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Amprenavir Inhibitory Quotient and Virological Response in Human Immunodeficiency Virus-Infected Patients on an Amprenavir-Containing Salvage Regimen without or with Ritonavir

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The efficacy of an amprenavir (APV)-containing therapy without (group A) or with (group B) ritonavir was assessed in patients with failure of previous protease inhibitor therapy for human immunodeficiency virus (HIV) infection. The mean minimal plasma APV concentrations in groups A and B were 58 and 1,320 ng/ml, respectively, corresponding to APV inhibitory quotients of 0.2 (range, 0.03 to 0.70) and 7.0 (range, 1.4 to 145), respectively. At week 24, 2 of 8 and 13 of 14 patients in groups A and B, respectively, had <200 HIV RNA copies/ml of plasma, including 4 of 5 patients infected with APV-resistant viruses.

In the context of the increasing prevalence of resistance to multiple drugs among human immunodeficiency virus (HIV) isolates, optimization of salvage therapy with all available tools, plasma drug concentration monitoring, and phenotypic and genotypic assessments are of crucial importance. However, the relationship between the predicted levels of viral drug resistance determined by in vitro phenotypic assays and the therapeutic response is unclear. Poor adherence, poor bioavailability, interindividual variabilities in pharmacokinetics, extensive serum protein binding, and drug-drug interactions may lead to underexposures to antiviral drugs and unfavorable outcomes (1–5). Recent in vitro studies have shown that amprenavir (APV) could conserve antiviral efficacy against HIV strains derived from patients experiencing failure of highly active antiretroviral therapy (HAART) by regimens that contain indinavir, ritonavir, nelfinavir, or saquinavir (9, 11). The 90% inhibitory concentration (IC_{90}) of APV corrected for protein binding (IC_{90c}) is approximately 140 to 280 ng/ml for wild-type viruses, whereas the expected minimal concentration of APV (administered at 1,200 mg twice daily [b.i.d.]) in plasma (C_{min}) is 280 ng/ml without the coadministration of a nonnucleoside reverse transcriptase inhibitor (NNRTI) (1, 3–5, 9). Condra et al. (1) suggested that improving the level of exposure to APV by increasing plasma APV levels may improve the response to therapy. The APV C_{min} is dramatically increased by the coadministration of low doses of ritonavir, even with reduced APV doses, leading to levels in plasma that are theoretically higher than the IC_{90c}s for some resistant HIV strains (1, 2, 10).

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To understand the relationship between APV susceptibility, APV C_{min}, and the virological response, we determined these parameters in patients who were naive for APV treatment, who had failed previous HAART, and in whom APV-containing salvage therapy was initiated. The first group (group A) consisted of patients starting APV at 1,200 mg b.i.d. in combination with efavirenz or nevirapine and one or two nucleoside reverse transcriptase inhibitors (NRTIs). Patients for whom the APV C_{min} was lower than 100 ng/ml at two consecutive determinations were offered ritonavir at 100 mg b.i.d. to increase plasma APV levels, with concomitant reduction of the APV dosage from 900 to 450 mg b.i.d. Due to the low APV C_{min} observed in patients in group A, additional patients starting on APV received APV at a dosage of 450 mg b.i.d. combined with ritonavir at 100 mg b.i.d.; this constituted the second group (group B).

Genotyping of the HIV type 1 (HIV-1) protease and reverse transcriptase genes was carried out at the baseline and at month 2 or 3 or at month 6 in patients with detectable viral loads at month 6 (6, 7). According to the recommendations in a European summary of product characteristics based on the results of studies with APV (at 1,200 mg b.i.d.) in APV-naive patients not treated with ritonavir (Amprenavir, European Summary of Product Characteristics, Glaxo-Wellcome, 2000), viruses were considered resistant to APV when at least three mutations at different codons among the M46I, M46L, I54L, I54M, I54V, V82A, V82F, V82I, V82T, I84V, and L90M mutations were detected (12).

