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Resistance Profiles Observed in Virological Failures After 24 Weeks of Amprenavir/Ritonavir Containing Regimen in Protease Inhibitor Experienced Patients

Anne-Geneviève Marcelin,[1]* Dissou Affolabi,[1] Claire Lamotte,[2] Hocine Ait Mohand,[3] Constance Delaugerre,[1] Marc Wirden,[1] Delphine Voujon,[1] Philippe Bossi,[3] Nadine Ktorza,[3] François Bricaire,[3] Dominique Costagliola,[4] Christine Katlama,[3] Gilles Peytavin,[2] Vincent Calvez,[1] and the Genophar Study Group

[1]*Department of Virology, Pitié-Salpêtrière Hospital, Paris, France*
[2]*Department of Clinical Pharmacy, Bichat-Claude Bernard Hospital, Paris, France*
[3]*Infectious Diseases, Pitié-Salpêtrière Hospital, Paris, France*
[4]*EMI 0214, Pitié-Salpêtrière Hospital, Paris, France*

Amprenavir (APV) is an HIV protease inhibitor (PI) used for the treatment of either naive or PI-experienced HIV-infected patients. Several genotypic resistance pathways in protease gene have been described to be associated to unboosted APV failure (I50V, V32I + I47V, I54L/M, or less commonly I84V, which may be accompanied by one ore more accessory mutations such as L10F, L33F, M46I/L). The aims of this study were to investigate the efficacy up to week 24 of an APV plus ritonavir containing regimen in PI experienced patients and to determine the genotypic resistance profiles emerging in patients failing to this therapy. Forty-nine, PI experienced but APV naïve patients were treated with APV (600 mg bid) plus ritonavir (100 mg bid). By intent-to-treat analysis, the median decrease in viral load (VL) was −1.32 log10 (min +0.6; max −2.8) and −1.46 log10 (min +0.5; max −2.8) 12 and 24 weeks after initiating APV plus ritonavir regimen, respectively. Twelve patients harboured a VL >200 copies/ml at week 24. Among these patients, the selection of mutations previously described with the use of APV as first PI (V32I, L33F, M46I/L, I50V, 54M/L, and I84V) was observed. However, in some cases, mutations classically described after the use of other PIs (V82F and L90M) were selected but always with APV-specific mutations. There was no relation between the resistance pathways selected with either APV or ritonavir plasma minimal concentration, but higher APV plasma minimal concentration were associated with a lower rate of resistance mutations selection. ***J. Med. Virol. 74:16–20, 2004.*** © 2004 Wiley-Liss, Inc.

KEY WORDS: failure; amprenavir; mutations; selection; plasma concentration

INTRODUCTION

Amprenavir (APV) is a protease inhibitor (PI) used for the treatment of antiretroviral-naive or -experienced HIV-infected patients [Noble and Goa, 2000; Tisdale et al., 2000]. When ritonavir is coadministred with APV, an approximately sixfold increase in APV plasma concentrations is achieved, without having a substantial impact on tolerability [Sadler et al., 2001; Marcelin et al., 2003; Nadler et al., 2003]. Reduced susceptibility to antiretroviral agents, such as PI, can arise both in vitro and in vivo, following selection and outgrowth of viral mutant strains and is associated with specific amino acid substitutions in the viral protease [Boden and Markowitz, 1998; Condra, 1998]. Additional compensatory mutations may also be selected in the protease substrate Gag cleavage sites [Doyon et al., 1996]. APV, a hydroxyethylamine sulfonamide, is an inhibitor of HIV-1 and HIV-2 proteases, with *Ki* of 0.6 and 19 nM, respectively [Tisdale et al., 2000]. In vitro selection experiments in which virus was passaged in increasing concentrations of APV, identified an isoleucine-to-valine substitution at protease position 50 (I50V) as a key marker of resistance development to this PI [Partaledis et al., 1995; Tisdale et al., 1995; Pazhanisamy et al., 1998]. The I50V mutation alone confers a two- to three fold decrease in susceptibility compared to the wild type virus. In the presence of other protease mutations,

