ANDROMEDA by Prosilico and log D outperform human hepatocytes for the prediction of intrinsic hepatic metabolic clearance of carboxylic acids

Urban Fagerholm

Prosilico AB, Lännavägen 7, SE-141 45 Huddinge, Sweden.

urban.fagerholm@prosilico.com; +46-70-1731302

ABSTRACT

Introduction Extrahepatic metabolism/conjugation is characteristic for carboxylic acid drugs. Other challenges for this group of compounds include deconjugation of their metabolites and low and varying unbound fraction in plasma (f_u). Thus, it is comparably difficult to estimate their *in vivo* intrinsic hepatic metabolic clearance (CL_{int}) and hepatic CL (CL_H) and to predict their *in vivo* CL_{int}, CL_H and CL. One objective was to investigate the laboratory variability of f_u and CL_{int} for carboxylic acids. Another objective was to compare human hepatocytes, measured log D and the software ANDROMEDA with regards to prediction of human *in vivo* CL_{int} of carboxylic acids.

Materials and Methods Measured unbound hepatocyte CL_{int}, non-renal CL (surrogate for CL_H), non-renal CL_{int} (surrogate for hepatic metabolic CL_{int}), log D and f_u data were taken from studies in the literature. ANDROMEDA (by Prosilico; version 1.0) was used for *in silico* predictions of CL_{int} for carboxylic acids not used in the training set of the CL_{int}-model.

Results and Discussion Mean and maximum differences between highest and lowest reported *in vivo* CL_{int} predicted from hepatocyte CL_{int} were 30- and 74-fold, respectively. Corresponding estimates for *in vitro* f_u were 16- and 50-fold, respectively. The data set with the apparently highest number of carboxylic acids contains 39 carboxylic acids with *in vitro* CL_{int} and log D (both measured at the same laboratory), *in vivo* CL_{int} and *in vitro* f_u. 18 carboxylic acids were excluded as their *in vitro* CL_{int} was below the limit of quantification. The correlation coefficient (R^2) for log hepatocyte predicted *in vivo* CL_{int} *vs* log *in vivo* CL_{int} was 0.34. The corresponding R^2 for log D *vs* log *in vivo* CL_{int} was 0.40 (0.47 for 64 carboxylic acids). The Q^2 (forward-looking R^2) between *in silico* (ANDROMEDA) predicted and measured log *in vivo* CL_{int} for 12 carboxylic acids was 0.86. The corresponding R^2 for hepatocytes and log D were 0.67 and 0.66, respectively. ANDROMEDA produced a lower maximum prediction error compared to hepatocytes and also predicted the *in vivo* CL_{int} for all carboxylic acids out of reach for the hepatocyte assay.

Conclusion Large interlaboratory variability was demonstrated for f_u and hepatocyte assays. Log D, and especially ANDROMEDA, outperformed the hepatocyte assay for the prediction of CL_{int} of carboxylic acids *in vivo* in man.

INTRODUCTION

Extrahepatic metabolism/conjugation is characteristic for carboxylic acid drugs. In addition to conjugation in the liver, the kidneys and intestines are important organs for conjugation of carboxylic drugs (Soars et al. 2002). This makes predictions of their *in vivo* intrinsic hepatic metabolic clearance (CL_{int}), hepatic CL (CL_H) and CL more difficult. Drug-conjugates are often excreted in bile and might undergo deconjugation (back to drug) and reabsorption in the intestines (such as for the carboxylic acid telmisartan), which adds complexity. Many carboxylic acids have low and varying unbound fraction in plasma (f_u), and this further increases the complexity and uncertainty.

One objective of the study was to investigate the laboratory variability of f_u and CL_{int} for carboxylic acids. Another objective was to compare human hepatocytes, measured log D and the software ANDROMEDA by Prosilico with regards to prediction of *in vivo* CL_{int} of carboxylic acids.

MATERIALS & METHODS

The literature was searched for studies with large(st) amount of data for measured unbound hepatocyte CL_{int}, non-renal CL (surrogate for CL_H), non-renal CL_{int} (surrogate for hepatic metabolic CL_{int}), log D and f_u. Useful data were found in the following references: Bowman and Benet 2019 (predicted *in vivo* CL_{int} with human hepatocytes; data from several studies), Keefer et al. 2023 (predicted *in vivo* CL_{int} with human hepatocytes in their laboratory, measured log D in their laboratory, and *in vivo* CL_{int}), Fagerholm et al. 2021 (f_u-data from many sources) and Sohlenius-Sternbeck et al. 2010 (predicted *in vivo* CL_{int} with human hepatocytes in their laboratory).

