2.122
Oxazepam	Training	0.0414	8.1541	5.8612	1.864	0	3	2	0	0	−0.7828	−9.4132	4.671	0.502	12.745
Phenacetin	Training	1.2833	1.4263	2.489	−2.2673	0	2	1	2	0	0.36157	−8.3627	4.354	−0.177	1.875
Pindolol	Training	0.6232	0.55811	3.4246	0.66913	0	3	1	2	0	0.31922	−8.1598	2.6	−0.979	−31.126
Prazosin	Training	0.4314	1.717	4.4537	−3.163	0	6	3	2	0	−0.41961	−8.2564	4.969	1.265	42.163
Prednisolone	Training	0.9395	11.034	3.7758	−5.5873	0.22492	5	3	2	0	−0.21078	−9.8862	7.3825	−5.949	−610.21
Propranolol	Test	1.2071	0.83231	3.6567	1.1554	0	3	2	2	0	−0.21	−8.4318	2.7973	−1.269	−41.438
Quinidine	Test	0.7267	8.7447	4.9096	−1.302	0	4	4	1	0	−0.59668	−8.8224	2.8826	−2.04	−25.554
Ranitidine	Training	0.4624	5.0738	3.423	0.59225	0	4	0	3	1	−0.44789	−9.0685	9.125	2.078	−46.659
Ritonavir	Training	0.0792	5.9237	6.3031	−1.7754	0	7	2	5	3	−0.48481	−9.3086	3.2513	−1.468	−148.38
Sildenafil	Training	0.7782	3.0017	5.1582	2.1934	0.048113	7	3	4	0	−0.88765	−8.7308	4.4542	3.134	149.25
Tenoxicam	Training	−1.1367	3.9244	3.3391	−4.2053	0.048113	5	2	1	0	−1.4426	−9.0902	1.7811	−0.237	11.236
Theophylline	Test	−0.1871	1.1439	3.6361	0.14464	0	3	1	2	0	−0.08797	−9.0707	6.1406	0.096	0.7
Timolol	Training	0.9624	8.2889	4.3221	2.7911	0.25	6	1	3	0	−0.281	−8.7	3.7264	0.778	−62.653
Tolbutamide	Test	−0.5229	1.6581	4.2903	2.5625	0.053791	3	1	2	0	−0.71571	−10.029	5.6313	4.978	309.47
Triprolidine	Training	0.9031	10.995	5.4405	1.0054	0	2	2	1	0	−0.07691	−8.966	1.8631	−0.117	24.779
Troglitazone	Training	0.9542	5.0331	3.8855	−0.0546	0.10206	5	3	4	0	−0.53697	−8.4748	2.1707	0.937	433.62
Verapamil	Training	1.1661	2.8798	5.0559	−3.1501	0.051031	6	2	7	1	0.19353	−8.532	4.4077	−2.646	−293.06
Warfarin	Test	−0.7959	1.475	5.1027	−5.0679	0	4	3	1	0	−1.0329	−9.317	7.9756	−0.377	−3.131
Zidovudine	Training	1.0934	9.2897	4.299	4.6341	0	5	1	1	0	−0.41289	−9.712	4.1154	−0.222	13.696

*X*1, cosmic torsional energy; *X*2, inertia moment 2 length; *X*3, dipole moment *Z* component; *X*4, Kier ChiV4 (cluster) index; *X*5, number of H-bond acceptors; *X*6, six-membered rings; *X*7, group count for methyl; *X*8, ADME violations; *X*9, energy of the lowest unoccupied molecular orbital (VAMP LUMO); *X*10, energy of the highest occupied molecular orbital (VAMP HOMO); *X*11, VAMP total dipole; *X*12: VAMP dipole *Z* component; x13, VAMP octupole *ZZZ*.

model (Table 2). However, oxaprozin appeared as an outlier in the model both for the entire data set and the test set. One possible explanation may be the relative position of oxaprozin in the data distribution, with the lowest $CL_{h,observed}$ in the data set (Table 2, further discussion in Section 3.2.2).

