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Inhibitory Quotient of Protease Inhibitors Using a Standardized Determination of IC_{50}

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ABSTRACT

Background: Studies have shown that IQ (C_{min}/IC_{50}) is associated with virologic response. This metric may have application in assessment of *in vitro* potency relative to achievable PI concentrations if determination of IC_{50} and method of protein binding correction are uniform.

Methods: Anti-HIV activity was assessed against HIV wild-type *(wt)* pNL4-3 strain in MT4 cells in media containing 10% fetal calf serum supplemented with 50% human serum. Protein-binding adjusted IC_{50} values for PIs were determined in at least two sets of triplicate measurements. Steady-state C_{min} from published reports was used to calculate IQ.

Results: Potency of PIs based on measured *in vitro* IC_{50} (mean±SD µg/mL) were: amprenavir (APV) 0.496±0.121; atazanavir (ATV) 0.016±0.005; indinavir (IDV) 0.071±0.028; lopinavir (LPV) 0.082±0.019; nelfinavir (NFV) 0.761±0.159; saquinavir (SQV) 0.456±0.114; and tipranavir (TPV) 4.7±0.8. Published mean (95% CI) C_{min} µg/mL values were {ritonavir represented by r}: APV/r 1200/200 mg QD, 1.36 (1.12–1.67) and 600/100 mg BID, 1.32 (1.02–1.86); ATV 400 mg QD, 0.16 (0.11–0.21); fos-APV 1395 mg BID, median 0.325 (na); fos-APV/r 1395/200 mg QD, 1.45 (1.16–1.81) and 700/100 mg BID, 2.12 (1.77–2.54); IDV/r 800/100 mg BID, 0.99 (0.58–1.40); LPV/r 800/200 QD, 2.46 (1.11–3.81) and 400/100 mg BID, 5.51 (4.22–6.80); NFV 1250 mg BID, 0.76 (0.61–0.92); SQV/r 1600/100 mg QD, 0.61 (0.37–0.84); and TPV/r 500/200 mg BID, median 19.51 (range, 0.43–42.83). Estimated mean and 95% CI for IQ were: APV/r 1200/200 QD [2.7, na] and 600/100 BID [2.7, na]; ATV 400 QD [10.0, 6.9–13.1]; fos-APV 1395 mg BID [0.7, na]; fos-APV/r 1395/200 QD [2.9, na] and 700/100 BID [4.3, na]; IDV/r 800/100 BID [13.9, 6.6–21.2]; LPV/r 400/100 BID [67.4, 48.1–86.7] and 800/200 QD [30.1, 13.8–46.4]; NFV 1250 mg BID [1.0, 0.7–1.3]; SQV/r 1600/100 QD [1.3, 0.8–1.9]; and TPV/r 500/200 BID [4.2, na].

Conclusions: Standardization of the assay to measure protein-binding adjusted IC_{50} improves consistency in assessment of IQ. This enables IQ to provide better quantification of the relative potencies of PIs *in vivo*. This metric is being validated in ongoing clinical trials evaluating IQ and antiretroviral response.

BACKGROUND

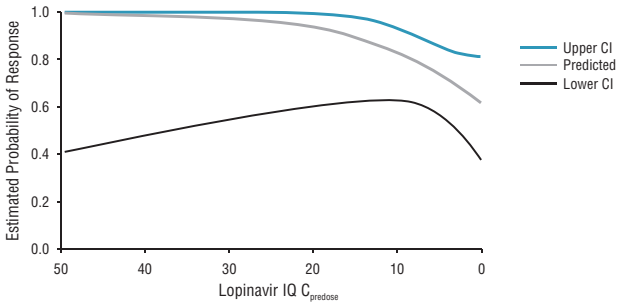
The inhibitory quotient (IQ) has been shown to be a useful pharmacodynamic predictor of protease inhibitor (PI) potency *in vivo*. Adopted from the antibiotic literature,[1] the IQ is best defined for PIs as the ratio of the minimum plasma drug concentration (C_{min}) to the drug concentration necessary to inhibit virus replication by 50% *in vitro* (IC_{50}). The clinical relevance of IQ in predicting virologic response has been demonstrated in several clinical studies using different PIs (Table 1 and Figure 1).[2–4]