ANDROMEDA by Prosilico (version 1.0) was used for *in silico* predictions of CL_{int} for carboxylic acids not used in the training set of the CL_{int}-model.

RESULTS & DISCUSSION

Laboratory variability

Seven carboxylic acids with measured f_u and predicted *in vivo* CL_{int} data from various sources were found - diclofenac, fenoprofen, gemfibrozil, ibuprofen, ketoprofen, montelukast and naproxen. Mean and maximum differences between highest and lowest reported *in vivo* CL_{int} for these predicted from hepatocyte CL_{int} were 30- and 74-fold, respectively. (Figures 1 and 2). Corresponding estimates for *in vitro* f_u were 16- and 50-fold, respectively (Figure 2). Particularly high $CL_{int} \bullet f_u$-differences were found for diclofenac (417-fold), fenoprofen (225-fold), gemfibrozil (910-fold), montelukast (167-fold) and naproxen (138-fold). Thus, quite large interlaboratory variability and low reproducibility was found for these selected carboxylic acids. This is believed to have a significant impact on human pharmacokinetic, exposure and dose predictions. The findings show general limitations with human hepatocytes and plasma protein measurements when predicting the pharmacokinetics of carboxylic acids.

Unfavorable human pharmacokinetics for one carboxylic acid predicted in one laboratory may be contradicted by results found by another laboratory (and vice versa), such as for diclofenac (CL_H predicted to be low to high; 144-fold difference between lowest and highest predicted CL_H), montelukast (CL_H predicted to be low to high; 1395-fold difference between lowest and highest predicted CL_H), gemfibrozil (CL_H predicted to be very low to low; 702-fold difference between lowest and highest predicted CL_H), fenoprofen (CL_H predicted to be very low to low; 205-fold difference between lowest and highest predicted CL_H) and naproxen (CL_H predicted to be very low to low; 135-fold difference between lowest and highest predicted CL_H).

The selection of data will have a major influence on established correlations between predicted and observed CL_H. A previous study showed that the correlation coefficient (R^2) for predicted and observed log CL_H may vary between near 0 and ca 0.8-0.9 depending on the choice of *in vitro* CL_{int} and f_u data (Fagerholm et al. 2022). Diclofenac, fenoprofen, gemfibrozil, ibuprofen, ketoprofen, montelukast and naproxen were included in a prediction study with human hepatocytes by Sohlenius-Sternbeck et al. 2010 (a R^2 of 0.72 for predicted *vs* observed log *in vivo* CL_{int} was reached). Fenoprofen and ketoprofen were also included in a human hepatocyte study by Yamagata et al. 2017 (a R^2 of 0.865 was reached). Different f_u-values for fenoprofen and ketoprofen were selected for these studies and used f_u-values for each of the carboxylic acids were different (up to 10-fold) than the calculated averages for these. In a study by Keefer et al. 2023, the *in vivo* CL_{int} and CL_H of montelukast were overpredicted by ca 400- and 30-fold (predicted very high CL_H and observed very low CL_H), respectively.

