PREDICTING HUMAN HEPATIC CLEARANCE USING HYPERNET NEURAL NETWORKS

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Abstract

Accurate prediction of human hepatic clearance of drugs plays a key role the development of new drugs. Doing so is challenging due to the complex nature of the human liver. Numerous hepatic mechanisms are involved in clearing drugs and toxins from bloodstream, some of which are not well understood. In this paper, we propose a simple learning algorithm based on Hypernet neural networks to predict in vivo human hepatic clearance of drugs. The algorithm uses a quadratic discriminant function. A set of 85 compounds was assembled from various sources. The feature space consists of 20 publicly available physicochemical properties calculated from compound molecular structures. In addition, in vitro and in vivo rat, and in vitro human clearance data were used as features. Prediction performance was poor when all 85 compounds were used. However, dividing the dataset into smaller normalized sets significantly improved the success rate. In particular, approximately 80% of the predicted values were successful when data from [13] was used (2-fold error = 20%, $r^2 = 0.775$).

1 INTRODUCTION

Anticipating the human hepatic clearance of drugs from blood plays an important role in new drug development. The industry spends over 800 million dollars over 10-15 years to develop a single new drug [1]. Even with tripling of R&D expenditures over the last decade, failure in the R&D process is still too high and new chemical entity development has been sluggish [2]. Accurate estimation of a drug's hepatic disposition is a crucial step in the drug development process. Computer aided identification of compound failures sooner in the process will help reduce development costs. Early, during new drug development, there is often a considerable over-supply of candidates. Suitable in silico methods can help narrow the list of candidate compounds before beginning expensive wet-lab evaluation and screening of those selected [3][4]. Compounds that are likely to have undesirable pharmacodynamic and pharmacokinetic properties need to be culled from the list. The current most widely used in silico methods rely on *correlational* techniques known as Quantitative Structure-Activity Relationship (QSAR). QSAR is the process by which chemical properties are quantitatively correlated with a well defined process, such as a biological activity. A QSAR's most general mathematical form is: *Activity = f (physiochemical properties and/or structural properties),* where *f* is a mathematical function. This kind of mapping does not consider the *mechanisms* involved in creating the biological activity. In this work we propose a method based on Hypernet Neural Networks [5] in order to predict the in vivo human hepatic clearance of compounds.

1.1 Biological Background

The liver is a complex biochemical factory. Cells within synthesize, modify and metabolize thousands of substances daily, which provides the body with essential substances such as proteins and fats. The liver is also responsible for eliminating toxins and drugs that find their way into blood. When detoxifying harmful substances, fat-soluble toxins must be converted to water-soluble toxins so they may exit the body through the urine or colon. Sometimes, up to a hundred enzymes are utilized to neutralize dangerous compounds. Some intermediates that result are known to be even more chemically active. The liver quickly remedies this problem by combining the active metabolite with other compounds that are water-soluble. After these two conversions, the toxin can safely exit the body. Health problems can result if these conversions fail. Drugs in the liver participate in actions such as transport, binding with proteins and metabolism in parenchymal cells of liver (hepatocytes). The rate of elimination, known as hepatic clearance, is different for different compounds. Clearance is a proportionality constant that relates the rate of drug elimination to blood or plasma concentrations. In new drug development, moderately slowly cleared compounds are more favorable than quickly cleared ones.

2 METHODS

2.1 Learning Algorithm

Consider the following quadratic discriminant function $D(\boldsymbol{x})$ [5]:

$$D(\boldsymbol{x}) = \sum_{i=1}^{m} w_i x_i + \sum_{i=1}^{m}\sum_{j=1}^{m} w_{i,j} x_i x_j + w_0$$

where x_i is the i^{th} component of the n-dimensional vector $\boldsymbol{x} \in \Re^n$, w_i and $w_{i,j}$ are weights.

The above equation can be written in the following matrix form:

$$D(\boldsymbol{x}) = \begin{bmatrix} x_1 & \dots & x_m & 1 \end{bmatrix} \begin{bmatrix} w_{1,1} & \cdots & w_{1,m} & w'_1 \\ \vdots & \ddots & w_{1,1} & \vdots \\ w_{m,1} & \cdots & w_{m,m} & w'_m \\ w'_1 & \dots & w'_m & w_0 \end{bmatrix} \begin{bmatrix} x_1 \\ \vdots \\ x_m \\ 1 \end{bmatrix}$$

where $w'_i = w_i / 2$. Thus,

$D(X) = X^{\mathrm{T}} W X$

where $X = \begin{bmatrix} x_1 & \dots & x_m & 1 \end{bmatrix}^{\mathrm{T}}$, and

$$W = \begin{bmatrix} w_{1,1} & \cdots & w_{1,m} & w'_1 \\ \vdots & \ddots & w_{1,1} & \vdots \\ w_{m,1} & \cdots & w_{m,m} & w'_m \\ w'_1 & \dots & w'_m & w_0 \end{bmatrix}.$$

Define Y = [X_1 X_2 … X_n], the data set matrix and C = [c_1 c_2 ... c_n], the set of labels such that X_i is a data point associated with the class label c_i. For a given data set matrix, Y and weight matrix W we define B = Y^TWY, where the diagonal of B = [c_1 c_2 ... c_n]. Solving for W yields: W = $(Y^+)^T$BY^+, where Y^+ denotes the pseudo inverse matrix of Y.

