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Physiologically-based pharmacokinetic (PBPK) models for prediction of saquinavir effect on midazolam pharmacokinetics

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Abstract:

Purpose: To optimize a PBPK model to describe saquinavir absorption and pharmacokinetics (PK) and with it, predict the effect of saquinavir on midazolam PK.

Methods: Absorption and PK of both drugs were simulated using GastroPlus™ 7.0 (Simulations Plus, Inc., Lancaster, CA). The program's Advanced Compartmental Absorption and Transit (ACAT™) model described the intestinal absorption and gut first-pass extraction (FPE), coupled with its PBPKPlus™ module for simulation of the PK distribution, liver FPE and systemic clearance. Human physiologies were generated by the program's internal Population Estimates for Age-Related (PEAR) Physiology™ module. Tissue/plasma partition coefficients were calculated using our modified Rodgers algorithm [1] based on tissue composition and *in vitro* and *in silico* (ADMET Predictor™ version 5.5, Simulations Plus, Inc., Lancaster, CA) physicochemical properties. Metabolic clearances of both drugs in gut and liver were based on *in vitro* enzyme kinetic constants for 3A4 from literature [2,3]. The PBPK model utilized built-in values for the expression levels of 3A4 in each gut enterocyte compartment as well as the average expression of 3A4 in liver. The effect of intestinal P-gp on saquinavir absorption was incorporated in the model with K_m and V_{max} values fitted to *in vivo* data. The PBPK models correctly described plasma concentration-time profiles of midazolam and saquinavir for various doses after *i.v.* and *p.o.* administration. The validated PK models were then used in dynamic simulations in the GastroPlus 7.0 DDI Module to predict the effect of CYP 3A4 deactivation by saquinavir on midazolam PK.

Results : Dynamic simulations correctly predicted the strong effect of saquinavir on midazolam PK. The model was able to distinguish between the contribution of gut and liver to the overall DDI as assessed by predicting the effect of saquinavir on PK of midazolam administered as either *i.v.* or *p.o.* dose.

References:

1. Lukacova V, Poster presentation, *AAPS* 2008,Altanta,GA
2. Paine MF, J Pharmacol Exp Ther 1997, 283: 1552-1562
3. Cazali N, Br J Clin Pharmacol 2003, 56: 526-536
4. Kupferschmidt HHT, Clin Pharmacol Ther 1995, 58: 20-28
5. Bornemann LD, Eur J Clin Pharmacol 1985, 29: 91-95
6. Kupferschmidt HHT, Br J Clin Pharmacol 1998, 45: 355-359
7. Mao J, Drug Metab Dispos 2011, 39: 591-602
8. Palkama VJ, Clin Pharmacol Ther 1999, 66: 33-39
9. Austin RP, Drug Metab Dispos 2002, 30: 1497-1503

Saquinavir Model:

Pharmacokinetics is described by a PBPK model (adult Western male, age=30 years, weight=76 kg).

Metabolic clearance (intestinal and hepatic) is described by CYP3A4 metabolism from *in vitro* Km and Vmax values [3]. Unbound Km was calculated from reported total Km and fu_{mic} estimated using Austin's algorithm [9] based on reported concentration of microsomal protein *in vitro*.

Renal secretion, which represents a minor portion of saquinavir systemic clearance, is estimated as the product of fraction unbound in plasma (0.03) and glomerular filtration rate.

Pgp effect on intestinal absorption is included with Km and Vmax values fitted against Cp-time profiles of saquinavir after *p.o.* administration with and without grapefruit juice (GFJ).

In accordance with literautre, simulation of *p.o.* administration with GFJ included effect of GFJ on intestinal CYP3A4 (62% deactivation) and no effect of GFJ on intestinal Pgp.

All simulations accounted for the time-dependent inactivation of intestinal and liver CYP3A4 by saquinavir using *in vitro* K_I and k_{inact} values [7]. Reported total K_i was converted to unbound K_I using fraction unbound in plasma (the *in vitro* experiment was done with hepatocytes suspended in plasma).

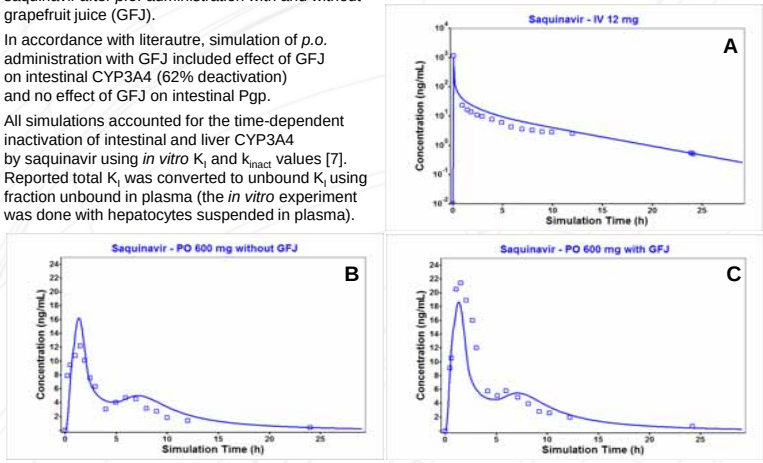


Figure 1. Simulated (lines) and observed (points) Cp-time profiles of saquinavir after A) *i.v.* administration of 12 mg bolus dose; B) *p.o.* administration of 600 mg dose without GFJ; C) *p.o.* administration of 600 mg dose with GFJ [6]. Common model parameters are identical across all simulations.

Midazolam Model:

Pharmacokinetics is described by PBPK model (adult Western male, age=30 years, weight=70 kg).

Metabolic clearance (intestinal and hepatic) is described by CYP3A4 metabolism with *in vitro* Km and Vmax values [2].