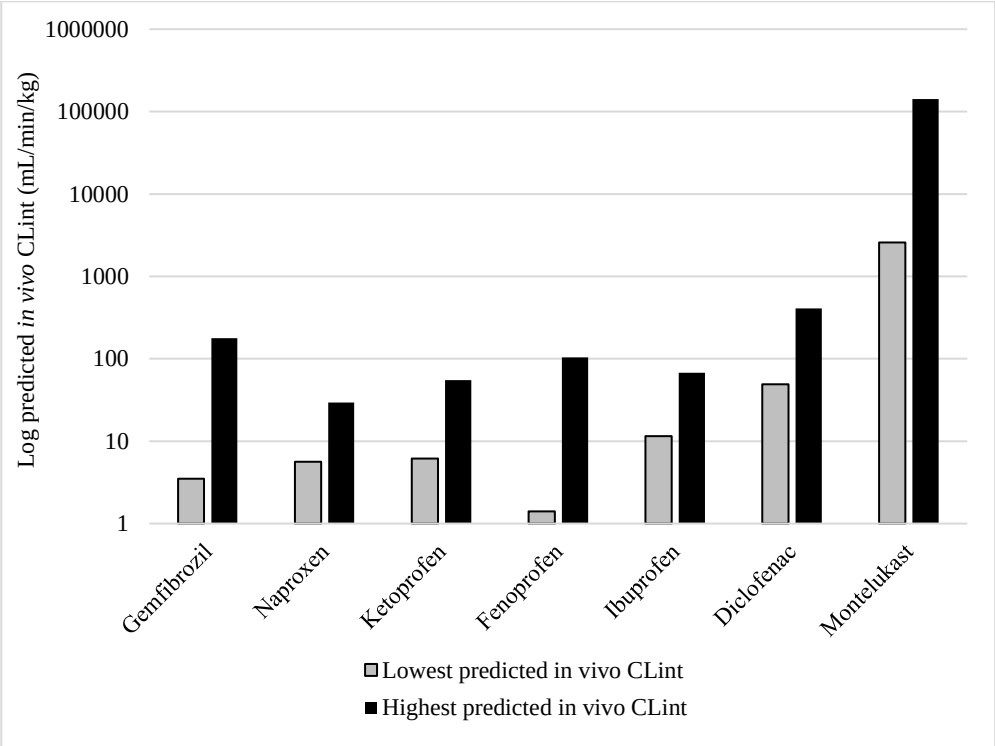

Figure 1. Lowest and highest reported predicted *in vivo* log CL_{int} for 7 carboxylic acids.

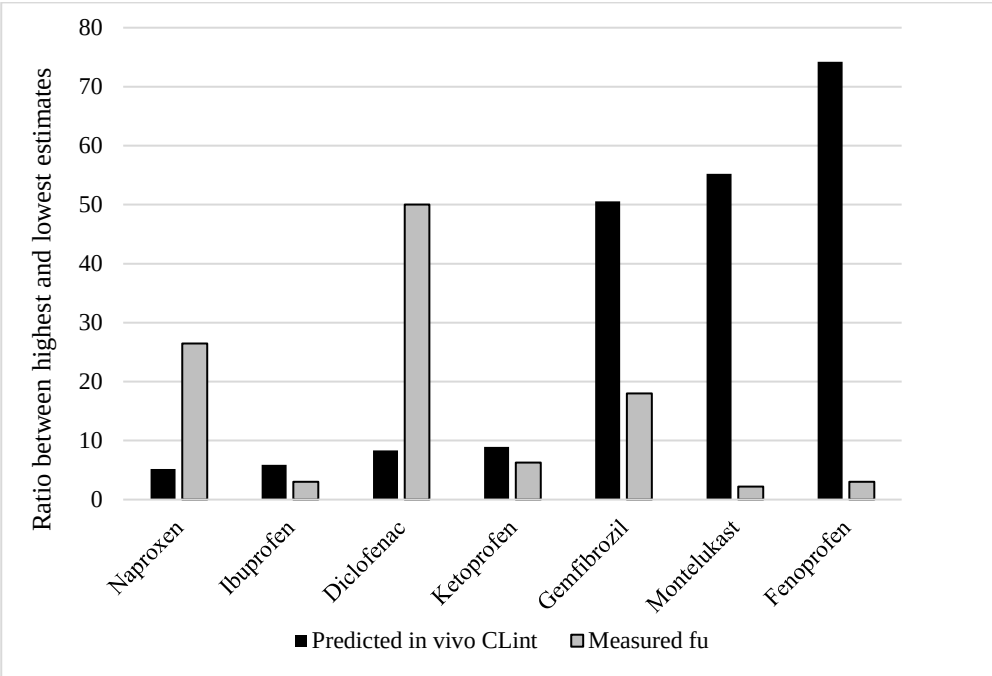

Figure 2. Ratios between lowest and highest reported predicted *in vivo* CL_{int} and *in vitro* f_u for 7 carboxylic acids.

Hepatocytes *vs* log D for prediction of *in vivo* CL_{int}

The data set with the apparently highest number of carboxylic acids (Keefer et al. 2023) contains 39 carboxylic acids with *in vitro* hepatocyte CL_{int} and log D (both measured at the same laboratory), *in vivo* CL_{int} and *in vitro* f_u. This data set has 748 measured *in vitro* CL_{int}-values, out of which 160 (21

%) are below the limit of quantification. 18 carboxylic acids were excluded as their *in vitro* CL_{int} was below the limit of quantification.