Table 1. IQ and Virologic Response

Reference	Protease Inhibitors		N	Virologic Response
Casado et al.[2]	IDV/RTV	800/100 mg BID	14	IQ > 1 associated with significant response
	IDV/RTV	400/400 mg BID	11	
	NFV/SQV	1250/1000 mg BID	27	
Shulman et al.[3]	IDV/RTV	400/400 mg BID	27	vIQ > 2 for IDV best predictor of viral load reduction (P < 0.001)
Hsu et al.[4]	LPV/r	400/100 mg BID	24	If LPV IQ ≥ 15, 16/16 pts had ≤ 400 c/mL and
		533/133 mg BID	26	if LPV IQ 4–15, 80% pts had ≤ 400 c/mL

(Where vIQ = virtual IQ, which is a function of baseline phenotypic resistance [estimated by virtual phenotype] and the predose plasma concentration. Drug abbreviations of the PIs are defined in the Methods.)

Figure 1. Estimated Probability (with 95% Confidence Bands*) of Virologic Response as a Function of Lopinavir IQ in PI-Experienced Patients Receiving LPV/r[4]



*The wide confidence bands observed in the high IQ region were a function of smaller number of samples with IQ in that range (e.g., sample sizes for IQ ranges of <4, 4–15 and >15 were 21, 15 and 16, respectively).

Becker et al. described several factors that may be unaccounted for in the determination of IQ and may subsequently limit the use of this pharmacodynamic parameter in comparing the potencies of the various PIs.[5] These factors include: whether inhibitory concentrations were measured in the presence of human serum and the concentrations of serum used; the specific cell line and strain of virus used to measure IC_{50}; whether C_{min} was calculated or measured in healthy volunteers versus HIV-infected individuals; and the dosing regimen of the drug (or agent) used to assess C_{min}. The authors concluded that to make valid comparisons between the PI drugs, it is necessary to compare data obtained using the same methodology.

OBJECTIVE

- To assess the IQ of PI drugs accounting for variance in both the observed plasma C_{min} and *in vitro* potency based on a standardized method of determining the protein-binding adjusted IC_{50} values.

MATERIALS AND METHODS

Protease Inhibitors

- Amprenavir (APV), atazanavir (ATV), fosamprenavir (fos-APV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), saquinavir (SQV), and tipranavir (TPV). If used in combination with ritonavir, the "boosted" PI is identified using the modifier "/r".
- ATV and TPV were synthesized according to published methods.

Determination of Protein-Binding Adjusted IC_{50}

- Anti-HIV activity was assessed against the pNL4-3 strain of wild-type HIV in MT4 cells in media containing 10% fetal calf serum (FCS) supplemented with 50% human serum (HS) using methodology described previously by Molla et al.[16] Briefly, the methodology used to determine anti-HIV activity can be summarized as follows:
  - Inhibition of HIV-induced cytopathic effect over a range of PI drug concentrations was monitored by uptake of MTT.
  - Protein-binding adjusted IC_{50} values for the PIs were determined in at least two sets of triplicate measurements.
  - Kempf et al.[17] have demonstrated that the attenuation of activity by 10% FCS supplemented with 50% HS approximates that predicted with 100% HS.

