Pharmacokinetic and pharmacodynamic determinants of early virological response to enfuvirtide-based regimens in HIV-positive patients

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Background: Early virological response (VR) to enfuvirtide-based salvage regimens at week 12 has been described as a predictor of long-term therapeutic success. The relationship between enfuvirtide plasma exposure and VR has not yet been investigated in the clinical setting. Our aim was to investigate the role of enfuvirtide plasma exposure as a determinant of early VR in the clinical setting.

Methods: Forty-two multidrug-experienced patients starting a salvage enfuvirtide-based regimen were prospectively evaluated over a 12 week period. HIV-RNA levels and enfuvirtide concentrations were regularly measured. VR was considered as achievement of viral load (VL) undetectability and/or a decrease of more than 1 log at week 12.

Results: Optimized background score (OBS) and enfuvirtide concentrations were associated with VL decrease at week 12. An OBS >2 and enfuvirtide >2100 ng/mL were associated with VR. The pharmacokinetic/pharmacodynamic (PK/PD) analysis confirmed this exposure–response relationship both in the total population and in different groups according to OBS <2 or ≥2. Higher estimates of IC50 were calculated for the OBS <2 group when compared with the OBS ≥2 group (7551 versus 2330 ng/mL, respectively), without a marked difference in I0 (0.31 versus 0.21 log) and Imax (−2.64 versus −3.33 log).

Conclusions: Enfuvirtide plasma exposure and OBS were found to significantly influence the magnitude and rate of early VR. The PK/PD modelling of enfuvirtide concentrations was different in our clinical setting, compared with previous data obtained under trial conditions. Therefore, optimization of enfuvirtide plasma exposure could deserve further evaluation as a determinant of therapeutic response in HIV-positive patients.

Keywords: entry inhibitor, efficacy, PK/PD

Introduction

Enfuvirtide is the first member of a class of antiretroviral agents used for the treatment of HIV-1 infection called fusion inhibitors. It has an extracellular mode of action that distinguishes it from the other available classes of antiretroviral agents, which all target viral replication inside the cell.1 In two large Phase III clinical trials (TORO-1 and TORO-2), subcutaneous administration of 90 mg enfuvirtide twice daily in combination with an optimized antiretroviral regimen was associated with undetectable plasma HIV-RNA at week 48 in a higher proportion of subjects than in those administered with optimized background therapy alone.1–3

The pharmacokinetic/pharmacodynamic (PK/PD) relationship of enfuvirtide has been explored by relying on data collected from the Phase III clinical trials (TORO-1 and TORO-2)1 and

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Determinants of enfuvirtide efficacy

from a Phase I/II functional monotherapy study (TRI-003).\(^5\) In TORO-1 and TORO-2 studies,\(^4\) antiretroviral response was found to be independent of drug exposure, suggesting that enfuvirtide concentrations at the approved dose of 90 mg twice daily were in the plateau portion of the dose–response curve of the drug. However, the PK/PD relationship of enfuvirtide in the clinical setting has not yet been assessed.

Therefore, the aim of this study was to investigate the role of enfuvirtide plasma exposure as a determinant of early virological response (VR) in the clinical setting.

Patients and methods

Multidrug-experienced patients starting a salvage enfuvirtide-based regimen [in association with one or two boosted protease inhibitors (PIs), two or three nucleoside reverse transcriptase inhibitors (NRTIs) and/or plus a non-NRTI (NNRTI)] in the context of the Enfuvirtide Expanded Access Program were prospectively enrolled in two centres of Northern Italy. The study was approved by the local Ethics Committee, and all patients gave informed consent. Patients were monitored regularly. Background regimen (BGR) optimization was guided by genotypic resistance tests. Only subjects with self-reported adherence of more than 90% in the last 7 days before each visit (not more than two drug intakes missed in the last 7 days) were considered.

Patients and methods

HIV-RNA (copies/mL) [viral load (VL)] and CD4\(^+\) (cells/mm\(^3\)) were assayed at baseline and at weeks 4 and 12. Enfuvirtide trough concentration (C\(_{\text{trough}}\)) was measured at the same time points.