The R^2 for log hepatocyte predicted *in vivo* CL_{int} *vs* log *in vivo* CL_{int} was 0.34 (n=39; Figure 3). The corresponding R^2 for log D *vs* log *in vivo* CL_{int} was 0.40 (same set of 39 carboxylic acids; Figure 4). The R^2 between log D *vs* log *in vivo* CL_{int} for 64 carboxylic acids was 0.47.

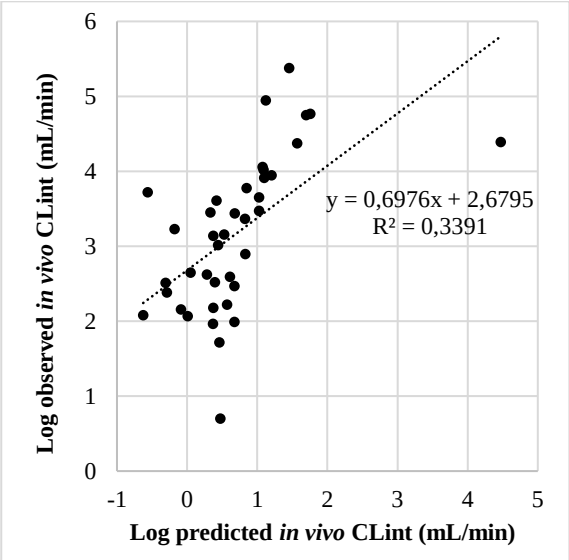

Figure 3. Relationship between log hepatocyte predicted *in vivo* CL_{int} and log observed *in vivo* CL_{int} for 39 carboxylic acids.

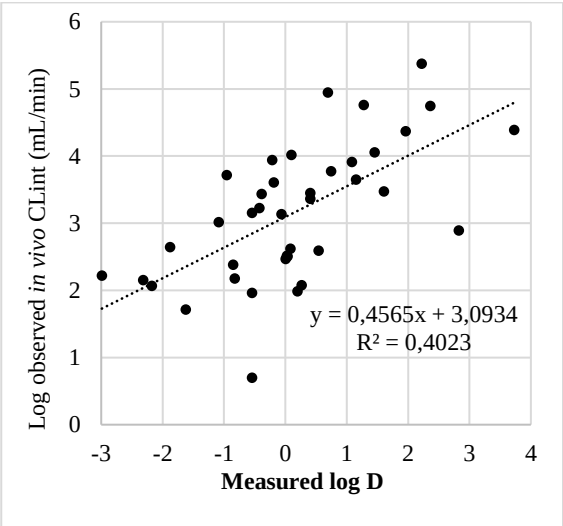

Figure 4. Relationship between measured log D and log observed *in vivo* CL_{int} for 39 carboxylic acids (same as in Figure 3).

With another smaller set of carboxylic acids (n=10), the R^2 for log hepatocyte predicted *in vivo* CL_{int} *vs* log *in vivo* CL_{int} was 0.28 (Sohlenius-Sternbeck et al. 2010). Consistent with findings above, the R^2 for log D *vs* log *in vivo* CL_{int} was higher (0.33).

Overall and for carboxylic acids, measured log D from one laboratory had a higher correlation *vs* log *in vivo* CL_{int} and wider range than human hepatocyte data from one laboratory.

It should be noted that the variability and selection of measured values could have influenced the results. The role of hepatic (vs extrahepatic) metabolism is not clear, and therefore, the degree of overestimation of hepatic CL_{int} for each carboxylic acid is unknown.

ANDROMEDA vs hepatocytes and log D for prediction of *in vivo* CL_{int}

The Q^2 (forward-looking R^2) between *in silico* (ANDROMEDA) predicted and measured log *in vivo* CL_{int} for 12 carboxylic acids not used in the training set of the CL_{int}-model was 0.86 (Figure 5). The corresponding R^2 (same set of carboxylic acids) for hepatocytes and log D were 0.67 and 0.66, respectively.