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*Correspondence to: Anne-Geneviève Marcelin, PharmD, PhD, Department of Virology, 83, boulevard de l'hôpital, 75013 Paris, France. E-mail: anne-genevieve.marcelin@psl.ap-hop-paris.fr

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especially M46I/L and I47V, reduction in susceptibility to APV can increase to greater than tenfold [Partaledis et al., 1995; Tisdale et al., 1995]. Protease substitutions L10F and I84V have been observed much more rarely in vitro [Partaledis et al., 1995]. The eventual replacement of I84V by I50V during continued in vitro selection suggests that the latter genotype is more viable in the presence of the inhibitor at concentrations achieved during these experiments. In vivo, development of resistance to APV has been mostly studied in PI naive patients and four pathways of APV resistance have been identified involving key protease substitutions related to APV trough plasma concentrations; either I50V, V32I + I47V, I54L/M, or less commonly I84V, which may be accompanied by one ore more accessory mutations (L10F, L33F, M46I/L) [Maguire et al., 2002]. However, limited data are available on the type of mutations selected under APV plus ritonavir therapy in PI experienced patients and the possible selection of cross-resistance mutations like V82A/F/T/S and L90M.

The aims of this study were to investigate the efficacy up to week 24 of an APV plus ritonavir containing regimen in PI experienced patients and to determine the genotypic resistance profiles emerging in patients failing to this therapy.

MATERIALS AND METHODS

Patients

Forty-nine PI-experienced but APV-naive patients, experiencing virological failure to antiretroviral therapy, were treated at baseline by two or three nucleoside reverse transcriptase inhibitors (NRTI) and APV (600 mg bid) plus ritonavir (100 mg bid). No non-NRTIs or other PIs than APV and ritonavir were used in the antiretroviral combinations. The choice of the NRTIs associated to APV plus ritonavir was driven by genotypic testing performed 4 weeks before starting regimen, following the French ANRS AC11 guidelines algorithm (www.hivfrenchresistance.org) [Delfraissy, 2000]. Baseline patient's characteristics are presented in Table I. The patients were followed prospectively at baseline, weeks 8, 12, and 24, including clinical examination, measure of plasma HIV-1 viral load (VL), CD4 cell count, and APV and ritonavir minimal plasma concentrations.

HIV-1 RNA Quantitation

Quantitation of plasma HIV-1 RNA was performed using the Amplicor Monitor assay (Cobas 1.5 test, Roche Diagnostics, Bazel, Switzerland) with a detection limit of 200 copies/ml.

Genotypic Resistance Testing

Baseline genotypic resistance testing was undertaken while patients were under the selective pressure of the previous failing PI containing regimen and in case of virological failure (VL > 200 copies/ml) at week 24. Plasma HIV-1 RNA was used for sequence analysis of the reverse transcriptase (RT) gene (codons 1–240) and protease gene (codons 1–99). HIV-1 RNA was purified from 1 ml ultracentrifuged (19,300*g* for 60 min) plasma using the High Pure Viral purification kit (Boehringer Mannheim, Mannheim, Germany). Plasma HIV-1 RNA was amplified by a one-step reverse transcription-PCR using the TITAN One Tube Reverse Transcription PCR Kit (Boehringer Mannheim) followed by a nested PCR with AmpliTaq Gold (Applied Biosystems, Foster City, CA). All primers used were described previously [Larder et al., 1991; Jung et al., 1992; Nijhuis et al., 1998]. Direct sequencing of the PCR product was performed using the D-Rhodamine Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). Sequencing reaction products were analyzed on an ABI 3100 Genetic Analyzer (PE Applied Biosystems). The sequences were analyzed using the Sequence Navigator software (PE Applied Biosystems) by comparing the sense and anti-sense strands of each fragment with the wild-type virus HXB2 sequence.