VR at week 12 was considered as reaching an undetectable VL (HIV-RNA <50 copies/mL) and/or a VL decrease >1 log compared with baseline. The intent-to-treat last-observation-carried-forward analysis of VR was used.

Genotypic resistance test and Virtual Phenotype\(^\text{TM} \) (Vph) were carried out at baseline. Optimized background score (OBS) was calculated as the number of drugs included in the BGR and reported to be active by Vph.

The interpretation of the genotypic resistance test was made according to the IAS-USA resistance guidelines (March/April 2005 version).

Enfuvirtide plasma concentrations were measured by using a validated HPLC method with fluorescence detection.\(^6\) In those subjects with more than one enfuvirtide C\(_{\text{trough}}\) measurement over the study period, all available values were averaged and then considered for analyses.

Linear regression and logistic regression analyses were performed as required. Receiver operating characteristics (ROC) test was used to calculate enfuvirtide C\(_{\text{trough}}\) cut-off.

Results

Forty-two multidrug-experienced subjects (age, 45; male, 34) were considered. Previous duration of antiretroviral therapy was 96 months (87–125), with administration of a median of 6 (5–6) NRTIs, 1 (1–2) NNRTI and 5 (4–5) PIs. Subjects initiated enfuvirtide in association with 4 (3–4) drugs as BGR, of which 2 (1–2) were considered as active drugs by Vph (OBS). Main co-administered drugs were tenofovir disoproxil fumarate (76.2%), lopinavir (71.4%), efavirenz (26.2%), saquinavir (19%), indinavir (4.8%) and atazanavir (4.8%).

The baseline genotypic resistance test showed a median of 8 (6–10) protease-associated resistance mutations and 5 (3–6) nucleoside-associated mutations. Log VL and CD4 cell count at baseline were 5.15 (4.66–5.48) and 45 cells/mm\(^3\) (15–109), respectively.

Enfuvirtide plasma concentrations were measured in 111 plasma samples from 42 subjects. Results from the PK analysis are shown in Table 1.

VL decrease at weeks 4 and 12 was $-0.73 \pm 0.22$ and $-0.43 \pm 0.22$, respectively. CD4 cell count increase was 61 (24–106) and 39 (5.5–89) cells/mm\(^3\) at weeks 4 and 12, respectively. VR was reached in 18/42 (42.9%) and 12/42 (28.6%) subjects at weeks 4 and 12, respectively, whereas HIV-RNA undetectability at the same time points was achieved in 0 and 8 (19%) subjects, respectively.

In the univariate linear regression analysis, higher VL decrease at week 12 was associated with higher OBS ($R = 0.365$, $P = 0.02$) and higher mean enfuvirtide concentrations ($R = 0.405$, $P = 0.008$). Both parameters remained as independent predictors in the multivariate analysis ($R = 0.34$, $P = 0.021$ and $R = 0.356$, $P = 0.017$, respectively).

The univariate logistic regression analysis showed that only OBS ($P = 0.014$) and mean enfuvirtide concentration ($P = 0.017$) predicted VR at week 12. Both parameters remained as independent predictors in the multivariate analysis ($P = 0.015$ and $P = 0.022$, respectively). Results from both analyses are presented in Table 2. No influence of any co-administered drug considered on VR was observed. The ROC test provided a cut-off for

<table>
<thead>
<tr>
<th>Week 2 $n^a = 32$</th>
<th>Week 4 $n = 28$</th>
<th>Week 8 $n = 26$</th>
<th>Week 12 $n = 25$</th>
<th>Meanb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (± SD)</td>
<td>2584 (1346)</td>
<td>2700 (1455)</td>
<td>2502 (1681)</td>
<td>2354 (1391)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>2389 (1605–3519)</td>
<td>2514 (2454–3373)</td>
<td>2095 (1151–3411)</td>
<td>2050 (1528–2964)</td>
</tr>
</tbody>
</table>

SD, standard deviation; IQR, interquartile range.

