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

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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 13- and 51-fold mean and maximum ratios were observed for highest and lowest reported hepatocyte predicted *in vivo* CL_{int}, respectively. Corresponding estimates for *in vitro* f_u were 18- 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

Six carboxylic acids with measured f_u and predicted *in vivo* CL_{int} data from various sources were found - Diclofenac, fenoprofen, gemfibrozil, ibuprofen, ketoprofen and naproxen. For predicted *in vivo* CL_{int}, mean and maximum ratios for highest and lowest reported values were 13- and 51-fold, respectively (Figures 1 and 2). Corresponding estimates for *in vitro* f_u were 18- and 50-fold, respectively (Figure 2). Particularly high $CL_{int} \bullet f_u$-ratios were found for diclofenac (417-fold), gemfibrozil (910-fold) and naproxen (138-fold). Thus, a quite large interlaboratory variability was found for these selected carboxylic acids. This is believed to have a significant impact on human pharmacokinetic, exposure and dose predictions.

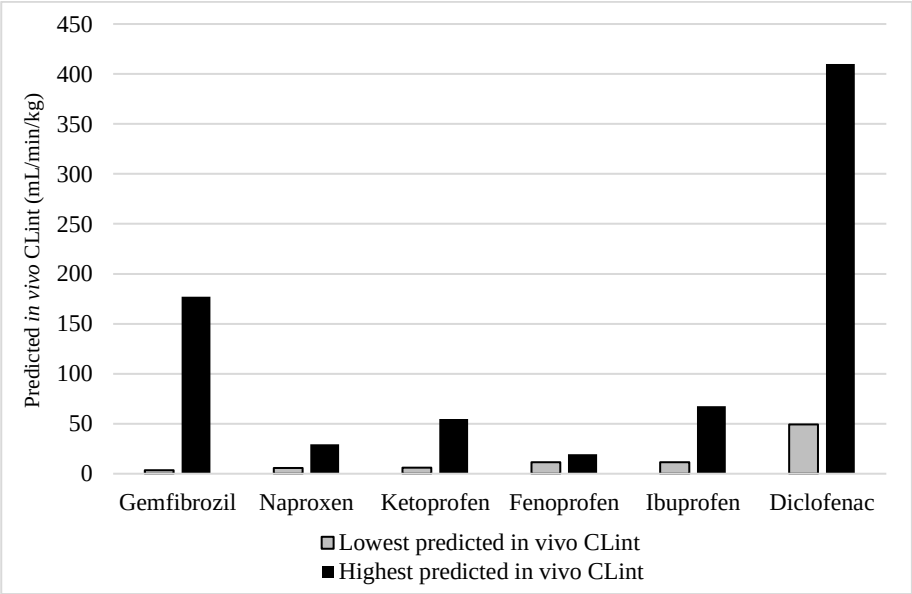

Figure 1. Lowest and highest reported predicted *in vivo* CL_{int} for 6 carboxylic acids.

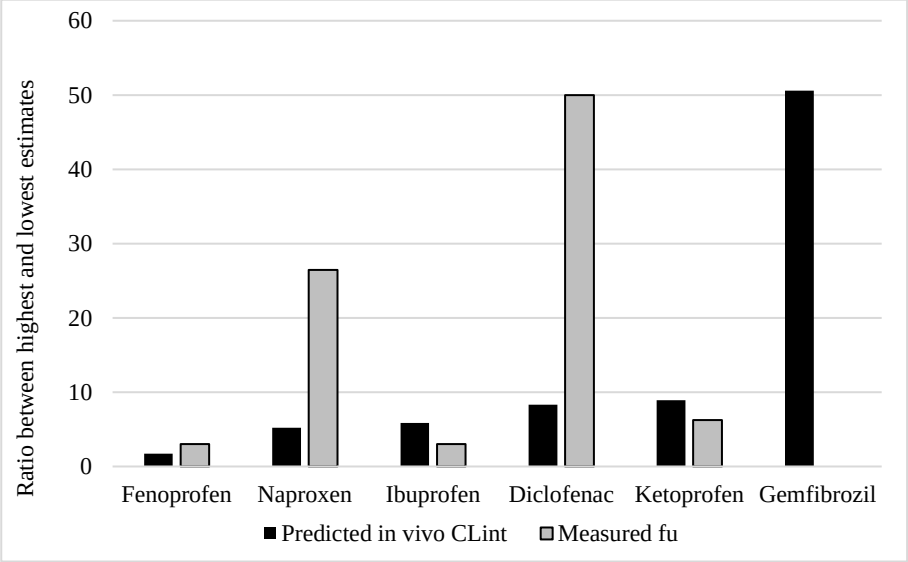

Figure 2. Ratios between lowest and highest reported predicted *in vivo* CL_{int} and *in vitro* f_u or 6 carboxylic acids.