Phenotyping was carried out at the baseline by recombinant virus assay (RVA) as described previously (8) and at month 6 in patients with detectable viral loads. The APV IC_{90c} was calculated by multiplying the raw IC_{90} by 7, the published fold attenuation of APV by 50% human serum in vitro (1, 4, 5). The IC_{90c} of APV for the RVA reference strain, strain NL4-3, was

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TABLE 1. Baseline and follow-up characteristics of 22 patients receiving APV without (group A) or with (group B) ritonavir containing salvage HAART

Characteristic	Group A (n = 8)	Group B (n = 14)
Baseline characteristics		
HIV RNA load (median $\log_{10}$ copies/ml)	4.9	4.5
Median CD4 count (no. of cells/mm^3)	120	200
Duration of previous protease inhibitor therapy (mo)	27	39
No. of patients naive for NNRTI therapy	7	11
Median corrected APV IC_{90c} [ng/ml (range)][a]	350 (70–1,260)	175 (21–1,162)
No. of patients with APV-resistant virus[b]		
Phenotypic	2	5
Genotypic	4	4
Follow-up characteristics		
Median APV C_{min} (1st month) [ng/ml (range)]	58 (5–260)	1,320 (980–3,015)
Median (range) APV inhibitory quotient[c]	0.2 (0.03–0.7)	7.0 (1.4–145)
No. of patients with <200 HIV RNA copies/ml/total no. of patients tested (wk 24)	2/8	13/14
Median CD4 count (no. of cells/mm^3) increase (wk 24)	40	80

[a] The IC_{90c} was determined by recombinant virus assay and is the IC_{90} corrected for protein binding by multiplication by 7 (1, 5).
[b] Phenotypic resistance was defined as an IC_{90c} greater than four times that for reference strain NL4-3. APV genotypic resistance was defined according to a summary of product characteristics (Glaxo-Wellcome, 2000).
[c] The inhibitory quotient was calculated for each patient as the mean C_{min} divided by the IC_{90c}.

120 ng/ml. Viruses for which the IC_{90c}s were higher than 480 ng/ml were considered resistant.

The C_{min} of APV was measured weekly during the first month and monthly up to month 6 by a validated high-performance liquid chromatography assay. For each patient, the mean APV C_{min} during the first month was determined by using the steady-state values (those on days 14, 21, and 30). For the patients in group A, only the levels in plasma determined before the addition of ritonavir were analyzed. For each patient, the APV inhibitory quotient was determined by calculation of the ratio of the mean APV C_{min} as defined above and the baseline IC_{90c}.

Group A consisted of 8 patients, and group B consisted of 14 patients. The characteristics of the patients are presented in Table 1. Four patients were NNRTI experienced and carried viruses with NNRTI resistance-associated mutations. Treatment with ritonavir was initiated in five patients in group A on days 14 (patients 2, 6, and 8), 21 (patient 3), and 30 (patient 1).

Three or more APV resistance mutations were detected in four patients from each group (Table 2). The median baseline APV IC_{90c} was higher for group A (350 ng/ml) than for group B (175 ng/ml). However, 2 of 8 patients in group A and 5 of 14 patients in group B carried phenotypically resistant viruses (Table 2). The median APV C_{min}s within the first month were 58 and 1,320 ng/ml for patients in groups A and B, respectively, and the median APV C_{min} between months 2 and 6 was 1,310 ng/ml for patients in group B. The median APV inhibitory quotient was 0.2 for patients in group A and 7 for patients in group B (Tables 1 and 2).

