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Re-evaluation of a Quantitative Structure Pharmacokinetic Model for Biliary Excretion in Rats

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Abbreviations: QSPKR, quantitative structure pharmacokinetic relationships; PSA, polar surface area; Pgp, p-glycoprotein; PD_b, percentage of dose eliminated in bile in parent form; CL_b, biliary clearance; MRP2, multidrug resistance protein 2; BCRP, breast cancer resistance protein

Abstract

Quantitative structure pharmacokinetic relationship (QSPKR) modeling can be used to predict the biliary clearance (CL_b) and percentage of dose eliminated in bile (PD_b) in humans prior to clinical studies. Recently, a QSPKR model based on in-house compounds was derived using simple physicochemical descriptors to predict the PD_b in rats (Luo et al., 2010). Our objectives were to evaluate the QSPKR model derived by Luo et. al. for the prediction of PD_b for our larger dataset of 164 compounds in the rat and for the 97 compounds in our human dataset (Yang et al., 2009). Re-analysis of the published QSPKR model by Luo et. al. revealed the model to be statistically insignificant. Thus, a new statistically significant QSPKR model, consisting of one less descriptor than the published model, was derived from the published data. The newly derived model performed as well as the published model in predicting the PD_b for the training and test sets from Luo et al. Conversely, the new model performed poorly in predicting the PD_b for our larger rat ($r^2=0.253$) and human dataset ($r^2=0.013$). The poor predictions for our datasets may be due, in part, to the quality and diversity of the data used to derive and test the model. Our re-evaluation suggests that hepatobiliary excretion is a process that cannot truly be captured by simple physicochemical descriptors when examining chemically dissimilar compounds. A simple approach involving a limited number of physicochemical predictors may be useful when examining a structurally similar series of compounds.

Introduction

Biliary excretion is a complex process that is difficult to experimentally measure in humans. Biliary excretion studies in humans are performed by collecting bile samples in patients, collecting duodenal fluid as a substitute for bile in healthy volunteers or by analyzing feces. Biliary excretion of drugs determined in patients may be influenced by changes in their physiology and by disease states. Analyzing feces can be inaccurate due to the presence of intestinal secretion, degradation of drug in the gastrointestinal tract, or enterohepatic cycling (Ghibellini et al., 2006).

Recently, quantitative structure pharmacokinetic relationship (QSPKR) models have been utilized to predict the biliary clearance and/or the percentage of dose eliminated in bile (Yang et al., 2009; Chen et al., 2010; Luo et al., 2010). In the first such model published by our group (Yang et al., 2009), we reviewed the literature for data on the percentage of dose eliminated in bile in parent form (PD_b) in rats (N = 164) and humans (N = 97) and biliary clearance (CL_b) of parent drug in rats (N = 55) and humans (N = 56). The data for the rat studies were only collected from publications where the animals were dosed intravenously. The human data were collected from publications predominantly where the drug was dosed intravenously, although there were a limited number of biliary excretion studies with oral administration where the data were corrected for the bioavailability of the compound. A simple set of 2-dimensional and 3-dimensional structural descriptors (n = 11) were calculated using the molecular modeling software SYBYL ver. 7.3 (Tripos, St. Louis, Mo). Molecular weight and logP were calculated using KOWWIN (Estimation Program Interface Suite, US EPA) and logD was calculated using the Marvin Sketch software (Chemaxon Inc, Budapest, Hungary). QSPKR models derived using these fourteen 2D and 3D structural descriptors poorly predicted CL_b and PD_b. Based on these initial results, the data was further analyzed using a set of 114 2D and 3D structural descriptors calculated using QSARis version 1.2 (SciVision, San Diego, CA). QSPKR models for CL_b and

PD_b for both rats and humans were derived using stepwise multiple linear regression. A QSPKR model for the training set of biliary clearance in rats was constructed with an r^2 of 0.689 and a cross validated correlation coefficienct (Q^2) of 0.618. Similarly, a simple QSPKR model was constructed for the training set in humans (N = 38) with an r^2 of 0.819 and a Q^2 of 0.722. Similar QSPKR models were generated for PD_b in both rats and humans. Splitting the dataset into subsets based on charge, or transporter substrate status (P-glycoprotein or MRP2) improved predictions. For example, a QSPKR model for the percentage of dose eliminated in bile for anionic compounds in rats (N = 61) was generated:

$$PD_b = 19.7*Gmax + 14.3*xch3 - 21.5*SdaaN + 1.28*Qyy - 4.42*SsssCH - 240.6$$

(r^2 of 0.784, p < 0.01; Q^2 of 0.737, p < 0.01)

Biliary clearance and percentage of dose eliminated in bile in both rats and humans was satisfactorily predicted by the QSPKR models (Yang et al., 2009).

