Population Pharmacokinetics of Saquinavir in rats after IV and IP administration. An approach to Saquinavir/r interaction

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1 Background and Objective

Saquinavir (SQV) is a protease inhibitor antiretroviral characterized by a low and variable oral bioavailability [1] and approved for human use in combination with Ritonavir (RTV) as a boosted regimen. Although the interaction between these two drugs has already been reported in previous studies [2, 3], so far the roles of liver and intestine in SQV first pass metabolism have not been clarify.

This study aimed to assess SQV pharmacokinetic disposition profile when IV administered and assess the hepatic first pass metabolism when IP administered. As to approach the influence of RTV in SQV disposition processes

2 Methods

Study design. Male Wistar rats were subjected to jugular vein cannulation. All the assays reported in the present study adhere to the Principles of Animal Care and were approved by the Faculty of Pharmacy Ethics Comission (Valencia, Spain). (N subjects= 46 and samples = 448).

Subjects were randomly allocated to six groups according to the dose and administration route (IV and IP). Blood samples were taken for 0.25-10h after IV administration and 0.25-24h after IP administration.

IV infusion: a 48 mg SQV dose alone (group 1) and a 24 mg SQV in combination with 6 mg RTV dose (group 9), were administered over 30 min.

IV bolus: 24 (group 2) or 12 (group 3) mg of SQV dose were administered.

IP administration: 24 (group 7) and 12 (group 8) mg doses of SQV were administered.

Analytical procedures. SQV and RTV levels in plasma were measured by validated high performance liquid chromatography (HPLC) with UV detection (λ= 235 nm) and a reversed phase column (Nova-Pack1 C18 Waters, 3.9x150 mm).

Pharmacokinetic calculations and statistical analysis.

a) Non-compartmental analysis using Winnonlin 2.1 was performed to assess non linearity and determine bioavailability. Subsequently, differences in mean parameter values among the groups were tested by means of a one-way ANOVA test or the U Mann-Whitney test, for the different doses and routes administered, when applicable.

b) Next, a stepwise population pharmacokinetic approach was performed by means of a non-linear mixed effects model, by use of the first order estimation method (FO), implemented in NONMEM, version V . Different subroutines were used, ADVAN 3, 11 and 9.

3 Results and Discussion

• Non compartmental analysis

❖*SQV IV administration*

The variance analysis did not found significant differences neither in *AUC∞/D*, nor in *Cl*, for the lowest doses. However, differences were found between group 1 and groups 2 and 3, (2.639, 1.596 & 1.450 mgh/L, respectively). The increase in the *AUC∞/D* for the largest dose, could be related to its lower *Cl*. In addition, significant differences were found in *Vdee* (1.703-G.1, 1.253-G.2 & 1.813-G.3 L) and in *Vd* for the smallest doses (1.650-G.2 & 3.050-G.3 L). Significant differences are found in lambda halflife, *t1/2λ*. **These results indicate that distribution and elimination phases are non linear.**

❖*SQV IP administration*

Non linear kinetics in the disposition processes were found, as *AUC∞/D* for the 12 mg dose was found to be twice that one of the 24 mg dose. However, this difference was not found between these two doses when the drug was administered by IV route. In addition, after IP administration *tmax* was six fold increased for the 24 mg dose and *Cmax/D* for the 12 mg dose resulted in four fold that one for the 24 mg dose.

The **IP bioavailability** was 82.47% (24 mg dose as reference). When comparing the *AUC∞/D* after the 12 mg dose administration, the IP route shows an increase in the parameter compared to the IV value, (2.476 and 1.450 mgh/L, respectively). The possible explanation would be a decreased in clearance by the IP route. So that it is difficult to know which fraction of dose escapes from liver, although it seems to be a high one. Hence, absolute IP bioavailability resulted in a 170.75%, which must be falsified by clearance.