Viral loads below 200 copies/ml were achieved at week 24 in 2 of 8 patients in group A, despite low APV C_{min}s, and in 13 of 14 patients in group B. One of the two patients in group A (patient 7) was prematurely switched from APV to another HAART containing dual protease inhibitors because he refused to receive ritonavir-APV on a delayed basis, and the other patient (patient 6) received ritonavir at day 14, which increased the inhibitory quotient from 0.08 to 3.8. Among the 13 patients in group B with virological responses, 3 carried virus predicted to be resistant according to their genotypes and 4 carried virus predicted to be resistant according to their phenotypes (Table 2). The 14th patient (patient 9) in group B, who was 1 of 5 patients infected with virus predicted to be resistant according to its phenotype, had a partial virological response (Table 2).

Seven patients had detectable viral loads at month 6. By month 3, three of four patients in group A and one patient (patient 9) in group B carried viruses which had acquired NNRTI resistance-associated mutation K103N, despite high plasma NNRTI levels. By month 6, four of four patients in group A carried viruses which had acquired NNRTI resistance-associated mutation K103N, despite high plasma NNRTI levels. Viruses from six of seven patients developed new mutations in the protease-encoding region (Table 3). The APV IC_{90c} increased by a mean of 8-fold (range, 2- to >20-fold) for viruses from all four patients whose viruses were phenotypically susceptible to APV at the baseline (Table 3). Virus strains from the three patients that were already resistant to APV at the baseline remained phenotypically resistant to APV. Patient 9 was the only patient whose virus did not develop any new mutation in the protease-encoding region and for which there was no increase in the IC_{90c} from the baseline value. His virus carried the S69S insertion in the reverse transcriptase-encoding region at the baseline. His viral load at month 6 was 1.3 logs below the baseline value; this was probably solely due to the continuing activity of APV-boosted ritonavir.

The significant decrease in the APV C_{min} induced by the coadministration of NNRTI led, in the patients in group A, to APV levels lower than the IC_{90c} (inhibitory quotient less than 1), the early acquisition of major NNRTI resistance-associated mutations, and virological failure, despite the addition of ritonavir within 1 month. Therefore, four patients carried viruses defined as being sensitive to APV but did not benefit from APV therapy. The APV underexposure during the first days of therapy, followed by what was functionally APV monotherapy during the months following the early acquisition of an NNRTI resistance-conferring mutation, led to the selection of

TABLE 2. APV genotypic and phenotypic resistance profiles before initiation, C_{min}s, and inhibitory quotients after initiation of APV-containing salvage therapy in patients previously treated with a protease inhibitor