Following the publication of the model by Yang et. al. (Yang et al., 2009), Chen et. al. (Chen et al., 2010) used literature data for 16 compounds and 40 proprietary compounds in their analysis to predict the percentage of dose eliminated in bile (PD_b) in rats. The compounds were split into a training set of 46 compounds and a test set of 10 compounds. A genetic algorithm based principal components regression analysis was used to derive a QSPKR model for percentage of dose eliminated in bile in rats using 2D molecular descriptors. The model performed well in predicting the PD_b for the training set for rats with a r^2 of 0.85 and a Q^2 of 0.79 and predicted the PD_b for the test set with a r^2 of 0.84 (Chen et al., 2010).

More recently, Luo et al. (Luo et al., 2010) generated a simple QSPKR model to predict the percentage of dose eliminated in bile in rats using three descriptors for a set of 50 in-house Bristol Myers Squibb compounds. Neither structures nor other physical chemical data are available for these compounds. The descriptors in the final model included the free energy of

aqueous solvation, polar surface area, and the presence or absence of a carboxylic acid group. This simple model was able to reasonably predict the biliary excretion of the 50 compounds (r^2 = 0.890). This QSPKR model was then used to predict the biliary excretion for a test set of 25 literature compounds and generated a r^2 = 0.730.

The objectives of our current study were two–fold: (1) to use our larger rat dataset of 164 compounds to evaluate the usefulness of the QSPKR model derived by Luo et. al. (Luo et al., 2010) to predict the extent of biliary excretion and (2) to evaluate the usefulness of this model to scale up and predict the biliary excretion of 97 compounds in our human dataset.

Methods

The first step was to re-evaluate the analysis in the published paper (Luo et al., 2010). In this publication, each descriptor (n = 8) was plotted against the dependent variable PD_b. The three descriptors with the highest correlation (free energy of aqueous salvation (Gsolv,aq), polar surface area (PSA), and presence or absence of carboxylic acid (Acid) were selected for the model and the PD_b values of 50 training set compounds were predicted. The data from the publication was digitized and a similar model was derived using SAS version 9.2 (SAS Institute Inc, Cary, NC). The model generated using SAS was almost identical to the published model (Published model: Biliary excretion = 0.245 x PSA + 50.289 x Acid − 0.616 x Gsolv,aq − 29.395 (r^2 = 0.89), SAS model: Biliary excretion = 0.250 x PSA + 50.374 x Acid − 0.603 x Gsolv,aq − 29.460 (r^2 = 0.89)), except that one descriptor in the derived model was not significant (Gsolv,aq, p = 0.083). Based on these results, a new QSPKR model was generated using stepwise regression: forward selection to include relevant descriptors (p < 0.05) followed by backward elimination to remove any descriptors that have become insignificant during the regression process (p > 0.05). The performance of the model was evaluated using 'leave one out' cross validation. In this internal validation method, the model is reconstructed with one

compound excluded and the biliary excretion of that compound is predicted by the model. This process was repeated until every compound had been excluded from the model once. The cross validation correlation coefficient, Q^2, was then calculated

$$Q^2 = \frac{\sum(y_{pred} - y_{obs})^2}{\sum(y_{obs} - y_{mean})^2}$$

External validation was performed by using the stepwise model to predict the biliary excretion of 25 compounds in the published test set.

To use the stepwise model on our rat dataset we first had to calculate the polar surface area. The PSA values were calculated using a fragmentation based approach (Ertl et al., 2000). The polar surface area calculated using this fragmentation approach showed good correlation (r^2 = 0.982) with the traditional 3D approach. Two different software applications that use the fragmentation algorithm were used to calculate the polar surface area; Pubchem (www.pubchem.gov) and Molinspiration (www.molinspiration.com). The stepwise model from the training set was used to predict the biliary excretion for the 164 compounds in our rat dataset. Internal validation was performed using the leave one out cross validation method. We also applied the stepwise QSPKR model to scale up and predict the percentage of dose eliminated in bile for our human dataset of 97 compounds.

The structural diversity of our rat dataset was determined using pharmacophore fingerprints with the Tanimoto dissimilarity coefficient (JChem Base, Chemaxon Ltd. Budapest, Hungary). For each compound in the dataset pharmacophore fingerprints are generated. Pharmacophore fingerprints used in the software are defined as the collection of all atom-atom pharmacophore feature pairs and their topological distances (www.chemaxon.com). The pharmacophore fingerprints for each compound is compared to the pharmacophore fingerprints for all other compounds in the dataset using the Tanimoto dissimilarity coefficient. A Tanimoto

dissimilarity coefficient of 1 between two compounds indicates that the compounds are dissimilar while a coefficient of 0 indicates the two compounds are exactly the same. Pairs of compounds with Tanimoto dissimilarity coefficients of less than 0.3 were considered to be very similar in structure.