Thus, *in silico* models for the entire data set and the training set were built with 49 and 36 drugs, respectively, as represented by Eqs. (3) and (4). In Eq. (3), all of the selected descriptors were auto-scaled to a mean value of zero and a variance of one to ensure that all descriptors have equal determinant strength to affect log CL_h. The correlations between log $CL_{h,observed}$ and predicted human log CL_h from *in silico* models (log $CL_{h,predicted,in\ silico}$, entire data set and training set) and from hepatocytes' model are shown in Figs. 1–3, respectively. Obviously, the *in silico* models exhibited high predictive performance, with $R^2 > 0.8$, RMSE < 0.3, $Q^2_{LOO} \geq 0.65$, significant *p* values (<0.0001), the slope (0.83 and 0.85) close to unity and the intercept (0.09 and 0.08) close to zero (Figs. 1 and 2). Consequently, *in silico* method only using molecular descriptors is capable of accurately predicting CL_h.

$$\begin{aligned}\log CL_h = {} & 0.3029X1^* - 0.1602X2^* + 0.2705X3^* - 0.1042X4^* \\ & + 0.1705X5^* + 0.1737X6^* + 0.1399X7^* \\ & - 0.1041X8^* + 0.3917X9^* + 0.2254X10^* \\ & + 0.2049X11^* - 0.2203X12^* + 0.1078X13^* \\ & + 0.5281 \end{aligned} \quad (3)$$

where $N = 49$, $R^2 = 0.83$, $Q^2_{LOO} = 0.71$, RMSE = 0.29, $F = 12.89$, and $p < 0.0001$.

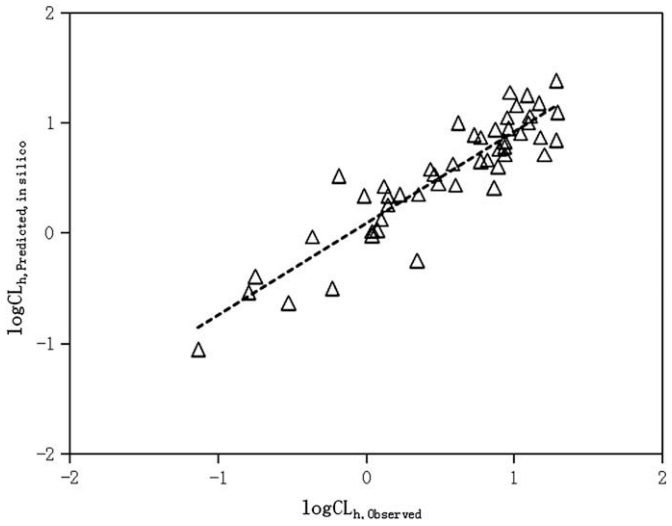

Fig. 1. Correlation between the predicted and observed human CL_h for a data set of 50 drugs from *in silico* model (entire data set: $n = 49$, excluding oxaprozin, $y = 0.83x + 0.09$, $R^2 = 0.83$).

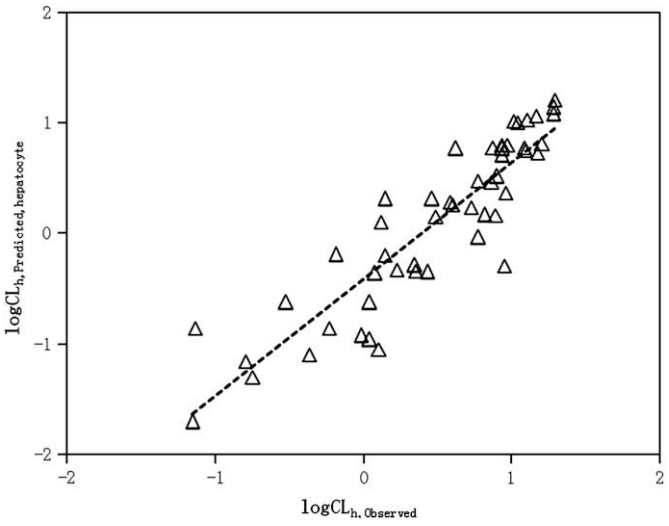

Fig. 3. Correlation between the predicted and observed human CL_h for a data set of 50 drugs from hepatocytes' incubation model ($n = 50$, $y = 0.76x + 0.41$, $R^2 = 0.81$).

$$\log CL_h = 0.0742X1 - 0.1325X2 + 0.0952X3 - 1.3804X4 + 0.0889X5 + 0.1766X6 + 0.0879X7 - 0.1613X8 + 0.8659X9 + 0.3447X10 + 0.1377X11 - 0.1113X12 + 0.0009X13 + 2.7883 \quad (4)$$

where $N = 36$, $R^2 = 0.85$, $Q^2_{LOO} = 0.65$, RMSE = 0.28, $F = 9.76$, and $p < 0.0001$.