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 correlation coefficient (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.

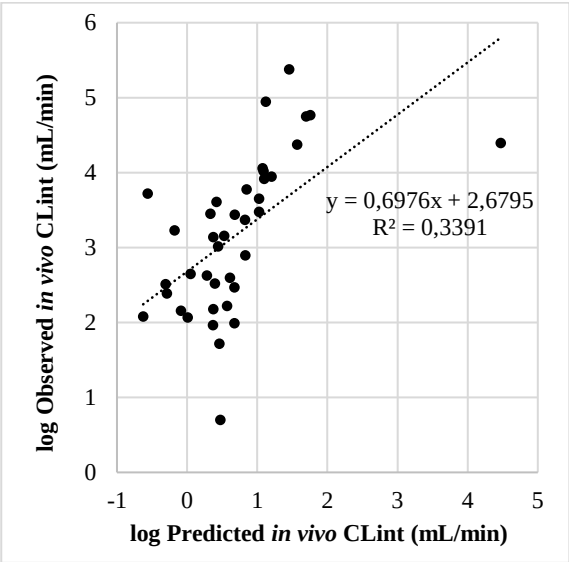

Figure 3. Relationship between log hepatocyte predicted *in vivo* CL_{int} and log observed *in vivo* CL_{int} for 39 carboxylic acids.

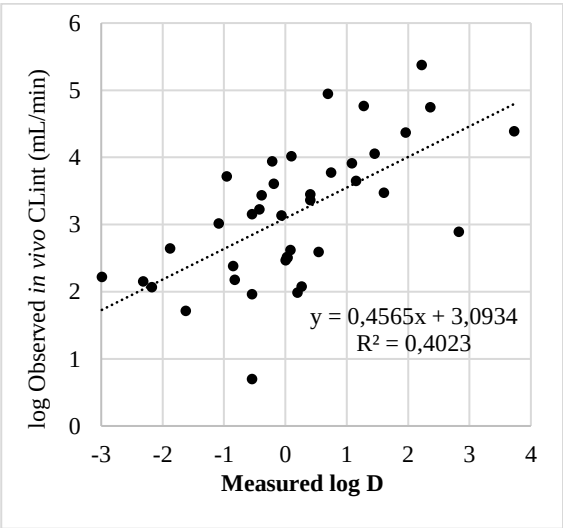

Figure 4. Relationship between measured log D and log observed *in vivo* CL_{int} for 39 carboxylic acids (same as in Figure 3).

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.

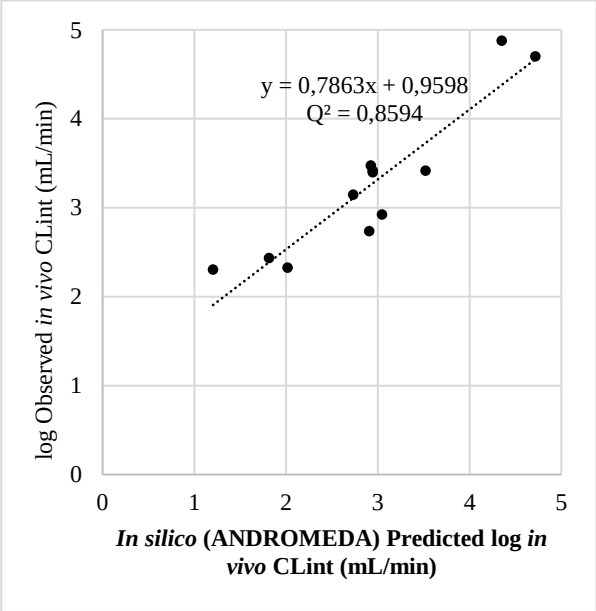

Figure 5. Relationship between *in silico* predicted (ANDROMEDA by Prosilico version, 1.0) and log observed *in vivo* CL_{int} for 12 carboxylic acids.

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 predicts the *in vivo* CL_{int} and CL_H of diclofenac, gemfibrozil 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.

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