ANDROMEDA by Prosilico Software Successfully Predicts Human Clinical Pharmacokinetics of 70 Drugs Out of Reach for *In Vitro* Methods

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ABSTRACT

Introduction *In vitro* measurements and predictions of human clinical pharmacokinetics (PK) are sometimes hindered and made impossible due to factors such as extensive binding to materials, low methodological sensitivity and large variability.

Methods The objective was to find compounds out of reach for *in vitro* PK-methods and (if possible) predict corresponding human clinical estimates using the ANDROMEDA by Prosilico software. *In vitro* methods selected for the investigation were human microsomes and hepatocytes for measuring and predicting intrinsic hepatic metabolic clearance (CL_{int}), Caco-2 cells for measuring apparent intestinal permeability (P_{app}) for prediction of fraction absorbed (f_a), plasma for measurement and estimation of unbound fraction (f_u), and water and buffers for measuring solubility (S) for prediction of *in vivo* dissolution potential (f_{diss}).

Results and Conclusion 73 non-quantifiable *in vitro* PK-measurements for 70 compounds were found in the literature: 40 for CL_{int}, 8 for P_{app}, 11 for f_u and 14 for S. ANDROMEDA was successful in predicting all corresponding clinical PK-estimates for the selection of compounds with non-quantifiable *in vitro* PK, and predicted estimates were generally in line with observed *in vivo* data and results/problems at *in vitro* laboratories. Thus, ANDROMEDA is applicable for predicting human clinical PK for compounds out of reach for laboratory methods.

INTRODUCTION

Various *in vitro* methods are used to measure and screen pharmacokinetic (PK) properties of drug candidates, including human microsomes and hepatocytes for measuring and predicting intrinsic hepatic metabolic clearance (CL_{int}), Caco-2 cells for measuring apparent intestinal permeability (P_{app}) for prediction of fraction absorbed (f_a), plasma for measurement and estimation of unbound fraction (f_u), and water and buffers for measuring solubility (S) for prediction of *in vivo* dissolution potential (f_{diss}).

In vitro measurements and *in vivo* predictions are commonly hindered and made impossible due to extensive binding to materials, low methodological sensitivity and large variability between laboratories and method set-ups. For example, the *in vitro* PK for about every other compound could not be quantified with human microsomes and hepatocytes (CL_{int}<limit of quantification (LOQ)) and Caco-2 cells (low recovery) in studies by Stringer et al. (2008) and Skolnik et al. (2010), respectively. This has recently also been investigated and shown by Fagerholm et al. (2022a-c).

Lack of preclinical PK data/information could jeopardize drug discovery and development, for example, causing systemic underexposures (and many dosing arms) or unwanted overexposures (side-effects) in first clinical trials with candidate drugs, resulting in selection of compounds with inadequate *in vivo* PK and making optimization of PK-properties difficult/impossible. Thus, it is important to improve and develop new methodologies so that useful PK-data can be produced for compounds that are out of reach for the conventional *in vitro* methods.

The ANDROMEDA software by Prosilico for prediction, simulation and optimization of human clinical PK has been applied and validated in many studies (Fagerholm et al. 2021a,b and 2022a,d,e). It has been shown to outperform (higher accuracy) laboratory methods in 13 comparisons out of 17 (76 %; on par in 12 % of cases) and to have a wider prediction domain (with ability to predict human clinical PK for compounds with good metabolical stability (low CL_{int}), extrahepatic elimination and low P_{app}, S and recovery) (Fagerholm et al. 2022a,e)).

The objective of this investigation was to - via literature search - find compounds out of reach for *in vitro* PK-methods (human microsome and hepatocyte CL_{int}, Caco-2 P_{app}, plasma f_u and aqueous S) and then to (if possible) predict corresponding human clinical estimates using ANDROMEDA.

MATERIALS & METHODS

The literature was searched for compounds with non-quantifiable human microsome and hepatocyte CL_{int} and Caco-2 P_{app}, uncertain f_u (below a certain limit) and reported insolubility in water (including lack of S-data). Useful data were found in the following references: microsome and hepatocvte CL_{int} (Stringer et al. 2008; Sohlenius-Sternbeck et al. 2010; Obach 1999; Riley et al. 2005; McGinnity et al. 2007; own non-published data), Caco-2 P_{app} (Irvine et al. 1999; Fagerholm and Björnsson 2005; Sköld et al. 2006; Alsenz and Haenel 2003), f_u (Fagerholm el al. 2021c; FDA drug labels) and S (Pham-The et al. 2013; Jiménez et al. 2022; Prosilico data bank).

ANDROMEDA software by Prosilico for prediction, simulation and optimization of human clinical PK) was applied to predict the main parameters for the study: *in vivo* CL_{int}, f_a (permeability-based f_a), f_u, and f_{diss} (at a default oral dose of 50 mg).

RESULTS, DISCUSSION & CONCLUSION

In total, 73 non-quantifiable *in vitro* PK-measurements for 70 compounds were found: 40 for CL_{int}, 8 for P_{app}, 11 for f_u and 14 for S (Table 1).

The minimum, median and maximum predicted CL_{int} were 103, 1083 and 35645 mL/min, respectively. 11 (28 %) of the compounds with *in vitro* CL_{int}<LOQ had an *in vivo* CL_{int}<500 mL/min, a limit that is slightly below the median *in vivo* CL_{int} for drugs (738 mL/min) and about 100-fold higher that lowest reported clinical values (Fagerholm 2022b,c; Varma et al. 2010). Most CL_{int}-predictions had an error of <2-fold, which in consistent with previous prediction results with ANDROMEDA (Fagerholm et al. 2022a,e). For these low CL_{int}-compounds it is common with contribution by elimination via excretion (Fagerholm 2022b). Thus, human microsomes and hepatocytes are generally insufficient for measuring and predicting elimination of such compounds. Validated prediction models for renal and biliary excretion are included in ANDROMEDA.