3.2. Comparison between in silico and hepatocytes' models

3.2.1. Predictive accuracy of all drugs

The observed and predicted values of CL_h, and the fold error of 50 drugs calculated from *in silico* model in this study and human hepatocytes' model in literature, are shown in Table 3. Comparison of prediction error between two models is summarized in Table 4. As can be seen, 78% of drugs with fold error <2, 10% of drugs with fold error >3 and AFE = 1.28 (Table 4) in our model, while for hepatocytes' model, 42% of drugs with fold error <2, 36% of drugs with fold error >3 and AFE = 2.55 (Table 4). Therefore, the predictability of our *in silico* model is superior to that of the hepatocytes' model in literature for the used 50 drugs [24].

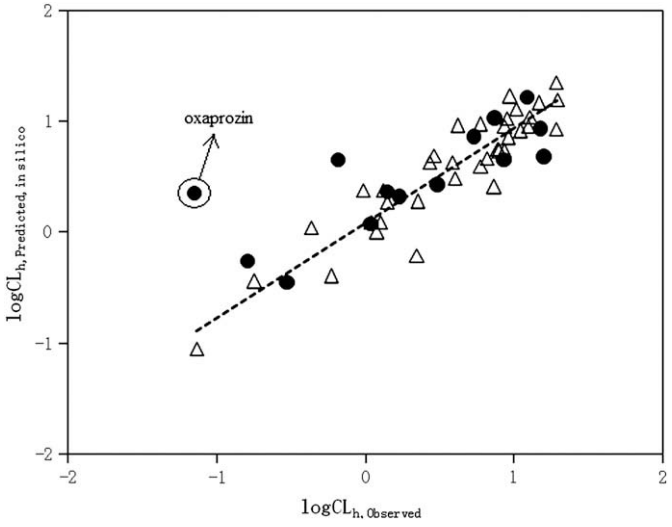

Fig. 2. Correlation between the predicted and observed human CL_h for a data set of 50 drugs from *in silico* model (training set: $n = 36$, $y = 0.85x + 0.08$, $R^2 = 0.85$; test set: $n = 13$, excluding oxaprozin, $R^2 = 0.73$, •: test set, △: training set).

The precision error plots for *in silico* and hepatocytes' models (Figs. 4 and 5) indicated that predicted values of CL_h from *in silico* model not only had high accuracy (AFE = 1.28) but also had no bias to over- or under-prediction. By contrast, most of the $\log(CL_{h,predicted}/CL_{h,observed})$ values were below the 1:1 correspondence line in the hepatocytes' model. In other words, the hepatocytes' model tended to under-predict CL_h. Possible reasons to such under-prediction included quality of tissue used for hepatocytes' preparations (cryopreserved human hepatocytes with low viability and activity, probably the main reason) [8]; active transport processes *in vivo* not reflected adequately *in vitro* [27,28]; neglect of the extra-hepatic elimination (such as gut wall metabolism [29]); use of liver extraction models that do not correctly reflect the drug dispersion in the human liver (under-prediction potential with the well-stirred model and over-prediction potential with the parallel-tube and dispersion models) [8].

3.2.2. Predictive differences among acidic, neutral and basic drugs

As shown in Table 4, the accurate prediction of CL_h for neutral and basic drugs was easier than acidic drugs in the hepatocytes' model [19]. Although our data of $CL_{h,predicted,hepatocyte}$ were obtained from human hepatocytes' incubations (corrected for both fu_b (unbound fraction in blood) and fu_{inc} (unbound fraction in incubation *in vitro*) according to the well-stirred liver model) [24], most of the $\log(CL_{h,predicted}/CL_{h,observed})$ values for acidic drugs were over the 2-fold error (Fig. 5) and the value of AFE for acidic drugs was larger than 3 (AFE = 3.39) (Table 4). This may be due to that values of fu_{inc} in literature [24] were partially predicted from log *P*/*D* (not resulted from experiments). Moreover, accurate prediction of fu_{inc} from log *P*/*D* for acidic drugs may be more difficult than neutral and basic drugs [30].

In contrast, the prediction accuracy for acidic, neutral and basic drugs was almost the same in our model (Fig. 4 and Table 4). Except several individual points, most of the $\log(CL_{h,predicted}/CL_{h,observed})$ values were distributed within the range of the 2-fold error lines (Fig. 4) and values of AFE for all drugs were smaller than 2 (Table 4).

