

## Short Communication

# Reevaluation of a Quantitative Structure Pharmacokinetic Model for Biliary Excretion in Rats

Received January 29, 2012; accepted April 20, 2012

### ABSTRACT:

Quantitative structure pharmacokinetic relationship (QSPKR) modeling can be used to predict the biliary clearance and percentage of dose eliminated in bile ( $PD_b$ ) in humans before clinical studies. Recently, a QSPKR model based on in-house compounds was derived using simple physicochemical descriptors to predict the  $PD_b$  in rats (*Drug Metab Dispos* 38:422–430, 2010). Our objective was to evaluate the QSPKR model derived 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 (*AAPS J* 11:511–525, 2009). Re-analysis of the published QSPKR model revealed the model to be statistically insignificant (*Drug Metab Dispos* 38:422–430, 2010). 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 (*Drug Metab Dispos* 38:422–430, 2010). In contrast, 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 reevaluation 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 used to predict the biliary clearance ( $CL_b$ ) 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  $CL_b$  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 two-dimensional (2D) and three-dimensional (3D)

structural descriptors ( $n = 11$ ) was calculated using the molecular modeling software SYBYL version 7.3 (Tripos, St. Louis, MO). Molecular weight and logP were calculated using KOWWIN (Estimation Program Interface Suite, U.S. Environmental Protection Agency), and logD was calculated using the Marvin Sketch software (Chemaxon Inc., Budapest, Hungary). QSPKR models derived using these 14 2D and 3D structural descriptors poorly predicted  $CL_b$  and  $PD_b$ . Based on these initial results, the data were 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 coefficient ( $Q^2$ ) of 0.618. Likewise, 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 (Pgp) or multidrug resistance protein 2 (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:  $PD_b = 19.7 \times G_{max} + 14.3 \times xch3 - 21.5 \times SdaaN + 1.28 \times Qyy - 4.42 \times SsssCH - 240.6$  ( $r^2$  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 were satisfactorily predicted by the QSPKR models (Yang et al., 2009).

After the publication of the model by Yang et al. (2009), Chen et al. (2010) used literature data for 16 compounds and 40 proprietary com-

This work was funded in part by Hoffmann La Roche Inc.  
Article, publication date, and citation information can be found at  
<http://dmd.aspetjournals.org>.  
<http://dx.doi.org/10.1124/dmd.112.044842>.

**ABBREVIATIONS:** QSPKR, quantitative structure pharmacokinetic relationship;  $CL_b$ , biliary clearance;  $PD_b$ , percentage of dose eliminated in bile in parent form; 2D, two dimensional; 3D, three dimensional; Pgp, p-glycoprotein; MRP2, multidrug resistance protein 2; PSA, polar surface area; SLC, solute carrier; BCRP, breast cancer resistance protein; J-104132, (+)-(5S,6R,7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopenteno[1,2-*b*]pyridine-6-carboxylic.

pounds in their analysis to predict the percentage of dose eliminated in bile 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. (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. (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.

### Materials and Methods

The first step was to reevaluate the analysis in the published article (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 solvation (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 were 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 =  $0.245 \times PSA + 50.289 \times Acid - 0.616 \times Gsolv,aq - 29.395$  ( $r^2 = 0.89$ ); SAS model: biliary excretion =  $0.250 \times PSA + 50.374 \times Acid - 0.603$  times] 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 are compared with 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, whereas 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. (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 (Fig. 1).

$$\text{Biliary excretion} = 0.401 \times \text{PSA} + 49.3 \times \text{Acid} - 27.1$$

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. (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 compared with the published model, and it performed as well as the published model in predicting the biliary excretion of the training and test sets.

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 25 compounds in the published test set for which structures are available in the literature. The PSA values calculated using Pubchem and Molinspiration were compared with those in the publication. The PSA values published in the article did not correlate well with the PSA values from Pubchem and Molinspiration, even though all three use the same algorithm. The published article used Scitegic Pipeline Pilot version 5.1 (Accelrys Inc.) to

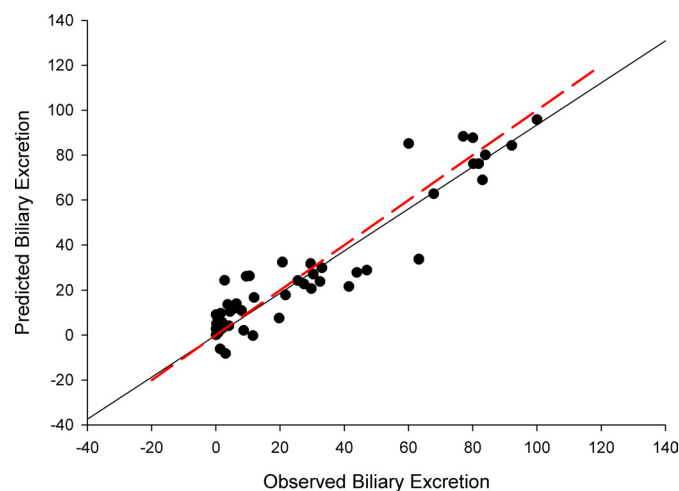


FIG. 1. Observed versus predicted biliary excretion for 50 compounds in the training set using stepwise regression model. The diagonal line is the regression line. The dashed line is the line of origin.