Values are expressed as ng/mL.

$^a$n, number of enfuvirtide $C_{\text{trough}}$ measurements available.

$^b$Calculated as the mean of all averaged individual concentrations.

\(n = \text{number of enfuvirtide } C_{\text{trough}} \text{ measurements available.}\)
of those without VR at this time point (OBS/C21 described as a predictor of long-term therapeutic success. In our VR to enfuvirtide-based salvage regimens at week 12 has been

Discussion

CGR, background regimen; OBS, optimized background score; PRO-RMs, protease-associated resistance mutations; NAMs, nucleoside-associated resistance mutations.

Table 2. Linear and logistic regression analyses of determinants of HIV-RNA decrease from baseline to week 12, and week 12 VR

<table>
<thead>
<tr>
<th></th>
<th>Linear regression analysis</th>
<th>Logistic regression analysis</th>
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<tbody>
<tr>
<td></td>
<td>univariate analysis: beta</td>
<td>multivariate analysis: beta</td>
</tr>
<tr>
<td></td>
<td>(95% CI), P value</td>
<td>(95% CI), P value</td>
</tr>
<tr>
<td>Total no. of drugs in</td>
<td>−0.143 (−0.97; 0.369),</td>
<td>1.7 (0.53; 5.4),</td>
</tr>
<tr>
<td>BGR</td>
<td>P = 0.36</td>
<td>P = 0.36</td>
</tr>
<tr>
<td>OBS</td>
<td>−0.356 (−0.85; −0.075),</td>
<td>2.75 (1.22; 6.18),</td>
</tr>
<tr>
<td></td>
<td>P = 0.02</td>
<td>P = 0.014</td>
</tr>
<tr>
<td>Baseline PRO-RMs</td>
<td>−0.017 (−0.18; 0.16),</td>
<td>0.97 (0.75; 1.27),</td>
</tr>
<tr>
<td></td>
<td>P = 0.9</td>
<td>P = 0.86</td>
</tr>
<tr>
<td>Baseline NAMs</td>
<td>0.07 (−0.18; 0.29),</td>
<td>0.86 (0.61; 1.24),</td>
</tr>
<tr>
<td></td>
<td>P = 0.63</td>
<td>P = 0.43</td>
</tr>
<tr>
<td>Baseline VL</td>
<td>0.139 (−0.46; 1.19),</td>
<td>0.31 (0.08; 1.22),</td>
</tr>
<tr>
<td></td>
<td>P = 0.38</td>
<td>P = 0.09</td>
</tr>
<tr>
<td>Baseline CD4+ count</td>
<td>−0.098 (−0.008; 0.004),</td>
<td>1.006 (0.99; 1.01),</td>
</tr>
<tr>
<td></td>
<td>P = 0.5</td>
<td>P = 0.19</td>
</tr>
<tr>
<td>Mean week 12 enfuvirtide</td>
<td>−0.405 (−0.69; −0.11),</td>
<td>2.15 (1.14; 3.9),</td>
</tr>
<tr>
<td>C_{tough}</td>
<td>P = 0.008</td>
<td>P = 0.017</td>
</tr>
<tr>
<td></td>
<td>−0.356 (−0.65; −0.069),</td>
<td>2.06 (1.1; 3.86),</td>
</tr>
<tr>
<td></td>
<td>P = 0.017</td>
<td>P = 0.02</td>
</tr>
</tbody>
</table>

BGR, background regimen; OBS, optimized background score; PRO-RMs, protease-associated resistance mutations; NAMs, nucleoside-associated resistance mutations.

Boldface text represents statistically significant variables.

enfuvirtide C_{tough} of 2100 ng/mL (100% sensitivity and 60% specificity). According to the latter, 12/12 (100%) subjects with VR had enfuvirtide C_{tough} above 2100 ng/mL, whereas 12/30 (40%) subjects with virological failure (VF) showed enfuvirtide C_{tough} above this threshold (χ² = 12.6, P < 0.0001). In the same way, subjects with an OBS ≥ 2 showed a better virological outcome. In fact, 10/12 (83%) subjects with VR at week 12 had OBS ≥ 2, whereas the latter was observed in only 14/30 (46.7%) of those without VR at this time point (χ² = 4.7, P = 0.04).