The predicted f_a for the compounds lacking P_{app}-data due to low recovery or sensitivity limitations were 0.41 to 1.00. For the hydrolysis sensitive naproxen-prodrug AZD3582 (naproxinod) an intrinsic f_a (in case of absence of gastrointestinal degradation) of 1.00 was predicted. The passive f_a of actively absorbed ACE-lisinopril was predicted to 0.01. Considering the active component a f_a of 0.25 was predicted. Most f_a-predictions had a maximum error of 1.1-fold.

Predicted f_u-values ranged between 0.0033 and 0.049 and were generally close to reported maximum f_u-estimates (<0.001 to <0.01). In 3 cases predicted estimates were lower than measured upper limits (<X), and in 6 cases they were above them.

For compounds with unmeasurable S, f_{diss} at the 50 mg oral dose level ranged between 0.01 (beta-carotene) and 1.00, with 7 (50 %) of compounds with f_{diss}<0.5. Incomplete f_{diss} was predicted for 12

(86 %) of these 14 poorly soluble compounds. As described in recent papers, there is very weak correlation between the clinical solubility/dissolution parameter f_{diss} and *in vitro* S (Fagerholm 2022b,c).

In conclusion, ANDROMEDA was successful in predicting all the corresponding clinical PK-estimates for the selection of compounds with non-quantifiable *in vitro* PK, and predicted estimates were generally in line with observed *in vivo* data and results/problems at *in vitro* laboratories. Thus, this software is applicable for predicting human clinical PK for compounds out of reach for laboratory methods.

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TABLE

Table 1 Compounds with non-quantifiable *in vitro* PK and corresponding *in silico* predicted estimates (n=73).

	CL_{int} (mL/min)		**f_u**		**f_a**		**S** *(in vitro) vs* **f_{diss}** *(in silico)*	
	In vitro[1]	*In silico*	*In vitro*	*In silico*	*In vitro*[2]	*In silico*	*In vitro*[3]	*In silico*
Acetaminophen	n.q.	676						
Betaxolol	n.q.	676						
Bosentan	n.q.	4385						
Chlorpheniramine	n.q.	288						
Chlorthalidone	n.q.	200						
Dapsone	n.q.	252						
Dofetilide	n.q.	355						
Flumazenil	n.q.	6026						
Fluvoxamine	n.q.	1365						
Furosemide	n.q.	103						
Galantamine	n.q.	589						
Glipizide	n.q.	679						
Granisetron	n.q.	2089						
Ketoprofen	n.q.	8128						
Ketorolac	n.q.	1259						
Levoprotiline	n.q.	6761						
Lidocaine	n.q.	497						
Lorazepam	n.q.	1023						
Melphalan	n.q.	3388						
Meptazinol	n.q.	1242						
Metoclopramide	n.q.	545						
Nalbuphine	n.q.	843						
Naloxone	n.q.	1208						
Ondansetron	n.q.	2399						
Oxprenolol	n.q.	2203						
Phenacetin	n.q.	1824						
Piperacillin	n.q.	222						
Pravastatin	n.q.	1096						
Prazosin	n.q.	5248						
Prednisolone	n.q.	1069						
Sulfinpyrazone	n.q.	417						
Tacrine	n.q.	1276						
Temazepam	n.q.	1585						
Tenoxicam	n.q.	200						
Theophylline	n.q.	104						
Timolol	n.q.	661						
Tolbutamide	n.q.	372						
Warfarin	n.q.	1542						
Zafirlukast	n.q.	35645						
Zaleplon	n.q.	1426						
Tivozanib			<0,01	0,0490				
Avacopan			<0,001	0,0099				
DDT			?	0,0067				
Ganaxolone			ca 0.01	0,0084				
Ibrexafungerp			<0,01	0,0167				
Lumefantrine			<0,01	0,0033				
Odevixibat			<0,003	0,0112				
Ponesimod			<0,01	0,0180				
Umbralisib			<0,003	0,0037				
Zafirlukast			<0,01	0,0064				
AZD3582[4]			?	0,0073				
Acyclovir					n.q.	0,41		
AZD3582[4]					n.q.	1,00		
Fenofibrate					n.q.	0,98		
Lisinopril					n.q.	0,25[5]		
Meclizine					n.q.	1,00		
Ranitidine					n.q.	0,64		

Sumatriptan					n.q.	0,74		
Terbutaline					n.q.	0,57		
ACBI1[6]							<0,72	0,98
Artemether							insol.	0,41
ARV-825[6]							<0,32	0,45
Azathioprine							insol.	1,00
Beta-carotene							insol.	0,01
Daridorexant							low	0,84
Lumefantrine							insol.	0,43
Mcl1 degrader-1[6]							<0,87	0,34
MD-224[6]							<0,23	0,39
Paricalcitol							insol.	0,51
Pranoprofen							insol.	1,00
Pyrantel							insol.	0,99
Rebamipide							low	0,70
Vitamin D2							insol.	0,32

[1] Non-quantifiable (including <LOQ) with human microsomes or hepatocytes.
[2] Non-quantifiable P_{app} with Caco-2.
[3] Solubility (µM).
[4] Naproxinod (naproxen prodrug).
[5] Including prediction of active uptake (passive f_a=0,014).
[6] PROTAC.