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Prevalence of subtype-related polymorphisms associated with *in vitro* resistance to attachment inhibitor BMS-626529 in HIV-1 'non-B'-infected patients

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Objectives: BMS-626529 is a member of the new drug class of HIV-1 attachment inhibitors currently in development. Mutations selected during *in vitro* experiments with BMS-626529 are located in the gp120 region: L116P, A204D, M426L, M434I-V506M and M475I. A differential antiviral activity of BMS-626529 was observed depending of the viral subtype. The aim of our study was to assess the prevalence of subtype-related polymorphisms previously described as being associated with *in vitro* resistance to BMS-626529 in patients infected with different HIV-1 'non-B' subtypes.

Patients and methods: The prevalence of substitutions in gp120 was assessed in 85 HIV-infected patients (not previously treated with attachment inhibitors and infected with HIV-1 'non-B' subtypes) by performing direct sequencing of the gp120 region.

Results: The most prevalent HIV-1 subtype was CRF02_AG ($n=46$, 54%). The M426L substitution was found in virus from 10 patients (11.8%), mainly in subtypes D and CRF02_AG. The M434I substitution was found in virus from 11 patients (12.9%), mainly in subtypes CRF02_AG and CRF06_cpx. None of the CRF02_AG viruses harboured both M426L and M434I substitutions.

Conclusions: In our series, the M426L substitution in the gp120 region was detected in 46% and 7% of subtype D and CRF02_AG samples, respectively, and might affect the activity of BMS-626529 against these specific subtypes. Further studies are needed to better describe associations between HIV-1 'non-B'-subtype-related polymorphism profiles and the level of phenotypic resistance to attachment inhibitor BMS-626529.

Keywords: HIV, antiviral, 'non-B' subtypes, primary resistance

Introduction

The HIV-1 attachment inhibitor BMS-626529 represents a potential new drug class for the treatment of HIV infection.[1,2] BMS-626529 binds to the gp120 glycoprotein of the viral envelope and interferes with virus attachment to the cellular receptor CD4. *In vitro* serial passage experiments identified four single and one double amino acid substitutions in gp120 that independently confer resistance to BMS-626529: L116P, A204D, M426L, M434I-V506M and M475I, all located around the CD4 binding site in gp120.[3] *In vivo*, the M426L substitution was previously described as being selected in patients included in an 8 day monotherapy trial with a former compound of the attachment inhibitor drug class BMS-488043, which was not further developed.[4] Concerning BMS-626529, *in vivo* preliminary results in 10 HIV-1-infected patients over 8 days in monotherapy reported a median HIV-1 RNA change ranging from -1.59 to -1.77 $\log_{10}$ copies/mL according to the dose.[1,3] In this latter study, the IC_{50} was described as predictive of the extent of HIV-1 RNA load decrease.[3] In addition, a differential antiviral activity of BMS-626529 was observed depending on the viral subtype, with a natural resistance of the CRF01_AE recombinant.[3]

The aim of our study was to assess the prevalence of subtype-related polymorphisms previously described as being associated with *in vitro* resistance to the HIV-1 attachment inhibitor BMS-626529 in patients infected with HIV-1 'non-B' subtypes.

Patients and methods

Eighty-five HIV-infected patients were included, all naive with regard to attachment inhibitor BMS-626529 treatment. All patients were infected with HIV-1 'non-B' subtypes. HIV-1 RNA was extracted from 1 mL of plasma. Two distinct fragments of the gp120 sequence, defined as fragment A and fragment B, were amplified with RT–PCR and nested PCR in order to assess the presence of the different mutations associated with *in vitro* resistance to BMS-626529. Thus the L116P and A204D mutations were assessed in fragment A and the mutations M426L, M434I, M475I and V506M were assessed in fragment B. Fragment A, of 1016 bp, was amplified using the following outer primers: CD4BSF1 (nt 5960–5977 according to the HIV-1hxb2 sequence) 5′-GCAATCTCCTATGGCAGG-3′ and CD4BSR1 (nt 6953–6976) 5′-ATTCCATGTGTACATTGTACTGTG-3′; and the following inner primers: CD4BSF2 (nt 6435–6454) 5′-ACACAT GCCTGTGTACCCAC-3′ and CD4BSR2 (nt 6945–6964) 5′-CATTGTACTGT GCTGACATT-3′. Fragment B, of 861 bp, was amplified using the following outer primers: CD4BSF6 (nt 7201–7226) 5′-AYATAAGACAAGCAYATT GTAAYVT-3′ and 6AS (nt 8042–8062) 5′- AGTGGTGCAAATGAGTTTTCC-3′; and the following inner primers: CD4BSF4 (nt 7356–7374) 5′-ATTGT GGAGGGGAATTTTTCTAC-3′ and CD4BSR4 (nt 7784–7809) 5′-TGCTGCT CCCAAGAACCC-3′. The reverse transcription step (Titan One-Tube RT–PCR kit, Roche, Mannheim, Germany) was carried out at 50°C for 30 min, followed by 94°C for 2 min for fragments A and B. PCR cycling parameters were 35 cycles (94°C for 30 s, 55°C for 30 s and 68°C for 90 s) and 68°C for 7 min for fragments A and B. Nested PCRs were performed with Taq polymerase (Applied Biosystems, Foster City, CA, USA) and 2.5 μL of inner primers, described above (10 mM), following the manufacturer's instructions. The PCR conditions consisted of initial denaturation at 94°C for 4 min, followed by 30 repeated cycles [94°C for 30 s, 60°C (fragment A) or 55°C (fragment B) for 30 s and 72°C for 60 s] and 72°C for 7 min. HIV-1 gp120 region sequencing reactions were performed using the inner primers of the nested PCRs. Sequencing reactions were run using the ABI Prism Dye Terminator kit on an automated sequencer (ABI Prism 3130, Applied Biosystems). The GenSearch software (PhenoSystems, Lillois, Belgium) was used to edit and align the nucleotide sequences.

