

# INFLUENCE OF BINDING TO HUMAN PLASMA PROTEINS BY PROTEASE INHIBITORS MAY BE OVERESTIMATED IN CURRENT IQ MODELS

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## ABSTRACT

**Background:** Binding of antiretrovirals to plasma proteins is a factor that has been associated with lack of efficacy of some drug candidates. Most HIV-Protease Inhibitors (PIs) bind to  $\alpha_1$ -acid glycoprotein (AAG). The effect of this binding is quantified by measuring the activity of the inhibitors against wild-type HIV-1 in the presence of AAG. Because the kinetics of the binding of PIs to AAG show a saturable interaction, the influence of AAG may be overestimated as it is determined at concentrations (wild type EC<sub>50</sub>) where the inhibitor free active fraction is low. Therefore we measured the influence of AAG on the anti-HIV potency of PIs at higher, physiologically achieved concentrations by using virus strains with different levels of resistance to each of them.

**Methods:** The influence of AAG, Human Serum Albumin (HSA) or Human Serum (HS) on the activity of PIs has been measured in a cell-based antiviral assay. Virus strains resistant to PIs have been obtained by the recombinant virus technology used in the Antivirogram assay.

**Results:** The anti-HIV activity of several PIs and the drug candidate TMC114 has been assessed in the presence of AAG (1mg/mL), HSA (45mg/mL) or HS (50%). With the exception of indinavir, which remains unaffected, all tested PIs show a decrease in potency against wild-type HIV-1 in the presence of AAG and HS, but not of HSA. The decrease ranges from 5- to 75-fold and is proportional to the AAG concentration. Virus strains with various levels of resistance against each of the PIs have been used in similar experiments with AAG. The results show that the decrease in potency observed in the presence of AAG for the tested PIs is inversely proportional to the EC<sub>50</sub> in the absence of the protein. At micromolar concentrations (1 - 5 $\mu$ M), saquinavir, ritonavir, nelfinavir, amprenavir and TMC114 show only a less than 5-fold decrease in potency in the presence of 1mg/mL AAG.

**Conclusions:** Binding of PIs to human plasma protein, and more precisely to AAG, is a factor often taken into account when determining the target trough levels for new drug candidates. We here show, however, that the influence of AAG decreases with increasing concentrations of drug, consistent with micromolar equilibrium dissociation constants for PIs binding to AAG. Hence at physiologically achieved, low micromolar plasma drug concentrations, binding to plasma proteins is a far less relevant factor to include in IQ calculations for the PIs tested than presumed before. Combination of this observation with the unique antiviral profile of TMC114 against multi-PI resistant HIV variants provides a further rationale for developing this compound also for use in PI-experienced patients.

## INTRODUCTION

- Human plasma protein binding has been associated with lack of *in vivo* efficacy of drug candidates
- It is often monitored by physico-chemistry methods (equilibrium dialysis, etc)
- It is an equilibrium reaction, characterized by affinity and dissociation constants
- Monitoring the anti-HIV activity of inhibitors in the presence of human plasma proteins allows for quantification of the functional effect of protein binding on the efficacy of drugs

## MATERIALS & METHODS

- Anti-HIV activity is determined in HIV- or mock- infected MT4 cells by the MTT method as described by Pauwels et al (1988).
- PI resistant viruses used for these studies have different origins:
  - HIV strains obtained by *in vitro* selection in the presence of various PIs
  - Recombinant clinical isolates constructed according to the Antivirogram™ method as described by Hertogs et al (1998).
- Influence of human serum proteins is monitored by performing the anti-HIV assay as described above in the presence of either of:
  - 50% heat-inactivated human serum (HS) instead of 10% foetal calf serum (FCS)
  - 1 mg/mL AAG and 10% FCS
  - 45 mg/mL human serum albumin (HSA) and 10% FCS

Pauwels et al (1988), J. Virol. Meth. **20**: 399-321  
Hertogs et al (1998), Antimicrob. Ag. Chemother. **42**: 269-27

## RESULTS

### Alpha<sub>1</sub> Acid Glycoprotein

- Alpha<sub>1</sub> acid glycoprotein (AAG) is an acute phase plasma protein, known to bind drugs.
- AAG is responsible for the decrease in activity of the current PIs exhibiting high plasma protein binding, when tested *in vitro* against wild type HIV, i.e at (very) low inhibitor concentrations
- As the binding to AAG is a saturable reaction (Cao et al, 2001), decrease in activity of the inhibitors in the presence of AAG might be minimized at clinically relevant concentrations, i.e. higher inhibitor concentrations.