TABLE I. Baseline Patients Characteristics

Number of patients	n = 49	Range
Gender	Male = 44 Female = 5	
Median age (years)	45	28–57
Median HIV-1 RNA viral load (log_{10} copies/ml)	4.13	3–5.2
Median CD4 cell count (cell/mm^3)	286	48–700
Median number of previous PI received[a]	2	1–6
RTV	15	
IDV	43	
SQV	6	
NFV	30	
r/SQV	24	
r/IDV	6	
Median number of PI resistance mutation[b]		
Total = major + minor	6	1–10
Major	2	0–4
Minor	4	1–7

[a]RTV, ritonavir; IDV, indinavir; SQV, saquinavir; NFV, nelfinavir; r/SQV, ritonavir plus saquinavir; r/IDV, ritonavir plus indinavir.
[b]2003 IAS table of resistance mutations (http://www.iasusa.org/resistance mutations/index.html).

PI Plasma Determinations

Blood samples were collected to determine APV and ritonavir plasma concentrations at steady state (at weeks 8, 12, and 24). Intervals between the last drug intake and sampling were recorded. APV minimal plasma concentrations (Cmin) were determined by reversed-phase high performance liquid chromatography coupled with fluorescence detection, after solid–liquid phase extraction as described previously [Sadler et al., 1999]. On the same samples, ritonavir Cmin were determined by reversed-phase high performance liquid chromatography coupled with ultra-violet detection, after liquid–liquid phase extraction as described previously [Marsh et al., 1997]. The APV and ritonavir methods were validated over plasma concentration ranges of 5–1,000 and 30–15,000 ng/ml with quantification limits of 5 and 30 ng/ml, respectively. The between-assay bias for APV and ritonavir were below 6 and 10% for all assays, respectively. APV and ritonavir were kindly provided by Glaxo Smith Kline Inc and Abbott Inc, respectively.

RESULTS

By intent-to-treat analysis, the median decrease in VL was −1.32 log10 (min +0.6; max −2.8) and −1.46 log10 (min +0.5; max −2.8) 12 and 24 weeks after initiating RTV/APV regimen, respectively.

Twelve patients harboured a VL > 200 copies/ml at week 24 and were included in the failure genotypic analysis. One sample was non-amplifiable. In five cases, there was no selection of new PI resistance mutation and in six cases, new PI resistance mutations were selected between baseline and week 24. Among the APV associated-resistance mutations previously described in first line PI, the mutation I54V/L was selected two times and the mutations V32I, I50V, and I84V once. The selection of accessory APV mutations was observed at positions L33F and M46I/L in three cases and L10I in one case. In addition, some mutations classically described with the use of other PIs were observed: the mutation L90M in two cases and the mutation V82F in one case. In all these three cases, these cross-resistance mutations were selected along APV-specific mutations, specifically, the I50V, V32I, and L33F respectively. Finally, a counter-selection of nelfinavir specific-mutations D30N + N88D was detected, with the replacement of mutated amino acid by wild type (patient 11). Protease gene substitutions observed in viruses from patients failing RTV/APV containing regimen are presented in Table II.

The median APV and ritonavir plasma Cmin measured at week 24 were respectively 1,543 ng/ml (min = 282; max = 2,760) and 300 ng/ml (min = 30; max = 1,000). Patients who failed to APV plus ritonavir therapy, without selecting new PI resistance mutations at week 24, have a trend to harbour higher median APV Cmin at week 24 (1,900 ng/ml) than patients who failed selecting new PI resistance mutations (1,600 ng/ml) ($P = 0.1$; Fig. 1). However, there was no difference of week 24 ritonavir Cmin between patients failing with or without resistance mutations. There was no relation between the APV or ritonavir Cmin with either the mutations linked classically to APV or to the other PIs.