Our CD4 binding site genotyping assay does not provide the possibility to combine in the same assay the detection of subtype-related polymorphisms associated with *in vitro* resistance to the HIV-1 attachment inhibitor BMS-626529 as well as CCR5/CXCR4 viral tropism.

Results

Among the 85 patients studied, most of them ($n=46$, 54%) were infected with a CRF02_AG recombinant virus. The subtype distribution of the 39 remaining patients was as follows: 13 (33%) subtype D, 8 (21%) subtype A, 4 (10%) subtype G, 5 (13%) CRF06_cpx, 2 (5%) CRF13_cpx, 2 (5%) CRF19_cpx, 2 (5%) subtype F, 1 (3%) subtype C, 1 (3%) CRF24_cpx and 1 (3%) CRF30_cpx.

The prevalence of subtype-related polymorphisms known to confer *in vitro* resistance to BMS-626529 is depicted in Table 1. No substitutions were detected at positions 116, 204, 475 or 506; the double-mutant M434I-V506M was not detected either. The M426L substitution was found in virus from 10 patients (11.8%), including six subtype D samples (46%), three CRF02_AG (7%) and one CRF30_cpx. Thus the M426L mutation was present at a higher frequency in subtype D when compared with all other subtypes ($P<0.001$, Fisher's exact test) or when compared with CRF02_AG ($P=0.002$). The M434I substitution was found in virus from 11 patients (12.9%), including 7 CRF02_AG (15%), 2 CRF06_cpx (40%), 1 subtype A (12.5%) and 1 CRF13_cpx. No difference in the frequency of the M434I substitution among subtypes was evidenced. None of the CRF02_AG subtype virus harboured both M426L and M434I substitutions. In addition, we found substitutions implying residues not described as associated with *in vitro* resistance to BMS-626529 at positions 204, 426, 434 and 506 in five (5.9%), two (2.4%), two (2.4%) and two (2.4%) viruses, respectively (Table 1).

Discussion

This study focused on gp120 sequences from HIV-1 'non-B' subtypes, mostly from the recombinant CRF02_AG, since it is the most prevalent 'non-B' virus in the French HIV-infected population, representing about 20% of new HIV infections.[5]

In our series the M426L substitution in gp120 was detected in 46% and 7% of subtype D and CRF02_AG samples, respectively,

Table 1. Percentage of viruses exhibiting gp120 mutations selected *in vitro* by BMS-626529 among different HIV-1 'non-B' subtypes ($n=85$)

		gp120 substitution, number (%)						
Subtype	Number	L116P	A204D	M426L	M434I	M475I	V506M	M434I-V506M
All subtypes	85	0 (0)	0 (0)	10 (11.8)	11 (12.9)	0 (0)	0 (0)	0 (0)
A	8	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)
C	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
D	13	0 (0)	0 (0)	6 (46.2)	0 (0)	0 (0)	0 (0)	0 (0)
F	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
G	4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CRF02_AG	46	0 (0)	0 (0)	3 (6.5)	7 (15.2)	0 (0)	0 (0)	0 (0)
CRF06_cpx	5	0 (0)	0 (0)	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)
CRF13_cpx	2	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)
CRF19_cpx	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CRF24_cpx	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CRF30_cpx	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Other residues[a]		0 (0)	5 (5.9)	2 (2.4)	2 (2.4)	0 (0)	2 (2.4)	0 (0)

[a]Substitutions implying residues not described as associated with *in vitro* resistance to BMS-626529: (i) residues L and T at position 204; (ii) residues P and V at position 426; (iii) residues L and T at position 434; and (iv) residue G at position 506.

and might affect the activity of BMS-626529 against these specific subtypes. Moreover, the M426L substitution, previously described as selected *in vivo* with a precursor of the attachment inhibitor drug class,[4] was associated with an 81-fold increase in resistance level to BMS-626529.[3] Analysis of gp120 subtype B sequences from the Los Alamos National Laboratory (LANL) HIV database showed a prevalence of the M426L substitution of 8.45%.[3] Interestingly, the prevalence was lower in 'non-B' subtypes, with a prevalence of M426L in this database of 4.3% and 3.4% in CRF02_AG and subtype D sequences, respectively. Thus differences were observed between the prevalence of the M426L substitution in subtype D sequences found in the LANL HIV database and in our study; however, only 13 subtype D strains were assessed in our study.

The M434I substitution was shown in 15% of CRF02_AG samples in our study; a similar prevalence (12.2%) is observed in CRF02_AG gp120 sequences in the LANL HIV database, but a lower frequency compared with the 2.5% in gp120 subtype B sequences. However, a mutation pattern described as associated with *in vitro* resistance to BMS-626529, the double mutant M434I-V506M, was a genotypic profile not found in our study. The impact on the phenotypic resistance level of the single M434I mutant in the CRF02_AG genotypic background is currently unknown and needs to be explored by site-directed mutagenesis. In addition, we can hypothesize that testing a higher number of viral strains from the different HIV-1 'non-B' subtypes would have revealed additional findings about the prevalence of subtype-related CD4 binding site polymorphisms.

Further studies are needed to better describe associations between HIV-1 'non-B'-subtype-related polymorphism profiles and levels of phenotypic resistance to attachment inhibitor BMS-626529.

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Transparency declarations

None to declare.

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