Results and Discussion

The final model generated by Luo et. al. (Luo et al., 2010) with three descriptors was not statistically significant (Gsolv,aq: $p > 0.05$) and a new stepwise multiple linear regression model was derived to predict the biliary excretion for the 50 compounds in the training set. The new QSPKR model contained two descriptors and was able to predict the biliary excretion with an r^2 of 0.880 (Figure 1).

$$\text{Biliary excretion} = 0.401*PSA + 49.3*Acid - 27.1 \qquad \text{Equation 1}$$

Prediction performance of this model was tested using leave one out cross validation and resulted in a Q^2 of 0.860 suggesting that this model can be used for further predictive purposes. This stepwise model was also used to predict the biliary excretion of 25 compounds in the test set of Luo et. al (Luo et al., 2010). The stepwise model ($r^2 = 0.722$) performed as well as the model in the publication ($r^2 = 0.735$). The new stepwise model is parsimonious and has statistically significant descriptors as compared to the published model and performed as well as the published model in predicting the biliary excretion of the training and test sets.

In order to apply the stepwise model to predict the biliary excretion for our rat dataset, we had to calculate the polar surface area. To determine which software to use to calculate the PSA for our rat dataset, we first calculated the PSA for the twenty five compounds in the published test set for which structures are available in the literature. The PSA values calculated using Pubchem and Molinspiration were compared to those in the publication. The PSA values published in the paper did not correlate well with the PSA values from Pubchem and

Molinspiration, even though all three use the same algorithm. The published paper used Scitegic Pipeline Pilot ver 5.1 (Accelyrs Inc.) to calculate the PSA. The company was contacted regarding the difference in the values and the difference was identified by the company as an error in the software. The newest version of the software was used by Accelyrs Inc. to calculate the PSA values for the 25 compounds in the test set and they compared well with those calculated using Molinspiration (r^2 = 0.99). Thus, the PSA values published for the test set compounds may be prone to error and we chose to calculate the PSA values using Molinspiration for our rat dataset.

The stepwise regression model generated using the training set and polar surface area values calculated using Molinspiration was used to predict the biliary excretion for the 164 compounds in our rat dataset. The model performed poorly with an r^2 = 0.253 (Figure 2). Splitting the full dataset in subsets of anion compounds or cation/neutral compounds, a strategy that was shown to improve predictions by Yang et. al. (Yang et al., 2009), failed to improve the relationship in this case. Similar results were obtained where we tried to apply the stepwise QSPKR model derived for rats (Equation 1) to predict biliary excretion in humans (r^2 = 0.013). There were significant under-predictions and over-predictions for the human dataset. For example, moxalactam was over-predicted almost 1000 fold (observed: 0.46%, predicted: 105%), while indocyanine green was under-predicted (observed: 80%, predicted: 19.1%). Splitting the full human dataset into subsets based on charge failed to improve the relationship.

The differences in the datasets (our data versus the published data) and descriptors used are important to explore to understand the reasons for the poor predictions of our datasets. The quality of the biliary excretion data used in the test set in the publication may be questionable. Some compounds were radioactively labeled and only the total radioactivity was measured with no correction made for radioactive metabolites in bile while another compound was administered in an *in situ* perfusion experiment. Re-analysis of the test set using the

stepwise model, after removing the poor quality data points, resulted in a good correlation as well (r^2 = 0.720), suggesting that the small size of the test set may be a bigger problem. The quality of the descriptors used in the modeling process can also affect the modeling process. We found that the polar surface areas calculated for the test set in the publication may be prone to error; similarly, the polar surface areas for the training set compounds may also be incorrect, which can affect the descriptors included in the final model.