**=> Monitor the influence of AAG on anti-HIV activity at various inhibitor concentrations by making use of virus strains with different levels of susceptibility to the different PIs.**

Cao et al (2001), Antiviral Therapy **6**, Sup 1:65

### Influence of Human Plasma Proteins on Activity of Current PIs Against Wild Type HIV-1

COMPOUND	FCS (10%)	AAG 1 mg/mL	HSA 45 mg/mL	HS 50%
INDINAVIR	1	1	2	3
SAQUINAVIR	1	5	3	5
NELFINAVIR	1	31	7	25
RITONAVIR	1	18	5	24
LOPINAVIR	1	26	5	5
AMPRENAVIR	1	25	2	12

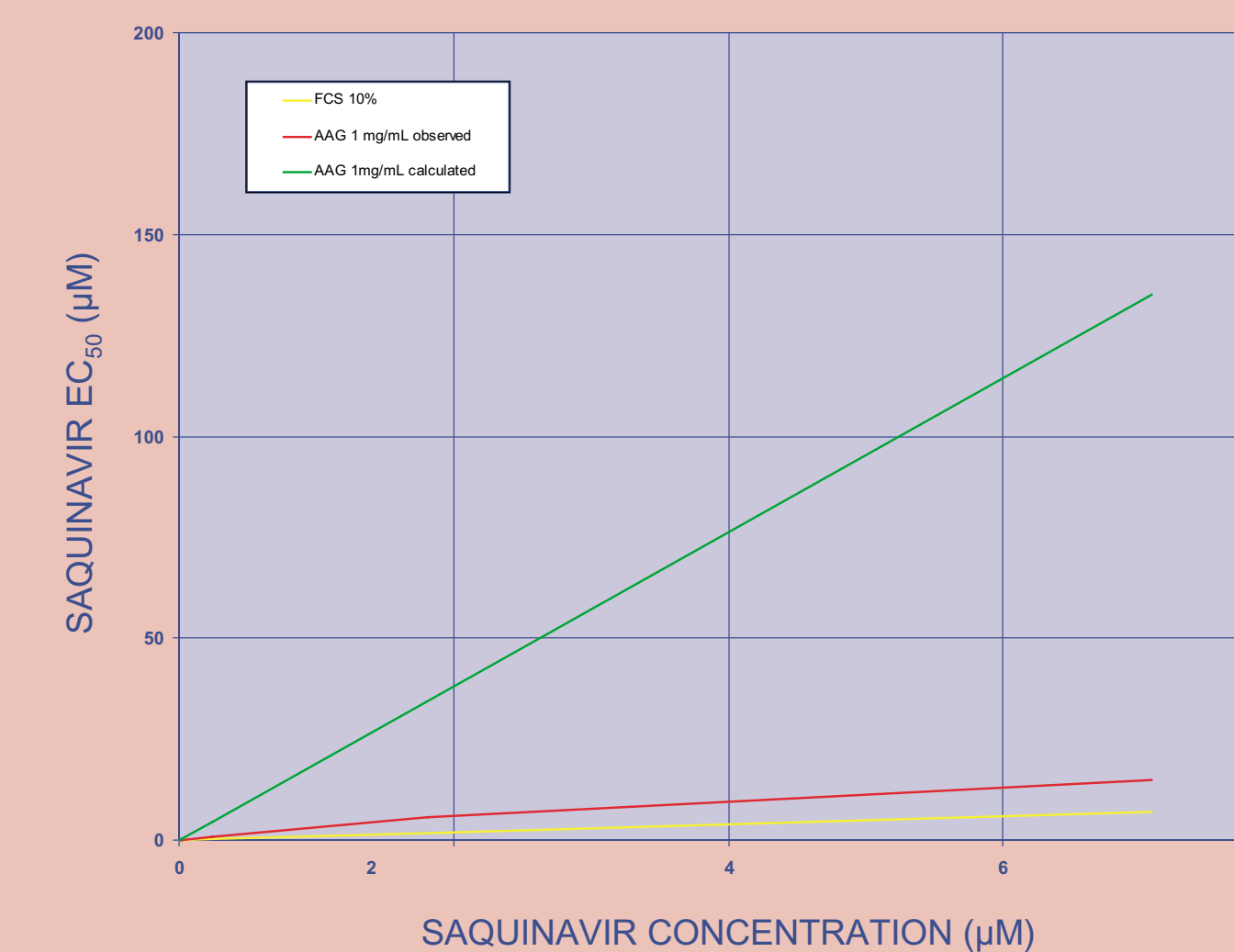
Results are expressed as the ratio between the EC<sub>50</sub> determined in the presence of the indicated human plasma protein and the EC<sub>50</sub> determined in the presence of 10% FCS, against wild type HIV-1 (LAI). Results are median of at least two determinations.