Based on the above matrix analysis, we propose the following learning algorithm.

Algorithm 1:

```
Initialize B as a diagonal matrix
with(c1, c2, ... cn) on the main
diagonal.
Until no changes
      W = (Y+)TBY+
      B = YTWY
      Reset diagonal of B = (c1, … ,cn)
```

2.2 Feature Selection

The following feature selection algorithm was used in order to determine important features. Basically, during each cycle, the algorithm looks at the prediction performance of the Hypernet NN absent one feature. The feature, without which the performance is maximum, is removed from the feature set. The process continues until most features are removed. Remaining features are considered most important.

Algorithm 2:

```
Initialize F as a set of all features.
Iterate until F is empty {
      Given a set of k features
      F = {f1, f2, … fk},
      For feature i (i = 1 … k) do {
          Remove the ith feature, fi.
          Measure the performance of
          the classifier, call it Pi.
      }

      Find j∈{1,…,k} such that Pj is
      the maximum performance measure.
      Remove feature fj from the
      feature set F.
}
```

2.3 Data Collection

2.3.1 Hepatic Clearance Data

The *in vivo* blood clearance and *in vitro* clearance data for 85 compounds, denoted $CL_{\text{in vitro, rat}}$, $CL_{\text{in vitro, human}}$, $CL_{\text{in vivo, rat}}$, and $CL_{\text{in vivo, human}}$, were obtained from literature for rats and humans ([10-18]). For all compounds selected, liver was assumed to be the main site of metabolism. Average $CL_{\text{in vitro, human}}$ and standard deviation values across different papers are shown in Table 1.

2.3.2 Physicochemical Properties

All physicochemical properties (PCPs) in this work were obtained from free and publicly available sources. MW (Molecular Weight), HBD (H-Bond Donor), HBA (H-Bond Acceptor), RBC (Rotatable Bond Count), TC (Tautomer Count), TPSA (Topological Polar Surface Area), HAC (Heavy Atom Count), Complexity, DASC (Defined Atom StereoCenter Count), UASC (Undefined Atom StereoCenter Count), DBSC (Defined Bond StereoCenter Count), CBUC (Covalently-Bonded Unit Count) were obtained from PubChem [6]. logP, apKa1 (acidic pKa), bpKa1 (basid pKa), logD (at pH=7.4) were calculated using ChemAxon [7]. Dipole moment and Ionization Potential (Ion. Pot.) were calculated using MOPAC [8][9]. Total Atom count (TAC) was calculated from molecular formula.

2.4 Calculation of Predicted CL values

The predicted CL values were calculated for each compound in a leave-one-out manner. Algorithm 1 was used to train the ANN by the z-scored data. Furthermore, a linear mapping was established to scale up the final ANN output. The resulting weight matrix, $W_{trained}$, and the linear mapping were used to predict CL for a given drug as follows.

$$y = X^T W_{trained} X$$

$$CL_{\text{predicted}} = ay + b$$

where X is the PCP vector of the drug.

The prediction performance of a trained ANN was measured using squared Pearson correlation coefficient (r^2) between predicted and observed CL values. In addition, the two-fold error ($E_{2\text{-fold}}$) was calculated for the pre-

dicted values. A successful prediction was considered one that had less than 2-fold error.

Table 1. In vivo human hepatic clearance of 85 compounds collected from six published papers. Average and standard deviation values (across papers) are shown.