DISCUSSION

The results indicate a sustained efficacy (−1.46 $\log_{10}$ copies/ml) up to week 24 of an APV plus ritonavir containing regimen in PI experienced patients. This is in accordance with the result of a clinical study in PI experienced patients treated with the prodrug of APV, the GW433908 (−1.50 $\log_{10}$ copies/ml at week 24) [De Jesus et al., 2003]. Among patients failing to this APV plus ritonavir containing regimen, we observed the selection of mutations previously described with the use of APV as first PI (V32I, L33F, M46I/L, I50V, 54M/L, and I84V) affecting primarily ritonavir and conferring limited cross-resistance to other PIs [Schmidt et al., 2000]. However, in some cases, mutations classically described with the use of other PIs, conferring PI cross-resistance (V82F and L90M) were selected but always with APV-specific mutations, suggesting that they were co-selected through the development of APV resistance. These mutations were not described previously with the use of unboosted APV in naive or PI experienced

TABLE II. Baseline Genotypic Profiles and Week 24 Selected Mutations Observed in Viruses From Patients Failing Amprenavir Plus Ritonavir Containing Regimen

Baseline genotype	Week 24 selected mutations
L10I, M36V, M46L, I54V, A71V, I84V, L90M	NA[a]
A71V G73S V77I L90M	None
L10I, M46I, A71V, G73S L90M	L33F, V82F
K20R, M36I, M46I, I47V, A71T, V82A, L90M	None
I54V, A71V, V77I, V82A, L90M	L33F, M46L, I84V
L33F, M46L, I54V, A71V, V82A, I84V L90M	L10I, V77I
L10I, K20M, M36I, I54A, A71V V82T	L33F, M46I, I50V, I54V, L90M
M36I, M46L, A71V, L90M	None
L10I, M46I, V82A	I54L
L10I	None
L10F, K20T, D30N, M36I, A71V, N88D	V32I, M46L, L90M
L10I, M46L, L63P, A71V, G73S, I84V, L90M	None

[a]NA, not available.

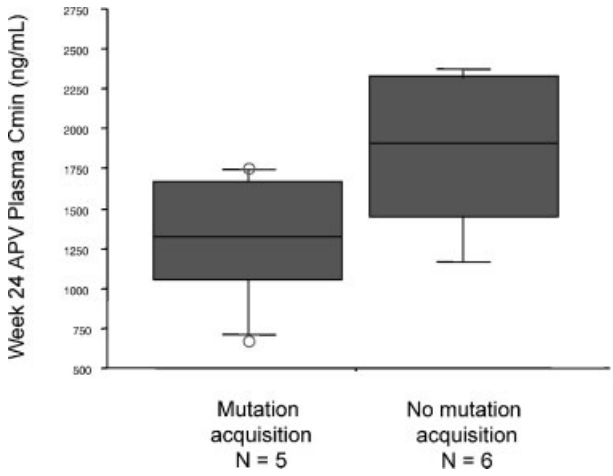

Fig. 1. Influence of week 24 APV Cmin on mutations selected at week 24.

patients [Tisdale et al., 2000; Maguire et al., 2002; Ait-Khaled et al., 2003]. These mutations could be selected either by APV itself or by ritonavir low dose, as they were previously described among patients failing to ritonavir monotherapy [Markowitz et al., 1995; Molla et al., 1996]. Previous studies have shown that substitutions at nine different codons can be selected by ritonavir, such as V82A/F/T/S, I84V, and L90M. The selection of mutations conferring PI cross-resistance could also be explained by different virus genetic background between naive and experienced patients that may affect the mutation patterns selected upon failure. Indeed, in treatment-experienced patients many viral variants with different mutation patterns exist as minority species and could constitute the backbone on which the new resistance mutation is acquired. Among subjects failing APV as their first PI, pathway selection have been described to be partly dependent on APV Cmin, with the I50V and I84V pathways associated with the highest levels of resistance being more frequent at higher APV Cmin, while lower APV concentrations in plasma were associated with selection of less APV resistant I54L- or I54M-containing genotypes [Elston et al., 2001]. In this study, in PI experienced patients, we did not find any relation between the type of mutations selected at week 24 and neither the APV nor ritonavir Cmin, but higher APV Cmin seems to be associated with a lower rate of resistance mutations selection. A similar phenomenon has been described previously for ritonavir with a delayed appearance of resistance mutations in patients with higher plasma levels of RTV [Molla et al., 1996]. These observations reinforce the impact of plasma drug concentrations in the rate of resistance development and the duration of response to antiretroviral therapy.

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