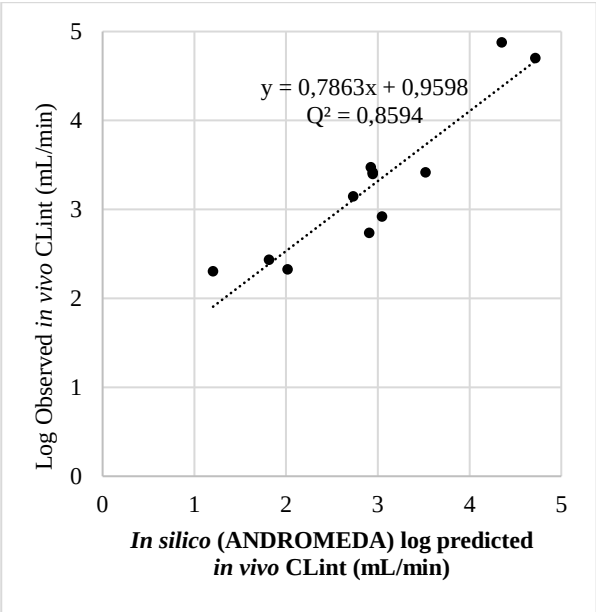

Figure 5. Relationship between *in silico* log predicted (ANDROMEDA by Prosilico version, 1.0) and log observed *in vivo* CL_{int} for 12 carboxylic acids.

ANDROMEDA produced a lower maximum prediction error compared to hepatocytes (12.5 *vs* 13.5-fold) and also predicted the *in vivo* CL_{int} for all carboxylic acids out of reach for the hepatocyte assay. Thus, in this comparative study it clearly outperforms human hepatocytes and log D. This is in line with a previous head-to-head study where ANDROMEDA reached a Q^2 of 0.48 and human hepatocytes a R^2 of 0.38 (Fagerholm et al. 2022).

Among advantages with ANDROMEDA is that it (in opposite to *in vitro* measurements) gives that same results upon repeated predictions. It also predicts the *in vivo* CL_{int} and CL_H of diclofenac, fenoprofen, gemfibrozil, montelukast and naproxen (which demonstrate very large interlaboratory variability) well.

The CL_{int} model in ANDROMEDA version 2 has 22 % higher relative Q^2 and 8 % lower relative RMSE compared to version 1, which indicates that even better performance is expected for carboxylic acids with an updated version.

CONCLUSION

Large interlaboratory variability was demonstrated for f_u and hepatocyte assays. Log D, and especially ANDROMEDA, outperformed the hepatocyte assay for the prediction of CL_{int} of carboxylic acids *in vivo* in man.

REFERENCES

Bowman CM, Benet LZ. 2019. Interlaboratory variability in human hepatocyte intrinsic clearance values and trends with physicochemical properties. Pharm Res. 36:113.

Fagerholm U, Spjuth O, Hellberg S. 2021. Comparison between lab variability and *in silico* prediction errors for the unbound fraction of drugs in human plasma. Xenobiot. 51:1095-1100.

Fagerholm U, Spjuth O, Hellberg S. 2022. The impact of reference data selection for the prediction accuracy of intrinsic hepatic metabolic clearance. J Pharm Sci. 111:2645-2649.

Fagerholm U, Hellberg S, Alvarsson J. Spjuth O. 2023a. *In silico* prediction of human clinical pharmacokinetics with ANDROMEDA by Prosilico: Predictions for an established benchmarking data set, a modern small drug data set, and a comparison with laboratory methods. Altern Lab Anim. 51;39-54.

Fagerholm U, Hellberg S, Alvarsson J, Spjuth O. 2023b. ANDROMEDA by Prosilico software successfully predicts human clinical pharmacokinetics of 300 drugs out of reach for *in vitro* methods. bioRxiv, Nov. 2023.

Keefer CE, Chang G, Di L, Tess DA, Osgood SM, Kapinos B, Racich J, Carlo AA, Balesano A. 2023. The comparison of machine learning and mechanistic *in vitro–in vivo* extrapolation models for the prediction of human intrinsic clearance. Mol Pharmaceut. 20:5616-5630.

Soars MG, Burchell B, Riley RJ. 2002. *In vitro* analysis of human drug glucuronidation and prediction of *in vivo* metabolic clearance. J Pharmacol Expl Ther. 301:382-390.

Sohlenius-Sternbeck A-K, Afzelius L, Prusis P, Neelissen J, Hoogstraate J, Johansson J, Floby E, Bengtsson A, Gissberg O, Sternbeck J, Petersson C. 2010. Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. Xenobiot. 40:637-649.

Yamagata T, Zanelli U, Gallemann D, Perrin D, Dolgos H, Petersson C. 2017. Comparison of methods for the prediction of human clearance from hepatocyte intrinsic clearance for a set of reference compounds and an external evaluation set. Xenobiot. 47:741-751.