Patient group and no.[a]	Previous protease inhibitor experience[b] (total duration [mo])	Previous treatment before APV initiation	Amino acid differences from clade B consensus[c]	
			Reverse transcriptase sequence (NNRTI resistance and S69S insertion)	Protease sequence
Group A				
With detectable viral load at week 24				
1	IDV,RTV,SQV,NFV (28)	d4T-3TC-NFV	None	L10I, **M46I**, A71V, N88D, **L90M**
2	IDV,NFV (24)	AZT-3TC-NFV	None	L10I, M36I, **I54V**, A71I, G73S, **V82A**, **L90M**
3	IDV,RTV,SQV (37)	3TC-ABC-ddI-HU	A98G, K101E, V108I, Y181C, G190A, S69S	L10I, K20M, M36I, **I54V**, L63P, A71V, G73S, **V82A**, **L90M**
4	SQV,IDV (33)	d4T-3TC-IDV	None	L10I, **M46I**, A71V, **I84V**, **L90M**
5	IDV,NFV,RTV,SQV (33)	d4T-ddI-RTV-SQV	None	L10I, **I54V**, A71V, V77I, **V82F**, **L90M**
8	RTV (22)	AZT-3TC-RTV	None	K20I, M36I, L63P, V82I, **L90M**
With undetectable viral load at week 24				
6	SQV, NFV (32)	AZT-3TC-NFV	None	**I54V**, A71V, V77I, **V82F**
7	NFV (10)	AZT-3TC-NFV	None	D30N, M36V/I, A71T, V77V/I
Group B				
With detectable viral load at week 24				
9	IDV (36)	d4T-ddI-IDV	A98G, S69S	L10I, M46M, **M46I**, **I54V**, L63P, **V82A**, V82S, **L90M**
With undetectable viral load at week 24				
10	RTV,IDV (39)	AZT-3TC-IDV	None	L10I, K20R, M36I, **I54A**, L63P, A71V, **V82T**, **V82A**, **V82S**, **L90M**
11	SQV,RTV,NFV (40)	AZT-3TC-NFV	A98G	L10I, **M46I**, L63P, A71A/T/V/I, G73S, V77I, **L90M**
12	IDV,SQV,NFV (39)	d4T-3TC-NFV	None	L10I, M36I, L63P, V77I, L90F
13	IDV,RTV,SQV (35)	d4T-3TC-RTV-SQV	None	L10I, **M46L**, **I54V**, L63P, A71I, **V82A**, **I84V**, **L90M**
14	IDV,RTV,NFV (36)	ddI-3TC-NFV-EFV	K103N, P225H	L63P, **V82A**
15	RTV,NFV,SQV (42)	ddI-SQV-NFV-EFV	S69S	M36I, **I54V**, L63A, **V82A**
16	IDV,RTV,SQV (42)	ddI-ABC-RTV-SQV	None	L10V, K20R, M36I, M46V, G48V, I54T, **I54V**, L63P, **V82A**
17	RTV,IDV,NFV (41)	AZT-3TC-IDV	None	L63P, V77I
18	IDV,NFV (46)	d4T-3TC-NFV	None	D30N
19	IDV,RTV (48)	d4T-3TC-RTV	None	L10I, K20R, M36I, **I54V**, L63P, A71V, **V82A**
20	NFV (26)	d4T-ddI-NFV	None	L10V/I, K20K/R, M36V, **I54L**, L63P, **L90M**
21	IDV,RTV,NFV (41)	AZT-3TC-NFV	None	L10V, L63P, A71T, N88D
22	IDV,RTV (36)	d4T-ABC-EFV	A98S, L100I, G190S, K101E, Y181C, S69S	L63P

[a] Group A patients received APV without ritonavir, whereas group B patients received ritonavir at 100 mg b.i.d.

[b] IDV, indinavir; NFV, nelfinavir; RTV, ritonavir; SQV, saquinavir; APV, amprenavir; AZT, zidovudine; ddI, didanosine; ddC, zalcitabine, 3TC, lamivudine; d4T, stavudine; ABC, abacavir; HU, hydroxyurea; EFV, efavirenz; NVP, nevirapine.

[c] For protease, all amino acid differences relative to the HIV-1 clade B consensus sequence are listed. Major genotypic APV resistance mutations are M46I, M46L, I54L, I54M, I54V, V82A, V82F, V82I, V82T, I84V, and L90M and are shown in boldface. Viruses with more than three major mutations at different codons were considered resistant to APV. For reverse transcriptase, only those differences associated with resistance to NNRTI and S69S insertions are listed.

[d] Fold resistance compared to that for reference strain NL4-3. An IC_{90c} greater than four times that for NL4-3 (480 ng/ml) was considered resistance and is shown in boldface.

[e] The IC90c was determined by recombinant virus assay and is the IC_{90} corrected for protein binding by multiplication by 7.

[f] The mean C_{min} was calculated by using the values at steady state (those on days 14, 21, 30) over the first month of treatment.

[g] The inhibitory quotient was calculated as the mean C_{min} divided by the IC_{90c}.

new mutations in the protease-encoding region and an increase in the APV IC_{90c} to levels for resistance, which dramatically reduced the remaining therapeutic options for these patients.