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 Accelrys 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 were used to predict the biliary excretion for the 164 compounds in our rat dataset. The model performed poorly with an  $r^2 = 0.253$  (Fig. 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. (2009), failed to improve the relationship in this case. Similar results were obtained when we tried to apply the stepwise QSPKR model derived for rats (eq. 1) to predict biliary excretion in humans ( $r^2 = 0.013$ ). There were significant underpredictions and overpredictions for the human dataset. For example, moxalactam was overpredicted almost 1000-fold (observed, 0.46%; predicted, 105%), whereas indocyanine green was underpredicted (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, whereas 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; likewise, 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

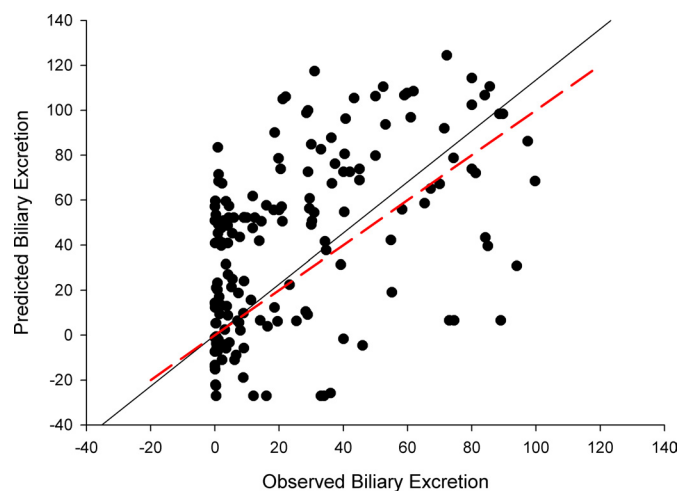


FIG. 2. Observed biliary excretion versus predicted biliary excretion for 164 compounds in the rat dataset. The diagonal line is the regression line. The dashed line is the line of origin.

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. (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 molecular mass, 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 article 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% (buto-prozine, diazepam, felodipine) to 99.7% (+)-(5S,6R,7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl) cyclopenteno[1,2-*b*]pyridine-6-carboxylic; 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 that 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 such as 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 solute carrier (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. (2010) using stepwise regression. The

stepwise model was simpler than the model in the published article 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. (2010) performed poorly for our large and diverse rat dataset that contains values for percentage of parent compound eliminated in the bile after intravenous 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. (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.

#### Acknowledgments

We thank Dr. Lisa Benincosa (Hoffmann LaRoche Inc., Nutley, NJ) for comments and support.

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#### References

- Callaghan R, Ford RC, and Kerr ID (2006) The translocation mechanism of P-glycoprotein. *FEBS Lett* **580**:1056–1063.
- Chen L, Li Y, Zhao Q, Peng H, and Hou T (2011) ADME evaluation in drug discovery. 10. Predictions of P-glycoprotein inhibitors using recursive partitioning and naive Bayesian classification techniques. *Mol Pharm* **8**:889–900.
- Chen Y, Cameron K, Guzman-Perez A, Perry D, Li D, and Gao H (2010) Structure-pharmacokinetic relationship of in vivo rat biliary excretion. *Biopharm Drug Dispos* **31**:82–90.
- Ertl P, Rohde B, and Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J Med Chem* **43**:3714–3717.
- Gandhi YA and Morris ME (2009) Structure-activity relationships and quantitative structure-activity relationships for breast cancer resistance protein (ABCG2). *AAPS J* **11**:541–552.
- Ghibellini G, Leslie EM, and Brouwer KL (2006) Methods to evaluate biliary excretion of drugs in humans: an updated review. *Mol Pharm* **3**:198–211.
- Loo TW, Bartlett MC, and Clarke DM (2003) Substrate-induced conformational changes in the transmembrane segments of human P-glycoprotein. Direct evidence for the substrate-induced fit mechanism for drug binding. *J Biol Chem* **278**:13603–13606.
- Luo G, Johnson S, Hsueh MM, Zheng J, Cai H, Xin B, Chong S, He K, and Harper TW (2010) In silico prediction of biliary excretion of drugs in rats based on physicochemical properties. *Drug Metab Dispos* **38**:422–430.
- Xing L, Hu Y, and Lai Y (2009) Advancement of structure-activity relationship of multidrug resistance-associated protein 2 interactions. *AAPS J* **11**:406–413.
- Yang X, Gandhi YA, Duignan DB, and Morris ME (2009) Prediction of biliary excretion in rats and humans using molecular weight and quantitative structure-pharmacokinetic relationships. *AAPS J* **11**:511–525.
- Zelcer N, Huisman MT, Reid G, Wielinga P, Breedveld P, Kuil A, Knipscheer P, Schellens JH, Schinkel AH, and Borst P (2003) Evidence for two interacting ligand binding sites in human multidrug resistance protein 2 (ATP binding cassette C2). *J Biol Chem* **278**:23538–23544.

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