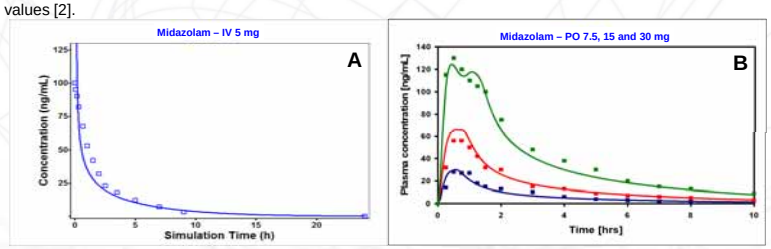


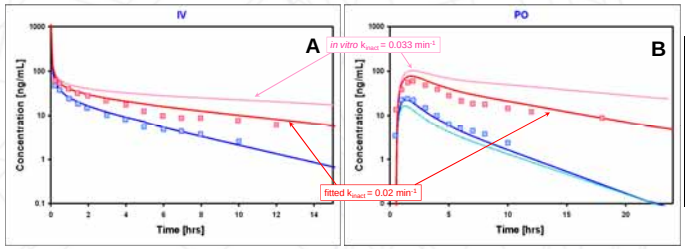
Figure 2. Simulated (lines) and observed (points) Cp-time profiles of midazolam after A) *i.v.* administration of 5 mg bolus dose [4]; B) *p.o.* administration of 7.5 mg (blue), 15 mg (red) and 30 mg (green) dose [5]. Common model parameters are identical across all simulations.

Saquinavir - Midazolam Interaction:

The previously developed absorption/PBPK model for midazolam (Figure 2) was used to simulate the Cp-time profile of midazolam administered *i.v.* and *p.o.* in the absence of saquinavir as reported in a different population [8]. The model predicted the Cp-time profile well for *i.v.* administration (Figure 3A, blue line), but underpredicted exposure after *p.o.* administration (Figure 3B, light-blue line). One possible explanation for the underprediction after *p.o.* administration in the Palkama study could be a lower average expression level of intestinal CYP3A4 in that population. Reducing the gut expression levels by 40% resulted in a good match for the *p.o.* administration of midazolam without saquinavir (Figure 3B, dark-blue line), and had no discernible effect on the *i.v.* simulation.

The model adjusted for the population in the Palkama study (reduced intestinal CYP3A4 expression) was then used to predict the exposure of midazolam when administered after saquinavir. 1200 mg of saquinavir TID was dosed 3-5 days before midazolam administration *i.v.* or *p.o.*

Using *in vitro* K_I and k_{inact} values [7] for saquinavir inactivation of CYP3A4 resulted in significant overprediction of saquinavir effect on midazolam PK. We explored the sensitivity of the DDI prediction to these parameter values. Decreasing k_{inact} by ~39% resulted in correct prediction of DDI. It is notable that the *in vitro* experiment was done with a maximum preincubation period of 20 minutes and *in vitro* k_{inact} of 0.033 min^{-1} translates to $t_{1/2}$ of ~ 21 minutes. The fitted k_{inact} value of 0.02 min^{-1} translates to $t_{1/2}$ of ~35 minutes. While this adjustment resulted in significant improvement in DDI prediction, it did not affect significantly pharmacokinetics of saquinavir itself (data not shown). Whether the design of the *in vitro* experiment (short preincubation time) might result in an overestimated k_{inact} will be further validated by exploring DDI predictions of saquinavir interaction with other CYP3A4 substrates.



$\frac{AUC_{inh}}{AUC_{ctrl}}$	*i.v.*	*p.o.*
Obs	2.5	5.1
Pred	*in vitro* k_{inact} (0.033 min^{-1})	
	3.9	13.7
	fitted k_{inact} (0.02 min^{-1})	
	2.2	6.6

Figure 3. Simulated (lines) and observed (points) Cp-time profiles of midazolam after A) *i.v.* administration of 0.05 mg/kg bolus dose with (pink and red) and without (blue) saquinavir pretreatment; B) *p.o.* administration of 7.5 mg dose with (pink and red) and without (blue) saquinavir pretreatment. The light blue line represents a simulation with typical intestinal CYP3A4 expression levels. Dark blue and red lines represent simulations with baseline intestinal CYP3A4 expression level reduced by 40%. Both figures show simulations with *in vitro* and with fitted k_{inact}. Administration of both drugs in simulation followed the reported study protocol: 1200 mg of *p.o.* saquinavir given TID for 5 days, 7.5 mg of *p.o.* midazolam given on day 3 or 5 of the study. Figures show only midazolam PK on the days of midazolam dosing. Common model parameters are identical across all simulations.

Conclusions:

- Physiological models describing absorption and pharmacokinetics were created for both drugs. Each model accurately described exposure (as assessed by comparison of simulated and observed Cp-time profiles) of the respective drug after several different dosing scenarios (Figures 1 and 2).
- Dynamic simulations utilizing PBPK models with detailed descriptions of both drugs' metabolism were used to explore the contribution of gut and liver metabolism to the overall DDI between saquinavir and midazolam.
- In agreement with the observed data, the model predicted higher effect of oral saquinavir on midazolam administered *p.o.* than on midazolam administered *i.v.* due to inhibition of intestinal first pass metabolism.
- Although the absolute DDI (as assessed by ratio of midazolam's AUC after saquinavir and placebo) is overpredicted for both dosing scenarios using *in vitro* parameters [7], the model correctly predicted a strong potential for drug-drug interaction between these two drugs. One possible explanation for overprediction of DDI may be overestimated maximum inactivation rate (k_{inact}) caused by *in vitro* experiment design.

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