3.3. Identification of important molecular descriptors affecting CL_h

A set of 13 descriptors was selected by Weka software to establish the *in silico* model (Table 2 and Eq. (3)). This result

Table 3
Values of observed and predicted CL_h of 50 drugs from human hepatocytes and *in silico* models

No.	Compounds	Chemical class	Data set	$CL_{h,observed}$	$CL_{h,predicted,in\ silico}$	$CL_{h,predicted,hepatocyte}$[a]	Fold $error_{in\ silico}$	Fold $error_{hepatocyte}$
1	Acetaminophen	N	Training	4	3.10	1.81	1.29	2.21
2	Buspirone	B	Training	19.2	22.37	12.11	1.17	1.59
3	Caffeine	N	Test	1.4	2.34	2.07	1.67	1.48
4	Carvedilol	B	Test	8.7	4.49	5.87	1.94	1.48
5	Chlorpromazine	B	Training	8.6	5.53	5.14	1.55	1.67
6	Desipramine	B	Training	10.3	13.00	10.39	1.26	1.01
7	Diazepam	N	Training	0.43	1.11	0.08	2.59	5.38
8	Diclofenac	A	Training	7.33	2.58	2.92	2.84	2.51
9	Diflunisal	A	Training	0.18	0.36	0.05	2.02	3.60
10	Diltiazem	B	Training	12.8	11.04	10.7	1.16	1.20
11	Etodolac	A	Training	1.31	2.40	1.28	1.83	1.02
12	Fenoprofen	A	Test	1.69	2.05	0.47	1.22	3.60
13	Furosemide	A	Training	0.59	0.41	0.14	1.45	4.21
14	Gemfibrozil	A	Test	3.09	2.65	1.43	1.17	2.16
15	Glipizide	A	Training	0.96	2.41	0.12	2.51	8.00
16	Granisetron	B	Training	11	8.22	10.17	1.34	1.08
17	Ibuprofen	A	Training	1.4	1.88	0.64	1.34	2.19
18	Imipramine	B	Training	9.46	16.90	6.33	1.79	1.49
19	Indomethacin	A	Training	2.24	1.91	0.46	1.17	4.87
20	Irbesartan	A	Training	3.85	4.31	1.93	1.12	1.99
21	Ketoprofen	A	Training	2.22	0.62	0.52	3.61	4.27
22	Lidocaine	B	Test	15	8.41	5.34	1.78	2.81
23	Lorazepam	N	Test	1.1	1.19	0.11	1.09	10.00
24	Methylprednisolone	N	Test	7.5	10.53	5.98	1.40	1.25
25	Metoprolol	B	Test	12.15	16.58	5.95	1.36	2.04
26	Midazolam	N	Training	6.6	4.63	1.48	1.43	4.46
27	Montelukast	A	Training	1.27	1.23	0.09	1.03	14.11
28	Naloxone	B	Training	19.5	15.82	16.16	1.23	1.21
29	Nifedipine	N	Training	7.8	5.69	1.46	1.37	5.34
30	Ondansetron	N	Training	5.9	9.37	3	1.59	1.97
31	Oxaprozin	A	Test	0.07	2.25	0.02	32.20	3.50
32	Oxazepam	N	Training	1.1	1.23	0.24	1.12	4.58
33	Phenacetin	N	Training	19.2	8.51	13.9	2.26	1.38
34	Pindolol	B	Training	4.2	9.15	5.89	2.18	1.40
35	Prazosin	N	Training	2.7	4.32	0.45	1.60	6.00
36	Prednisolone	N	Training	8.7	8.99	6.32	1.03	1.38
37	Propranolol	B	Test	16.11	4.70	6.46	3.43	2.49
38	Quinidine	B	Test	5.33	7.18	1.73	1.35	3.08
39	Ranitidine	B	Training	2.9	4.96	2.07	1.71	1.40
40	Ritonavir	N	Training	1.2	1.01	0.44	1.19	2.73
41	Sildenafil	N	Training	6	3.93	0.93	1.53	6.45
42	Tenoxicam	A	Training	0.073	0.09	0.14	1.24	1.92
43	Theophylline	N	Test	0.65	4.54	0.65	6.98	1.00
44	Timolol	B	Training	9.17	7.13	2.32	1.29	3.95
45	Tolbutamide	A	Test	0.3	0.36	0.24	1.20	1.25
46	Triprolidine	B	Training	8	5.40	3.31	1.48	2.42
47	Troglitazone	A	Training	9	10.60	0.51	1.18	17.65
48	Verapamil	B	Training	14.66	14.68	11.65	1.00	1.26
49	Warfarin	A	Test	0.16	0.55	0.07	3.47	2.29
50	Zidovudine	N	Training	12.4	9.05	5.66	1.37	2.19