The structural diversity of the training set used to derive the model is very important. QSPKR models that are derived with structurally similar compounds or congeneric series usually perform poorly when applied to a different congeneric series or a structurally dissimilar dataset. Another important factor is the chemical space of the test set. If the chemical space the test set covers is very different from the chemical space of the training set, the QSPKR model derived using the training set will poorly predict the biliary excretion for compounds in the test set. Luo et. al. (Luo et al., 2010) tested the structural similarity of the compounds in the training set and in the test set using the Tanimoto similarity index and reported that both datasets are structurally dissimilar and similarity was found only among compounds from the same discovery program. However, most of the compounds in the training set are high molecular weight compounds (median MW - 484 Da), an attribute associated with increased biliary excretion. We do not have access to the structures of the compounds in the training set from the published paper to test whether the chemical space of the training set in the publication covers the chemical space of our rat dataset. We evaluated the diversity of the 164 compounds in our rat dataset using the Tanimoto dissimilarity coefficient, where structurally similar compounds will have a coefficient of 0. Structural similarity was only observed among compounds within the same class. The compounds in our rat dataset also show diversity based on physicochemical properties. The percentage of dose eliminated in bile for the 164 compounds ranged from 0% (butoprozine, diazepam, felodipine) to 99.7% (J-104132), the molecular weight ranged from 117

(triethylmethylammonium) to 1255 (actinomycin D), and the cLogP values ranged from -2.83 (ouabain) to 16.5 (cosalane). Overall, our larger and structurally diverse rat dataset which may cover a larger chemical space may represent one reason for the poor predictions of our rat dataset.

Finally, the complexity of biliary excretion may be a reason for the poor predictions using simple descriptors like polar surface area and absence or presence of a carboxylic acid group. Drug in the portal circulation must first cross the basolateral membrane to enter hepatocytes. This process for some drugs is mediated by transporters of the SLC family, such as OATP1B1. Inside hepatocytes, drug can be metabolized, effluxed back across the basolateral membrane into the portal circulation, or be transported across the canalicular membrane into bile. Some of the above mentioned processes are mediated by transporters from the ABC family. For example, MRP2 (ABCC2), P-gp (ABCB1), and BCRP (ABCG2) are transporters located on the canalicular membrane of hepatocytes that efflux drugs into bile. Binding affinity for SLC or ABC proteins, and the rate limiting step in this process for any specific compound, can be difficult to capture through simple descriptors. Although lipophilicity and hydrophobicity have been implicated as possible structural features necessary for binding to a transporter, there are no consensus models in the literature for predicting whether a drug is a substrate for a particular transporter (Gandhi and Morris, 2009; Xing et al., 2009; Chen et al., 2011). Another complexity associated with biliary excretion is that several transporters including P-gp and MRP2 have multiple binding sites, and substrates may bind to multiple transporters (Loo et al., 2003; Zelcer et al., 2003; Callaghan et al., 2006). These may be additional factors contributing to the poor predictions of our QSPKR models.

Previously, in our laboratory, we have used simple descriptors including molecular weight, logP, and Connolly surface area among others with stepwise multiple linear regression and partial least squares regression to predict the biliary clearance and percentage of dose

eliminated in bile in rats and humans. Both regression techniques using the simple descriptors poorly predicted both biliary clearance and percentage of dose eliminated in bile as parent compound. In our experience, simple descriptors poorly predict the biliary excretion in rats and humans.

In conclusion, a QSPKR model was generated for the training set compounds presented by Luo et al. (Luo et al., 2010) using stepwise regression. The stepwise model was simpler than the model in the published paper and was able to predict the biliary excretion of the training set compounds (from (Luo et al., 2010)) well. Quality of the descriptors used to derive QSPKR models, usually assumed to be error free, represents an area that requires careful evaluation in the development of QSPKR models. The QSPKR model from Luo et. al. (Luo et al., 2010) performed poorly for our large and diverse rat dataset that contains values for percentage of parent compound eliminated in the bile after iv administration in rats. We also used the stepwise regression model to predict the biliary excretion for the 97 compounds in our human dataset, but the model performed poorly with an r^2 of 0.013. Possible reasons for the poor prediction may reflect the greater diversity of compounds in our dataset; but the exact reasons would be difficult to elucidate without access to the chemical structures of the compounds used by Luo et. al. (Luo et al., 2010). Although biliary excretion can be reasonably predicted using 2D and 3D physicochemical predictors (Yang et al., 2009), a simple approach using measures of polarity and size may not perform well for a chemically diverse range of compounds.

Authorship contributions

Participated in research design: Gandhi, Morris

Conducted experiments: Gandhi

Contributed new reagents or analytical tools: Morris

Performed data analysis: Gandhi

Wrote or contributed to the writing of the manuscript: Gandhi, Morris

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Footnote

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Figure Legends

Figure 1 - Observed versus predicted biliary excretion for 50 compounds in the training set using stepwise regression model. The diagonal line is the regression line. The dashed line is the line of origin.

Figure 2 - Observed biliary excretion versus predicted biliary excretion for 164 compounds in the rat dataset. The diagonal line is the regression line. The dashed line is the line of origin.