### Influence of AAG on the anti-HIV activity of PIs at Various Inhibitor Concentrations

CONCENTRATION RANGE	SQV	RTV	IDV	NFV	APV	LPV	TMC114
CLINICALLY RELEVANT CONCENTRATIONS 5 $\mu$ M and more	2	1					4
500 nM to 5 $\mu$ M	3	4	2	5	5	4	7
SUB-THERAPEUTIC CONCENTRATIONS 50 to 500 nM	4	8	1	9	14	20	12
IN VITRO TESTING CONCENTRATIONS (WILD TYPE HIV) 5 to 50 nM	5	7	2	11	38	64	24
Less than 5 nM	19						85

**At clinically relevant inhibitor concentrations, the influence of AAG on anti-HIV activity of PIs is minimized, thereby showing the saturation of the interaction of the drugs with the protein.**

### Influence of AAG on the anti-HIV activity of Saquinavir, a Highly Protein-Bound PI



Saquinavir and Indinavir were tested against different HIV-1 strains with various susceptibility (EC<sub>50</sub>) to the inhibitor in the indicated concentration range:

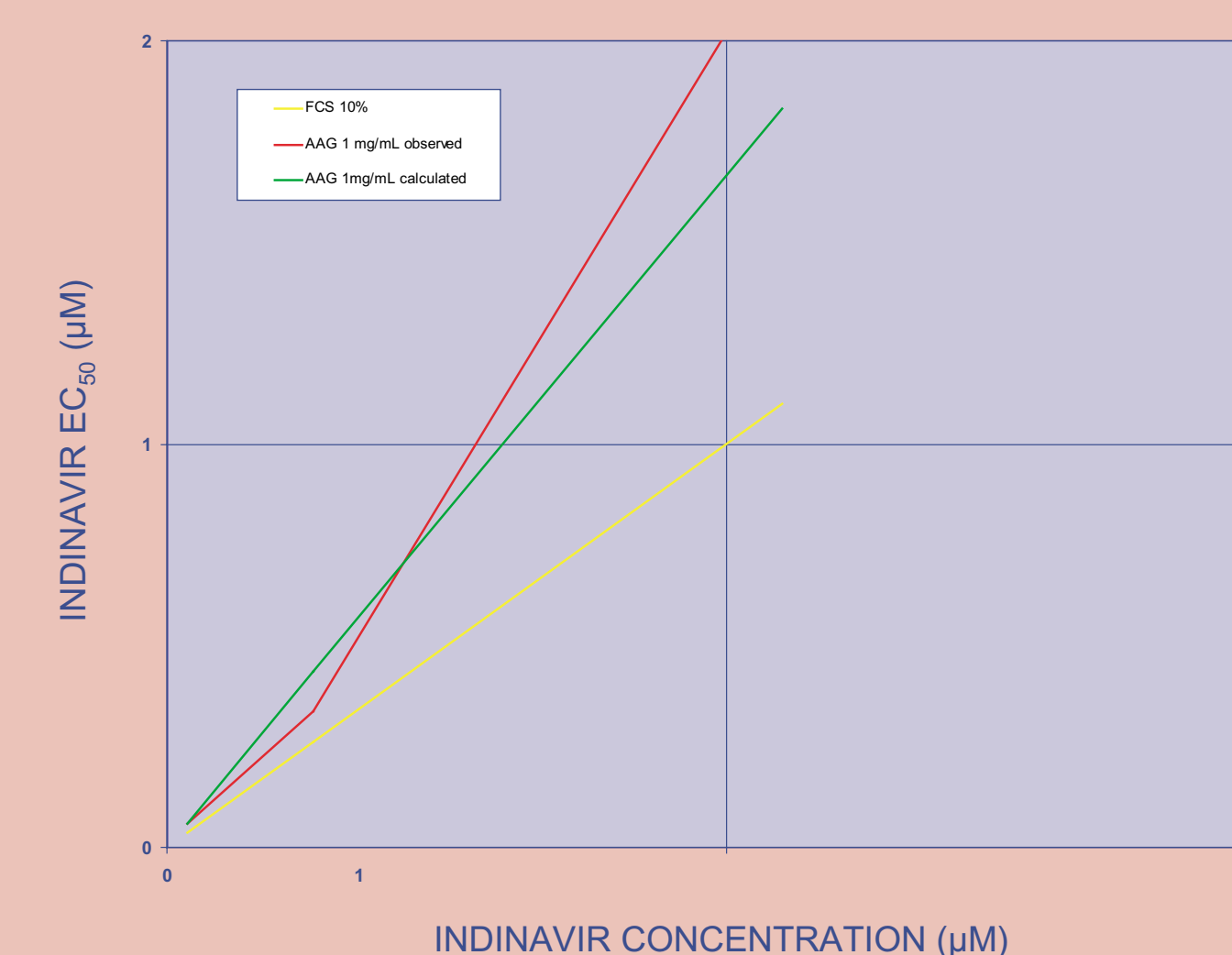
The yellow curve represents the data obtained in the presence of 10% FCS.

The green curve represents the data calculated for the different strains based on the decrease observed for SQV and IDV activity against wild type HIV-1 in the presence of AAG (1mg/mL)

The red curve represents the data obtained for the different strains in the presence of AAG (1mg/mL)

**=> Influence of AAG on the anti-HIV activity of SQV decreases with increasing inhibitor concentrations.**

### Influence of AAG on the anti-HIV activity of Indinavir, a Lowly Protein-Bound PI



**=> Influence of AAG on the anti-HIV activity of IDV remains constant with increasing inhibitor concentrations.**

## CONCLUSIONS

- Alpha<sub>1</sub>-acid glycoprotein decreases anti-HIV activity of highly protein-bound PIs, when measured at low inhibitor concentrations.
- Because the kinetics of the binding of PIs to AAG show a saturable interaction, the influence of AAG may be overestimated as it is mostly determined at concentrations (wild type EC<sub>50</sub>) where the inhibitor free active fraction is low.
- In an experimental setting using PI resistant viruses with various susceptibilities to the inhibitors, we have shown that the influence of AAG decreases with increasing concentrations of drugs.
- This is consistent with micromolar equilibrium dissociation constants for PIs binding to AAG.
- At the clinically achieved plasma levels for the current PIs the binding of drugs to plasma proteins / AAG is a less relevant parameter than was presumed formerly in the determination of IQ.
  - The "protein binding factor" to be included in the calculation will need to be correlated to the drug plasma concentration, rather than be derived from a "plasma protein binding corrected EC<sub>50</sub>", as determined on wild type HIV.
- The determination of the target plasma levels for next generation PIs, with good activity against multi-PI resistant HIV, such as TMC114, will need to take into account both:
  - The potency of the compound on resistant HIV, and
  - The plasma levels at which the influence of plasma proteins / AAG on the potency of the compound is minimized.

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