[a] Data of $CL_{h,predicted,hepatocyte}$ obtained from human hepatocytes' incubations in the absence of fetal calf serum (corrected for both fu_b (unbound fraction in blood) and fu_{inc} (unbound fraction in incubation *in vitro*) according to the well-stirred liver model) [24].

Table 4
Comparison of the predictive accuracy of *in silico* model with *in vitro* human hepatocytes' model

Model	Chemical class	*N*	Test criteria		
			AFE	$E_{2\text{-fold}}$ (%)	$E_{3\text{-fold}}$ (%)
In silico	Total	50	1.28	22	10
	A[a]	17	1.95	35	18
	B[b]	17	1.5	12	6
	N[c]	16	1.6	19	6
Hepatocytes	Total	50	2.55	58	36
	A	17	3.39	76	53
	B	17	1.72	35	12
	N	16	2.87	63	44

[a] Acid drugs.
[b] Basic drugs.
[c] Neutral drugs.

suggested that these descriptors have significant effects on CL_h and allow for mechanistic interpretation of the model in part. In general, these molecular descriptors related to molecular reactivity and molecular interactions of drugs.

Energies of the HOMO and LUMO are very popular quantum chemical descriptors. It has been shown that these descriptors play a major role in governing many chemical reactions and the formation of many charge transfer complexes [31]. The energy of HOMO is directly related to the ionization potential and characterizes the susceptibility of the molecule toward attack by electrophiles. The energy of LUMO is directly related to the electron affinity and characterizes the susceptibility of the molecule toward attack by nucleophiles. Both HOMO and LUMO energies have been considered as indicators of drug activity [32]. As can be expected, the larger the VAMP HOMO/LUMO values, the more possibility to suffer metabolism and the larger the CL_h. Especially, VAMP HOMO and VAMP LUMO are positively correlated with CL_h (Eq. (3)), which is consistent with the expectation above.

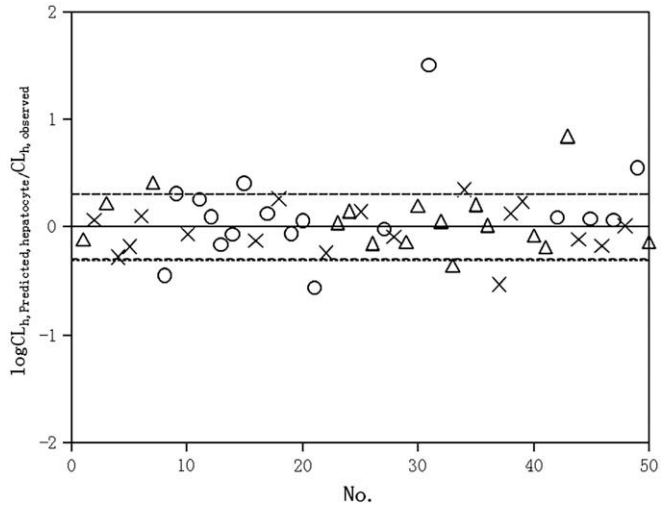

Fig. 4. Predictive accuracy of human hepatic metabolic clearance (CL_h) values predicted from *in silico* model (short dash lines represent 2-fold error between $CL_{h,predited,in\ silico}$ and $CL_{h,observed}$, ○: acid drugs; △: neutral drugs; ×: basic drugs).

Cosmic torsional energy means the energy it takes to overcome torsional strain, or the difference in energy between eclipsed and staggered conformations. It has been revealed that the torsional energy correlates with the molecular stability [33], and the lower the torsional energy, the more stable the molecule. This conclusion can be used to explain the positive effect of the torsional energy on CL_h (Eq. (3)).

