Evaluation of the reliability and applicability of human unbound brain-to-plasma concentration ratios

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ABSTRACT

Background Blood-brain barrier permeability (BBB P_e) and unbound brain-to-plasma concentration ratio ($K_{p,uu,brain}$) are relevant parameters describing the brain uptake potential of compounds. BBB efflux by transporter proteins, mainly MDR-1 and BCRP, is an essential factor determining $K_{p,uu,brain}$. $K_{p,uu,brain}$-values are commonly estimated *in vivo* in rats and monkeys and predicted using *in silico* methodology. Such estimates can be used to predict corresponding human clinical values.

Objective The objective of the study was to evaluate the reliability and applicability of human clinical $K_{p,uu,brain}$-data for understanding and predictions of brain uptake in man.

Methodology $K_{p,uu,brain}$ in rats, monkeys and humans, measured and *in silico* predicted MDR-1 and BCRP substrate specificities and *in silico* predicted passive P_e were used for the analysis. *In silico* predictions were done using the ANDROMEDA by Prosilico ADME/PK-prediction software.

Results and Discussion Rat and monkey $K_{p,uu,brain}$-values were highly correlated (R^2=0.74; n=17). Based on this finding a correlation between rat and human $K_{p,uu,brain}$ was expected. However, no correlation between rat and human $K_{p,uu,brain}$ was found (R^2=0.01; n=13). There was no (as also anticipated) correlation between passive P_e and human $K_{p,uu,brain}$ (R^2=0.04; n=16) and compounds with measured or predicted efflux did not have lower $K_{p,uu,brain}$ than compounds without efflux. The compound with highest $K_{p,uu,brain}$ in man (2.8) is effluxed and predicted to have high passive P_e and has no apparent efflux at the rat BBB. The MDR-1 substrate with highest $K_{p,uu,brain}$ in rat (2.4) has very low $K_{p,uu,brain}$ in man (0.15) is predicted to have high passive P_e.

Conclusion Results indicate that available human $K_{p,uu,brain}$-data are too uncertain to be applicable for validation of predictions and understanding of clinical brain uptake of drugs and drug candidates.

INTRODUCTION

Blood-brain barrier permeability (BBB P_e) and unbound brain-to-plasma concentration ratio ($K_{p,uu,brain}$) are relevant parameters describing the brain uptake potential of compounds (Summerfield et al. 2008; Syvänen et al. 2009; Chen et al. 2011; Sato et al. 2021; Loryan et al. 2022). BBB efflux by transporter proteins, mainly MDR-1 and BCRP, is an essential factor determining $K_{p,uu,brain}$ (Chen et al. 2011; Sato et al. 2021; Loryan et al. 2022).

$K_{p,uu,brain}$-values are commonly estimated *in vivo* in rats and monkeys and predicted using *in silico* methodology (Summerfield et al. 2008; Syvänen et al. 2009; Chen et al. 2011; Sato et al. 2021; Loryan et al. 2022). Provided that there are sufficiently strong correlations between preclinical estimates/models and human clinical values, human $K_{p,uu,brain}$-values and brain uptake potential can be predicted using such estimates.

The objective of the study was to evaluate the reliability and applicability of human clinical $K_{p,uu,brain}$-data for understanding and predictions of brain uptake in man.

METHODOLOGY

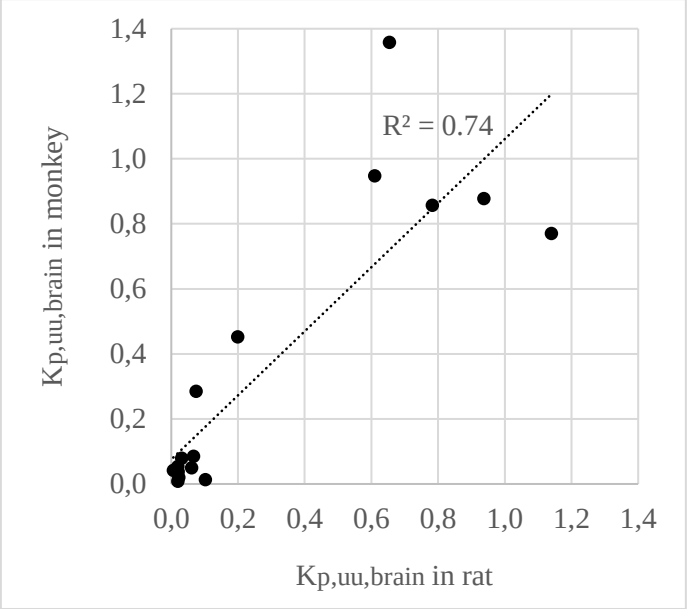
$K_{p,uu,brain}$ in rats, monkeys and humans, measured and *in silico* predicted MDR-1 and BCRP substrate specificities and *in silico* predicted passive P_e were used for the analysis. Sato et al. 2021 was the main source of rat, monkey and human $K_{p,uu,brain}$-values and MDR-1 and BCRP substrate specificities. Additional rat and human $K_{p,uu,brain}$-values were taken from Summerfield et al. 2008.

In silico predictions of passive P_e and substrate specificity for MDR-1 and BCRP (in cases where measured values were not available) were done using the ANDROMEDA by Prosilico ADME/PK-prediction software (Fagerholm et al. 2022).