Number	Compound	Average $CL_{in\ vitro,\ human}$ (mL/min/kg)	References
1	Acebutolol	4.10	[10]
2	Alprazolam	2.70	[13]
3	Antipyrine	0.49 ± 0.02	[11-13][16,17]
4	Atenolol	1.00	[10]
5	Bepridil	5.30	[10]
6	Betaxolol	3.90	[10]
7	Bisoprolol	1.30	[10]
8	Bosentan	3.70 ± 0.00	[12][16,17]
9	Bromocriptine	15.00	[10]
10	Caffeine	2.08 ± 0.52	[10-13][16,17]
11	Carbamazepine	0.40	[10]
12	Carvedilol	8.70	[10]
13	Cetirizine	1.00	[10]
14	Chlorpheniramine	1.30	[10]
15	Chlorpromazine	8.60	[11]
16	Chlorprothixene	12.00	[11]
17	Cimetidine	3.20	[10]
18	Clozapine	7.40	[10]
19	Codeine	7.50	[10]
20	Cyclosporin A	4.70	[10]
21	Desipramine	11.15 ± 1.20	[10,11]
22	Dextromethorphan	6.00	[10]
23	Diazepam	2.38 ± 4.49	[10-13][17]
24	Diclofenac	20.04	[13]
25	Diltiazem	13.27 ± 3.41	[10-13][17]
26	Diphenhydramine	9.60	[10]
27	Dofetilide	3.70	[13]
28	Doxepin	14.00	[10]
29	Ethinylestradiol	5.40	[10]
30	Famotidine	2.10	[10]
31	Felodipine	14.20 ± 5.54	[12,13][17]
32	FK1052	20.44	[13]
33	FK480	19.51	[13]
34	Flunitrazepam	7.18	[13]
35	Fluoxetine	7.80	[10]
36	Furosemide	0.64	[11]
37	Granisetron	11.00	[10]
38	Hexobarbital	5.87	[13]
39	Ibuprofen	8.66 ± 11.19	[11,13]
40	Imipramine	14.80 ± 6.01	[10][11][13]
41	Indinavir	17.86	[13]
42	Isradipine	8.00	[10]
43	Lidocaine	15.04	[13]
44	Lorazepam	1.08 ± 0.05	[10-12][17]
45	Methylprednisolone	7.50	[11]
46	Metoprolol	12.27 ± 1.04	[10][13]
47	Mibefradil	7.00 ± 0.00	[12][16][17]
48	Midazolam	10.91 ± 4.61	[10-13][16,17]
49	Mofarotene	11.00 ± 0.00	[12][16][17]
50	Morphine	18.00	[10]
51	Nadolol	0.80	[10]
52	Naloxone	21.25 ± 5.68	[10-12][17]
53	Nicardipine	11.49 ± 7.78	[12][17][13]
54	Nifedipine	7.75 ± 1.06	[10][11]
55	Nilvadipine	20.22 ± 0.38	[12][17][13]
56	Nitrendipine	19.13 ± 0.75	[12][10][17]
57	Omeprazole	13.71 ± 8.78	[10][13]
58	Ondansetron	9.31 ± 4.82	[10][13]
59	Oxazepam	1.10 ± 0.00	[12][11][17]
60	Phenacetin	14.28	[13]
61	Phenytoin	3.35	[13]
62	Pindolol	4.20	[10]
63	Pirenzepine	1.00	[10]
64	Prazosin	2.70	[10]
65	Prednisolone	8.70	[11]
66	Propofol	11.00	[10]
67	Propranolol	14.59 ± 2.69	[10-13][16,17]
68	Ranitidine	2.90	[10]
69	Remikiren	19.80 ± 0.28	[12][17]
70	Ritonavir	1.20	[10]
71	Scopolamine	11.00	[10]
72	Sildenafil	6.00	[11]
73	Sulpiride	1.50	[11]
74	Temazepam	1.30	[10]
75	Tenoxicam	0.04	[11]
76	Terbutaline	1.00	[11]
77	Theophylline	0.62 ± 0.02	[11,12][16,17]
78	Tolbutamide	1.03 ± 1.12	[11][13]
79	Tolcapone	2.70 ± 0.00	[12][16][17]
80	Triazolam	7.95 ± 7.71	[10][13]
81	Triprolidine	8.00	[10]
82	Verapamil	14.50 ± 0.71	[10][11]
83	Warfarin	1.87 ± 2.58	[11][13]
84	Zileuton	6.00	[10]
85	Zolpidem	18.33	[13]

3 RESULTS

The result of running the feature selection algorithm on 39 compounds from [13] is shown in Table 2. In the beginning, when all features where included, the prediction performance was poor (r^2 =0.02). At the end of each iteration thereafter, one feature was chosen such that once

it was removed, the prediction performance (r^2) was maximum. The first feature in Table 2 is TAC (Total Atom Count). It means that removing TAC from the feature set resulted in a better performance than removing any other feature. We concluded that TAC was the least important feature. It was removed from the feature set thereafter. One feature per iteration was removed. The four most important features, which remained until the end, were Hydrogen Bond Donor (HBC), Hydrogen Bond Acceptor (HBA), Rotatable Bond Count (RBC), $CL_{in\ vitro,\ human}$. When these four features were used, the highest prediction performance was achieved (r^2=0.775).

Table 2. Results of the feature selection algorithm. At the end of each iteration, one feature was chosen such that absent that feature, the prediction performance (r^2) was maximized. Abbreviations are defined in section 2.3.2.