Inhibitory Quotient

$$IQ = \frac{\text{mean } C_{min}}{\text{mean protein-binding adjusted } IC_{50} \text{ (}wt\text{ HIV)}}$$

Statistical Analysis

IQ is typically reported as a point estimate. However, pharmacokinetic and *in vitro* antiviral parameters are measured with some degree of variability. As such, we report corresponding confidence limits for IQ. Assuming that pharmacokinetic (C_{min}) and *in vitro* antiviral (IC_{50}) parameters are independent random variables, the point estimate (mean) and variance for IQ were derived using first-order Taylor approximations.[18] In particular, the point estimate and variance for IQ are given by:

$$\text{Mean} = \mu_x / \mu_y \quad \text{and} \quad \text{Variance} = ((1 / \mu_y^2) * \text{Var X}) + ((\mu_x^2 / \mu_y^4) * \text{Var Y}),$$

where μ_x and μ_y represent point estimates for C_{min} and IC_{50}, respectively. In addition, Var X and Var Y represent variance estimates for C_{min} and IC_{50}, respectively. The lower (LL) and upper (UL) 95% confidence limits for IQ are then given by:

$$LL = \text{Mean} - 1.96 * (\text{Variance})^{1/2} \quad \text{and} \quad UL = \text{Mean} + 1.96 * (\text{Variance})^{1/2}$$

Summary statistics for C_{min} were computed on the arithmetic scale for all PIs with the exception of amprenavir and fosamprenavir, which were computed on the logarithmic scale and then converted (transformed) to the arithmetic scale. Given that summary statistics for amprenavir and fosamprenavir were computed on a different measurement scale than the other PIs, 95% confidence limits for the IQ of amprenavir and fosamprenavir have not been reported.

RESULTS

- Table 2 lists the IC_{50} values for various PIs determined in a standardized *in vitro* assay using the pNL4-3 wild-type strain of HIV in MT4 cells in media containing 10% FCS supplemented with 50% HS.
  - The free fractions of the PI drug concentrations under these conditions are similar to the free fractions in human plasma.
- In this assay, the *in vitro* IC_{50} values ranged by more than 100-fold from 0.16 (ATV) to 4.7 (TPV) µg/mL.

Table 2. Protein-Binding Adjusted IC_{50} Values for Protease Inhibitors Measured *In Vitro* Against Wild-type HIV

Protease Inhibitor	Mean ± SD IC_{50} (µg/mL)
ATV	0.016 ± 0.005
IDV	0.071 ± 0.028
LPV	0.082 ± 0.016
SQV	0.456 ± 0.114
APV	0.496 ± 0.121
NFV	0.761 ± 0.159
TPV	4.7 ± 0.8

- Steady-state C_{min} values of the PIs were obtained from published reports and are listed in Table 3.
- C_{min} was defined as the concentration at the end of a regularly scheduled dosing interval.
  - For example, C_{min} of a PI administered once daily (QD) = 24 hour post-dose concentration and twice daily (BID) = 12 hour post-dose concentration.

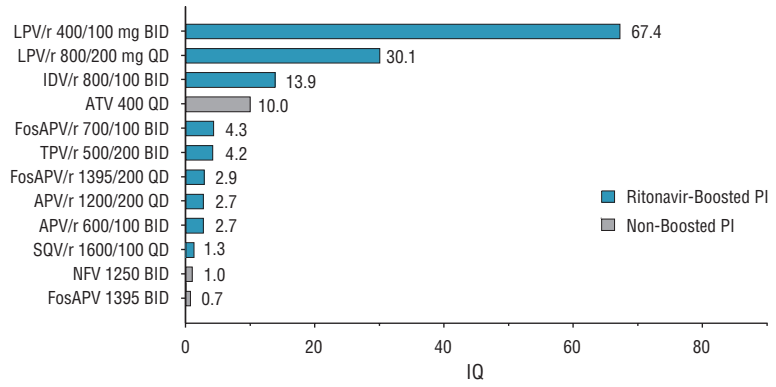
Table 3. Steady-State C_{min} Values Used to Estimate IQ