In the sigmoid I_{max} inhibitory model, the maximum HIV-RNA decrease was estimated to be −1.64 log, with an estimated IC_{50} of 2037 ng/mL. Modelling showed that those subjects with OBS ≥ 2 reached a slightly higher I_{max} than those with OBS < 2 (−3.33 versus −2.64 log reduction, respectively). Moreover, subjects with OBS ≥ 2 had a higher IC_{50} than those with an OBS < 2 (2330 versus 7551 ng/mL).

Discussion

VR to enfuvirtide-based salvage regimens at week 12 has been described as a predictor of long-term therapeutic success. In our study, two factors were found to be associated with such early VR to enfuvirtide-based salvage regimens. Subjects with a higher number of active drugs in the BGR (OBS ≥ 2) showed a higher VL decrease and a higher probability of VR at week 12, thus confirming the findings from previous studies. This emphasizes the importance of combining enfuvirtide with drugs retaining significant residual antiretroviral activity. Current availability of recent or new compounds highly effective in salvage regimens (tipranavir, darunavir, maraviroc, raltegravir and etravirine) should lead to a better optimization of the OBS. Moreover, this is the first clinical report showing enfuvirtide plasma exposure as a predictor of VR in the clinical setting. An enfuvirtide concentration cut-off (2100 ng/mL) for VR has been identified. This exposure–response relationship could be relevant in this class of antiretroviral drugs that have an extracellular mechanism of action. In fact, in contrast to other classes of drugs, such as NRTIs, NNRTIs or PIs, plasma concentrations of enfuvirtide directly reflect the drug concentrations at the site of action.

This association was also confirmed by PK/PD modelling. Although the difference in I_{max} was not very pronounced in the comparison between models obtained from those with OBS < 2 and those with OBS ≥ 2 (−2.64 log versus −3.33 log, respectively), a significantly higher estimate of IC_{50} was predicted for subjects with OBS < 2 when compared with the latter (7551 versus 2330 ng/mL). This finding suggests that the interaction between OBS and enfuvirtide C_{tough} could be synergistic.

From a clinical point of view, there are interesting possible implications. First of all, enfuvirtide plasma exposure in the clinical setting is supposed to be lower than that reported under trial conditions, as confirmed by others. Moreover, in our previous report, a significant decrease in enfuvirtide plasma exposure was found to be associated with nodule formation after subcutaneous injection. In this context, new needle-free devices for subcutaneous injection, which reduce the frequency and/or severity of injection site reactions, thus reducing intra- and interpatient variability, are promising.

A main limitation of our study is the sample size. Although two OBS subgroups were balanced (18 versus 24 subjects), the small number of observations and the lack of higher enfuvirtide concentrations could have impaired the capacity of the model of predicting VL decrease associated with higher concentrations in subjects with OBS < 2. However, in patients with OBS ≥ 2, the problem is probably not relevant because I_{max} values are over the concentration range attained in these subjects. Another limitation regarding the design of our study is that plasma exposure of concomitant PIs was not considered among the possible determinants of VR.

386
In conclusion, an exposure–response relationship between enfuvirtide \( C_{\text{trough}} \) and early VR was found in the clinical setting, and a possible enfuvirtide \( C_{\text{trough}} \) cut-off was calculated for VR. Moreover, the PK/PD modelling showed that, in contrast to previously published data, the mean observed value for enfuvirtide \( C_{\text{trough}} \) was in the maximum shape region of the response–concentration curve, in which minimal changes of enfuvirtide exposure could be associated with considerable changes in probability of VL decrease. Therefore, optimization of enfuvirtide plasma exposure could deserve further evaluation as a determinant of therapeutic response in HIV-positive patients.

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**Transparency declarations**

None to declare.

**References**