Iteration	Feature	r^2
	none	0.019
1	TAC	0.125
2	UASC	0.195
3	apKa	0.256
4	MW_10	0.288
5	Dipole	0.349
6	$CL_{in\ vivo,rat}$	0.424
7	CBUC	0.470
8	MW	0.497
9	$CL_{in\ vitro,\ rat}$	0.489
10	DASC	0.488
11	logP	0.507
12	DBSC	0.572
13	Ion. Pot.	0.519
14	TPSA	0.448
15	logD	0.533
16	bpKa	0.624
17	TC	0.531
18	Complexity	0.619
19	HAC	0.775
20	HBD	0.699
21	HBA	0.774
22	RBC	0.740
23	$CL_{in\ vitro,\ human}$	-

The prediction results are shown in Figure 1. 20.5% of the predicted values were outside the 2-fold error range ($E_{2\text{-}fold}$ = 20.5%).

Based on the resulting Wtrained matrices and the leaner mappings, the following equations were used to predict in vivo human hepatic clearance:

$$CL_{predicted} = 38.45\ y + 11.07$$

where

$$y = 0.022\ x_1^2 + 0.034\ x_1x_2 + 0.1\ x_1\ x_3 + 0.05\ x_1\ x_4 + 0.026\ x_1 + 0.022\ x_2^2 + 0.05\ x_2\ x_3 + 0.058\ x_2\ x_4 + 0.035\ x_2 + 0.017\ x_3^2 + 0.016\ x_3\ x_4 + 0.04\ x_3 + 0.017\ x_4^2 + 0.134\ x_4 + 0.002,$$

and x_1, x_2, x_3, and x_4 are the z-scores as defined below:

x_1 = (Hydrogen Bond Donor – 1.44) / 1.55,

x_2 = (Hydrogen Bond Acceptor – 4.64) / 2.72,

x_3 = (Rotatable Bond Count – 4.8) / 4.69,

$x_4 = (CL_{in\ vitro,\ human} - 6.8)/\ 6.0$.

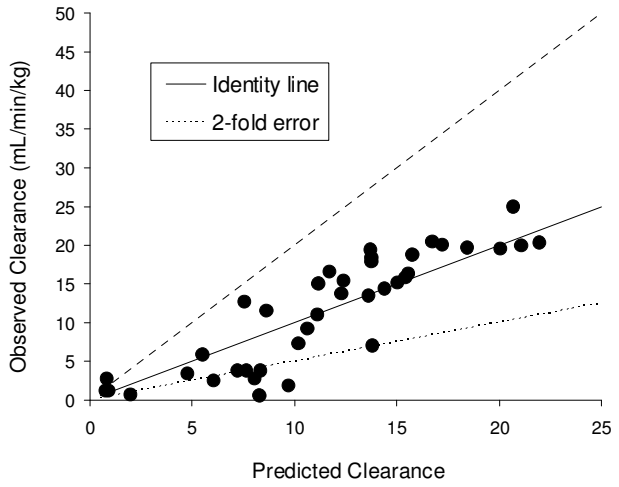

Figure 1. Prediction results for 39 compounds from [13]. r^2 = 0.775, $E_{2\text{-}fold}$ = 20.5%.

4 CONCLUSIONS

In this paper, we used the Hypernet neural network learning algorithm to predict in vivo human hepatic clearance. A set of 85 compounds was assembled (Table 1). Compounds represent a wide range of metabolic stability, metabolic enzyme usage and protein binding. The data set is heterogeneous. The hepatic clearance values are collected from six different published papers. The values come from diverse protocols, measurement methods and assays. To homogenize and normalize the combined data, we need detailed information about the experimental protocols, but that is not be easily obtained. Even with that information, it is not straight-forward to normalize the data. Consequently, the predictions were poor when all 85 compounds were used. However when normalized data from one source were used the prediction results were significantly enhanced. For the results presented in this paper, 39 compounds from [13] were used. Approximately 80% of the predicted values were successful and led to r^2 value of 0.775. A prediction was considered successful if it was within the 2-fold error range of the ob-

served value. The success rate was decreased to approximately 56% when all 85 drugs were used.

Drug clearance may vary greatly from one person to another, due to varying contributions of different influencing factors such as age, sex, race, body weight, body size, etc. As such, accurate prediction of hepatic metabolic clearance in humans requires careful attention to inter-individual variability, potential complexities (e.g. active transport processes) and the pharmacokinetic properties of the test compounds.

The method presented herein does not take into account the complex mechanisms involved in the process of hepatic drug clearance. Future work involves combining that knowledge with traditional classification methods (such as the one presented in this paper). The synthetic method of modeling and simulation [19] can facilitate that process. Doing so is expected to improve our insight into drug clearance processes, which in turn improves our ability to anticipate the metabolic and biological fates of compounds of interest.

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