❖*SQV/r IV administration*

Variance analysis shows significant differences in all SQV pharmacokinetic parameters, when a 24 mg dose of SQV alone is compared to the combined doses administered by IV route. *AUC∞/D* results in 1.596 when administered alone and 10.984 h/L when coadministered, indicating that **RTV decreases SQV clearance in six times.**

• Compartmental analysis

To describe the disposition processes where the empirical modelling had shown non linear phenomena, at first a two compartment model with Michaelis-Menten elimination process was considered, not being enough to explain IV data. So, taking into account that SQV is bound to plasma proteins in a 97%, a dynamic and saturable plasma protein binding was considered, so it could give a possible explanation of the bias in clearance between groups (*ClG9<ClG1<ClG3<ClG2*). A 24 mg dose could show saturation of the binding to plasma proteins could deliver more drug available to be eliminated, thereby clearance would be increased compared to the 12 mg dose one. Whereas the 48 mg dose could be saturating, in turn, both processes, the plasma protein binding and the elimination process. *Table 1* shows the obtained results for the selected model.

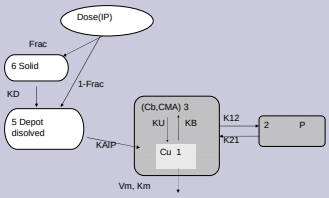

Figure 1. Structure of the selected model. Where *Frac* is the precipitated fraction at IP cavity, *KD* dissolution constant, *KAIP* the first order IP absorption constant rate. Cb and Cu, bound and unbound SQV concentrations. Ku and Kb the unbound and bound constants to plasma proteins, respectively. Vm and Km, the Michaelis-Menten maximum velocity and constant, respectively.

Model: (ADVAN9) Parameters FMO: 334.773		IIV (ω)	
Vm (mg/h)	56.7	ω_{Vm}	1.8E-04
Km (mg/L)	57.7	ω_{Km}	FIXED 0
FKm (G9)	4.88	ω_{K12}	FIXED 0
K12 (h^{-1})	2.77	ω_{K21}	0.391
K21 (h^{-1})	1.12	ω_{Vc}	0.708
Vc (L)	0.244	ω_{Kb}	0.352
Kb (L/mg.h)	2.71E-06	ω_{Ku}	0.940
Ku (h^{-1})	0.137	ω_{QMA}	0.557
QMA (mg)	9720		
KaIP (h^{-1})	1.05		
BIO	1		
FR8	0.951		
KDG7 (h^{-1})	0.0436		
KDG8 (h^{-1})	0.596		
σ exp			
σ1 (CV)	0.134		
σ1 (SD) (mg/L)	0.031		

Table 1. *Population pharmacokinetic parameters, ω y σ obtained with the selected model.*

The interaction between SQV-RTV is shown through Km, which is approximately 5x larger for group 9, indicating that there is a lose of SQV affinity towards the metabolic enzyme when RTV is co-administered, resulting in an inhibition of SQV metabolism, which agrees with the non-compartmental results. The maximum amount of drug which can bind to proteins (QMA) is 9720 mg, this value is overestimated, however the lack of reliability of this parameter does not invalidate the model, indicating that the binding to plasma proteins produces a delay in the distribution process, so that it is performing as a peripheral compartment.

After incorporating the IP data, taking into account the low solubility of the drug, a precipitation of the drug in the IP cavity was considered, being the dissolution limitative factor for absorption. The model considers that the same amount of drug is dissolved when administering each dose, being the precipitated fraction a different one, depending on the administered dose.

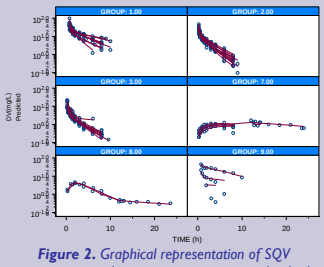

Figure 2. *Graphical representation of SQV experimental concentration vs time and individual predicted concentrations.*

4 Conclusion

Data analysis showed non linear disposition processes for SQV and located drugs interaction at the elimination process, so that RTV inhibits SQV metabolism.

Opposite to the expected result, IP data showed that SQV low bioavailability was not mainly due to its hepatic first pass metabolism, escaping most part of the drug from liver. However, further studies, involving an oral administration are required to properly describe the processes involved in SQV presystemic losses.

REFERENCES

(1)Guiard-Schmid, J.B. et al. Antimicrob Agents Chemother, 47(3), 986-990. 2003.
(2)Shibata, N. et al. J Pharm Sci, 2002. 91(3): p. 680-9
(3) Zeldin, R.K.. et al. J Antimicrob Chemother, 2004. 53 (1): p. 4-9.

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