RESULTS & DISCUSSION

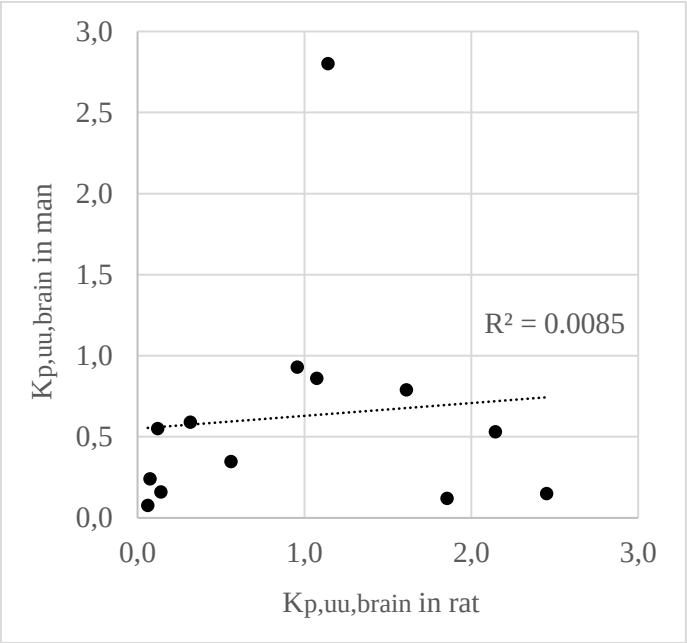
Rat and monkey $K_{p,uu,brain}$-values were highly correlated (R^2=0.74; n=17; Figure 1). Based on this finding, a correlation between rat and human $K_{p,uu,brain}$ was expected.

Figure 1. The correlation between rat and monkey $K_{p,uu,brain}$-values (n=17; Sato et al. 2021).



In Sato et al. 2021 and Summerfield et al. 2008 there are rat and human $K_{p,uu,brain}$-data for 21 compounds. There was, however, no correlation between rat and human $K_{p,uu,brain}$ (R^2=0.01; n=13; Figure 2; using data from Sato et al. 2021, R^2=0.00 using data from Sato et al. 2021 and Summerfield et al. 2008).

Figure 2. The correlation between rat and human $K_{p,uu,brain}$ (n=13; Sato et al. 2021).



A higher dependency of efflux on $K_{p,uu,brain}$ was anticipated at low P_e, and therefore, a positive correlation between human passive P_e and $K_{p,uu,brain}$ was expected. There was, however, no correlation between passive P_e and human $K_{p,uu,brain}$ (R^2=0.04; n=16). Furthermore, compounds with measured or predicted efflux did not have lower $K_{p,uu,brain}$ than compounds without efflux. The compound with highest $K_{p,uu,brain}$ in man (lamotrigine; 2.8) is effluxed and predicted to have high passive P_e and has no apparent efflux at the rat BBB. The compound with the second highest $K_{p,uu,brain}$ in man (flumazenil; 1.7) is effluxed and predicted to have high passive P_e. The MDR-1 substrate with highest $K_{p,uu,brain}$ in rat (olanzapine; 2.4) has very low $K_{p,uu,brain}$ in man (0.15) is predicted to have high passive P_e. Cefotaxime is the compound with the lowest $K_{p,uu,brain}$ in man (0.007) and it was predicted to have the lowest passive P_e (corresponding to less than 1 % oral uptake) and maybe BCRP-efflux (undetermined regarding BCRP-specificity).

At least 14 of the 16 compounds with human $K_{p,uu,brain}$-estimates in Sato et al. 2021 had measured and/predicted MDR-1 and/or BCRP-efflux. 14 of them were also predicted to have high passive P_e.

Results indicate that available human $K_{p,uu,brain}$-data are too uncertain to be applicable for validation of predictions and understanding of clinical brain uptake of drugs and drug candidates. An explanation could be methodological differences. Human data were obtained post-mortem (reduced/no blood flow and transporter activity) and in PET-studies using radioligands (non-equilibrium; radioligand degradation), while animal data were commonly produced in microdialysis (equilibrium) studies. Uncertainties in measurements of unbound fractions in plasma/blood and brain tissue and laboratory variability (on average ca 2-fold differences between highest and lowest reported values in the rat) are also possible contributors.

The high correlation between rat and monkey $K_{p,uu,brain}$ (R^2=0.74), and high degree of species homology for MDR-1; 85 % between rats and man and 93-97 % between monkeys and man; Syvänen et al. 2009) indicates that data obtained these two species are likely to correlate to corresponding true human values to a similar extent. A 15 % difference in MDR-1 homology and relatively high expression of MDR-1 and comparably low expression of BCRP in rats demonstrate that significant true $K_{p,uu,brain}$-differences between these two species may also occur sometimes. Measurements and/or predictions of MDR-1 and BCRP-substrate specificities and ratios are useful for improving the certainty of human $K_{p,uu,brain}$-predictions. The new 3-dimensional brainavailability-matrix (passive BBB P_e-class *vs* brain binding-class + efflux/non-efflux and $K_{p,uu,brain}$) is also applicable for a better overview (Fagerholm et al. 2022).

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