Conversely, the concomitant administration of ritonavir to the APV regimen led to inhibitory quotients greater than 1 in all patients in group B, even those infected with viruses defined as genotypically and/or phenotypically resistant. Thus, even if some level of resistance to APV was manifest in viruses from some patients, it was possible to overcome that resistance by providing higher levels of exposure to APV. It is important to appreciate the fact that resistance to antivirals is not absolute but is measured as a continuous scale of a reduction of drug susceptibility. As such, any clinically relevant interpretation of genotypic or phenotypic data generated in vitro must consider the concentrations achievable in plasma in vivo.

In the present evaluation, determination of the relative efficacy of each component of the antiretroviral combination was not performed. At the initiation of therapy, viruses from four patients in group B had an S69S insertion and/or mutations that conferred resistance to NNRTIs. In these patients, the APV-associated reverse transcriptase inhibitors probably had low levels of antiviral efficacy. Nevertheless, favorable virolog-

TABLE 2—*Continued*

APV		APV-containing salvage therapy	APV	
Fold IC_{90c} resistance[d]	IC_{90c} (ng/ml)[e]		Mean C_{min} (ng/ml)[f]	Inhibitory quotient[g]
1.5	175	ddI-EFV-APV	65	0.4
2.9	350	ddI-HU-EFV-APV	55	0.2
2.9	350	AZT-3TC-ADF-EFV-APV	60	0.2
4.7	560	ABC-EFV-APV	260	0.5
10.6	1260	ddI-HU-EFV-APV	70	0.06
1.5	175	d4T-ddI-EFV-APV	5	0.03
3.8	455	d4T-ddI-EFV-APV	35	0.08
0.6	70	ddC-3TC-EFV-APV	50	0.7
4.1	490	AZT-3TC-ABC-EFV-RTV-APV	1,550	3.2
4.1	490	d4T-ddI-EFV-RTV-APV	1,010	2.1
4.1	490	ddI-3TC-EFV-RTV-APV	1,285	2.6
0.2	21	AZT-ddI-EFV-RTV-APV	3,015	145
9.8	1,162	AZT-3TC-EFV-RTV-APV	1,590	1.4
1.2	140	ddI-3TC-EFV-RTV-APV	1,460	10.4
1.5	175	AZT-ddI-NVP-RTV-APV	1,000	5.7
2.8	329	AZT-ABC-EFV-RTV-APV	1,190	3.6
0.6	70	ddI-NVP-RTV-APV	1,685	24
0.3	35	ddI-EFV-RTV-APV	1,365	39
1.4	175	ddI-3TC-EFV-RTV-APV	1,355	7.7
4.9	595	3TC-EFV-RTV-APV	910	1.5
0.9	105	d4T-ddI-EFV-RTV-APV	985	9.3
1.2	140	3TC-EFV-RTV-APV	1,160	8.3

ical responses were observed in three of these four patients, which may be explained by the high APV inhibitory quotient. Determination of the optimal inhibitory quotient required to reduce the viral load to undetectable levels should consider the efficacy of each molecule included in the combination. Moreover, knowledge and/or prediction of inhibitory quotients for all antiviral drugs could aid in the selection of the optimal combination therapy, not only in terms of the antivirals se-

TABLE 3. Genotypic and phenotypic APV resistance profiles at week 24 and genotypic NNRTI resistance profile at week 8, 12, or 24 after initiation of APV-containing HAART in seven patients experiencing virological failure compared to the profiles at the baseline

Patient group and no.	Sequence mutations acquired compared to sequence at baseline		Fold APV IC_{90c} resistance compared to that at baseline
	In reverse transcriptase for NNRTI resistance-encoding region	In protease-encoding region	
Group A			
1	K103N, V108I	L47V, L63P, V77I	2
2	K103N, VA98S	K20I, L63P	2
3	None	M46I, I84V	>20
4	K103N, G190S	L63P	2
5	None	F53L, L63T	1
8	K103N, Y188L	I50V	>20
Group B			
9	G190S	None	1

lected but also in terms of the determination of the optimal dose of each component required to achieve maximum antiviral efficacy without compromising tolerance.

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