The dipole moment, representative of the whole molecular charge distribution, is the most obvious and most often used quantity to describe molecular polarity. As we know, the polarity of a drug is important for drug metabolism process (metabolites are generally more polar than their parent compounds) [34]. And VAMP total dipole, dipole moment *Z* component, VAMP octupole *ZZZ* and VAMP dipole *Z* component are also identified as significant descriptors in our model.

Number of H-bond acceptors, which has long been recognized as important descriptor in drug actions, was also included in our model. Many researches have identified that the number of H-bond acceptors is a key pharmacophore of phase I [35–37] and phase II [38] metabolic enzymes. The drugs with more H-bond acceptors are more possible to become the substrates of metabolic enzymes. In our model (Eq. (3)), the number of H-bond acceptors is positively correlated with CL_h, which is in accordance with the results of pharmacophore generation studies.

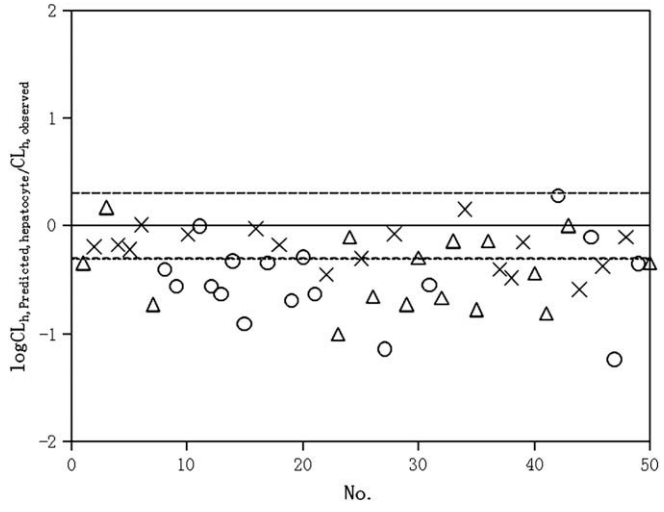

Fig. 5. Predictive accuracy of human hepatic metabolic clearance (CL_h) values predicted from hepatocytes' incubation model (short dash lines represent 2-fold error between $CL_{h,predited,hepatocyte}$ and $CL_{h,observed}$, ○: acid drugs; △: neutral drugs; ×: basic drugs).

Table 5
Comparison of the prediction accuracy of drugs with low, medium and high CL_h

$CL_{h,observed}$ class	*N*	*In silico* model		Hepatocytes' model	
		cp[a]	cp%	cp	cp%
Total	50	35	70	33	66
Low	27	24	89	0	0
Medium	17	8	47	5	29
High	6	3	50	1	17

[a] Correctly predicted.

Moreover, six-membered rings, group count for methyl, ADME violations (the number of violations of the Lipinski's rule-of-five for each structure), inertia moment 2 length (geometrical descriptors) and Kier ChiV4 (cluster) index (connectivity index) were selected simultaneously as contributors in the *in silico* model to CL_h prediction (Eq. (3)).

3.4. Model limitations

One obvious weakness of the present model is the scarcity of the data used in the study. The size of data set (36 for training set and 14 for test set) may not be large enough to evaluate the generalization ability of the prediction model. Therefore, the leave-one-out and test set validation procedures have been employed.

A further shortcoming of the study lies in the skew distribution of $CL_{h,observed}$ in the entire data set. As shown in Table 5, 27 drugs of the entire data set (54%) were classified as low $CL_{h,observed}$, while 17 drugs (34%) and six drugs (12%) were categorized as medium and high $CL_{h,observed}$, respectively. And the prediction for drugs with low CL_h was better than those with medium or high CL_h. The reason may be that the drug candidates with lower $CL_{h,observed}$ have the high probability to be developed as marketed drugs, and the drug candidates with higher $CL_{h,observed}$ have the low probability to be drugs. For this reason, the training set and test set were split by the cluster analysis of $CL_{h,observed}$ to ensure the representativeness of the test set as far as possible. In summary, a larger data set, composed of drugs with uniform distribution of CL_h in the low, medium and high values, is necessary for further validating the *in silico* approach in drug CL_h prediction.

4. Conclusions

A first-principle, structure-based model using molecular descriptors merely was developed successfully for the prediction of human hepatic metabolic clearance, predictive performance of which was superior to the hepatocytes' model in the study. VAMP LUMO, VAMP HOMO, cosmic torsional energy and number of H-bond acceptors were identified as the important molecular descriptors to affect CL_h.

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