PI Regimen		Subjects* (n)	Mean C_{min} (95% CI) (µg/mL)	Reference
APV/r	1200/200 mg QD 600/100 mg BID	HIV+ (15) HIV+ (12)	1.36 (1.12–1.67) 1.32 (1.02–1.86)	Wood et al.[6]
ATV	400 mg QD	HIV- (16)	0.16 (0.11–0.21)	O'Mara et al.[7]
FosAPV	1395 mg BID	HIV+ (28)	Median 0.325 (na)	Wood et al.[8]
FosAPV/r	1395/200 mg QD 700/100 mg BID	HIV- (22) HIV- (24)	1.45 (1.16–1.81) 2.12 (1.77–2.54)	Wire et al.[9] Wire et al.[10]
IDV/r	800/100 mg BID	HIV+ (6)	0.99 (0.58–1.40)	van Heeswijk et al.[11]
LPV/r	800/200 mg QD 400/100 mg BID	HIV+ (17) HIV+ (19)	2.46 (1.11–3.81) 5.51 (4.22–6.80)	Bertz et al.[12] Bertz et al.[12]
NFV	1250 mg BID	HIV- (12)	0.76 (0.61–0.92)	Kurowski et al.[13]
SQV/r	1600/100 mg QD	HIV- (10)	0.61 (0.37–0.84)	Kilby et al.[14]
TPV/r	500/200 mg BID	HIV- (9)	Median 19.51 (0.43–42.83)	McCallister et al.[15]

*Subjects: HIV+, HIV-infected subjects; HIV-, healthy volunteer subjects

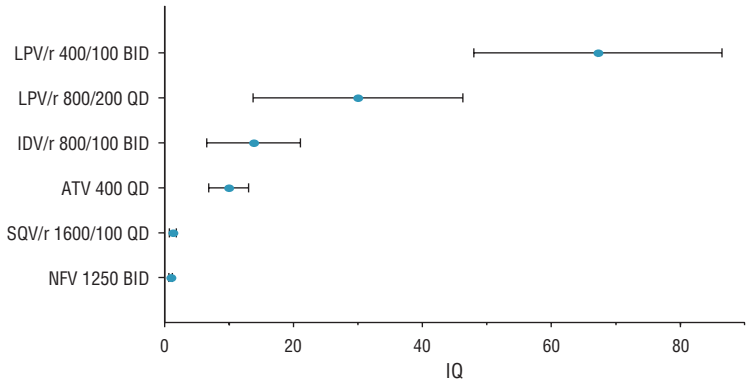
- The standardized IC_{50} values (Table 2) were combined with C_{min} values from the literature (Table 3) to provide mean IQ estimates for various ritonavir-boosted and non-boosted PI regimens as shown in Figure 2.
- Mean IQ estimates ranged from 0.7 to 67.4 with LPV/r (400/100 mg BID) providing the highest estimated IQ of all PIs assessed and ATV (400 mg QD) providing the highest estimated IQ for the non-boosted PIs assessed.

Figure 2. Estimated Mean IQ for Protease Inhibitors



- For PI regimens with available data, the 95% CI for the estimated IQ values were derived taking into account variability observed with C_{min} and IC_{50} (Figure 3).
- The lower 95% CI for LPV/r 400/100 mg BID exceeded the upper value for the other PIs reported.

Figure 3. Estimated Mean and 95% CI for IQ*



*C_{min} values for amprenavir and fosamprenavir were reported as geometric means, which precluded estimation of the 95% CI for IQ of these PIs.

DISCUSSION/CONCLUSIONS

- Standardization of the assay to measure protein-binding adjusted IC_{50} improves consistency in assessment of IQ. This enables IQ to better quantify the relative potencies of PIs *in vivo*.
- LPV/r 400/100 mg BID and 800/200 mg QD provided the highest estimated IQ of all PIs included in this assessment.
- ATV had the highest estimated IQ of the non-boosted PIs included in this assessment.
- One potential limitation of this investigation is that not all C_{min} values were obtained from HIV-infected individuals.
- A second potential limitation is that regimens with different dosing frequencies (i.e., BID and QD) were compared. The relationship of IQ and clinical response with respect to this parameter has not been explored.
- The predictive value of IQ as a metric of antiretroviral response needs further validation in prospective, randomized, controlled clinical trials (e.g., ACTG 5126) since therapeutic outcome is a consequence of many factors, not just drug potency and pharmacokinetics.

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