Clinical and pharmacological aspects of induction-maintenance therapy in HIV-1 positive patients: the ADAM study
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Chapter 8

Concentrations of stavudine, lamivudine, nelfinavir, and saquinavir in plasma, cerebrospinal fluid, and seminal plasma of HIV-1-infected individuals.


-submitted for publication-
Abstract

In the ADAM study, a study investigating the feasibility of induction maintenance therapy, a combination of four antiretroviral agents was used as an induction therapy for 26 or 50 weeks. In 12/65 HIV-1-infected patients participating this study, concentrations of stavudine, lamivudine, nelfinavir, and saquinavir, were assessed in plasma, cerebrospinal fluid (CSF) and semen after 26 or 50 weeks of continuous treatment. Five of them could not obtain semen on the requested day. Two patients refused a lumbar puncture. In all but one patient, the HIV-1 RNA concentration in both plasma and CSF had declined below 400 copies/mL at the time of sampling. The median plasma concentration of stavudine, lamivudine, saquinavir, and nelfinavir was 84 ng/mL, 359 ng/mL, 387 ng/mL, and 1190 ng/mL, respectively. Of the protease inhibitors, only saquinavir was detected at a low concentration in seminal plasma. Neither nelfinavir nor saquinavir was detected in the CSF. Concentrations of stavudine and lamivudine in seminal plasma were higher than in CSF: 104 vs. 49 ng/mL for stavudine (NS) and 1329 vs. 100 ng/mL for lamivudine (p=0.006). For lamivudine, seminal plasma/blood plasma ratios were significantly higher than CSF/blood plasma ratios. These data support the hypothesis that poor penetration of the central nervous system and the male genital tract by antiretroviral drugs can contribute to differences in viral dynamics in these compartments.
Introduction

Antiretroviral therapy decreases the levels of human immunodeficiency virus type 1 (HIV-1) in plasma, cerebrospinal fluid (CSF) and semen.\(^1\)\(^-\)\(^3\) However, the decline of HIV-1 RNA concentrations and the evolution of virus in the CSF or semen during therapy has not always been comparable to those in plasma, indicating viral compartmentalisation.\(^4\)\(^-\)\(^5\) A poor penetration of the male genital tract and the central nervous system by antiretroviral drugs is suggested to contribute to the differences in the viral dynamics in these compartments.

Compared to other antiretroviral drugs, the penetration of the protease inhibitors (PIs) into the CSF is relatively poor. In general, PIs are highly bound to plasma proteins and are not very lipid-soluble, impeding the entry of these drugs into the CSF.\(^6\) Reported concentrations of ritonavir, saquinavir and nelfinavir in CSF are low or below the lower limit of quantification.\(^7\)\(^-\)\(^8\) As an exception (possibly due to limited binding to plasma proteins), indinavir has been reported to enter the CSF in concentrations above the \textit{in vitro} 95\% inhibitory concentration.\(^9\) NRTIs penetrate the CSF, although the reported concentrations in CSF are usually lower than in plasma.\(^10\)

The penetration into the genital tract seems to vary between the different PIs. Taylor et al. showed good penetration of indinavir in contrast to poor penetration of ritonavir and saquinavir into the seminal plasma.\(^11\) Recently, Pereira et al. reported the concentrations of zidovudine and lamivudine in the semen and plasma of nine patients. Zidovudine concentrations and especially lamivudine concentrations in semen were higher than in plasma and were associated with a reduction of HIV-1 RNA concentrations in both seminal and blood plasma.\(^12\)

In the ADAM study, a study investigating the feasibility of induction maintenance therapy, a combination of four antiretroviral drugs was used as an induction therapy for 26 or 50 weeks.\(^13\) In this study, plasma, CSF, and semen were obtained in a subset of patients and the concentrations of stavudine, lamivudine, nelfinavir, and saquinavir were investigated.
Chapter 8

Methods

The ADAM study

In the ADAM study, an open-label randomised-controlled study, the feasibility of induction maintenance therapy in antiretroviral treatment was investigated. A quadruple induction regimen, consisting of stavudine, lamivudine, saquinavir, and nelfinavir, was used for 26 weeks in antiretroviral therapy-naive patients. At week 26, patients were randomised to either maintenance therapy or the prolongation of the quadruple drug regimen. In the latter group, a second randomisation was performed after 50 weeks of treatment. Patients, methods and the efficacy results of the induction-maintenance regimen in the ADAM study have been described elsewhere.\(^\text{13}\)

As part of the ADAM study, patients were asked to participate in a sub-study to assess the concentrations of the antiretroviral medication used in plasma, CSF and semen. The institutional review boards of all participating institutions approved this sub-study and written informed consent was obtained.

Patients

At the time of assessment, patients had to be using a quadruple drug regimen consisting of stavudine, (40 mg BID, or 30 mg BID if body weight < 60 kg), lamivudine (150 mg BID), saquinavir hard-gelatin-capsules (600 mg TID) and nelfinavir (750 mg TID). When saquinavir soft-gelatin-capsules became available (November 1\(^\text{st}\) 1997), all patients using saquinavir hard-gelatin-capsules (600 mg TID) switched to saquinavir soft-gelatin capsules (800 mg TID). Patients were instructed to take their medication with food.

Samples

On the day of sampling, patients were instructed to collect semen in a 50 mL sterile collection tube. Within 6 h of collection, semen was frozen at -70°C until testing. On the same day, a lumbar puncture was performed at a random time point. Part of the obtained CSF was used for an immediate assessment of the number of cells and the concentration of proteins and glucose. The other part was stored at -70°C until analysis. Immediately after the lumbar puncture, heparinized blood was obtained by venipuncture for an assessment of stavudine, lamivudine, saquinavir, and nelfinavir concentrations. The time of last medication intake and the time of sample withdrawal were recorded.
Drug concentrations in plasma, CSF, and seminal plasma

The concentrations of stavudine and lamivudine in plasma, CSF, and seminal plasma were quantified simultaneously using an HPLC-assay. Stavudine and lamivudine were extracted from plasma, CSF, and seminal plasma using silica extraction columns prior to isocratic, reversed-phase HPLC with ultraviolet detection at 270 nm. The method has been validated over the range of 10-5,000 ng/mL using a 500 μL sample volume. Simultaneous quantification of saquinavir and nelfinavir in plasma, CSF, and seminal plasma was performed using an HPLC-assay, as previously published. Briefly, sample pre-treatment consisted of solid-phase extraction. Seminal plasma and CSF samples were diluted with blank human plasma (1:1 v/v) prior to isocratic ion-pair, reversed-phase HPLC and were detected at 239 and 210 nm, respectively. The lower limit of quantification for saquinavir and nelfinavir was 25 and 50 ng/mL, respectively, using a 600 μL sample volume. The assay was linear up to concentrations of at least 25 μg/mL.

The HIV-1 RNA concentration in plasma, CSF, and seminal plasma was measured using a commercially available PCR assay with a variable lower limit of detection (Amplicor HIV Monitor Test, Roche Diagnostic Systems Inc., Branchburg, NJ, USA).

Statistical analysis

All data were tabulated. Median and interquartile ranges were calculated. If the value of the HIV-1 RNA concentration or the drug concentration was below the lower limit of quantification, the value of the cut-off point was used for all calculations. Seminal plasma/blood plasma concentration ratios and CSF/blood plasma concentration ratios were used as a measure for drug penetration into these tissues.
Chapter 8

Results

Patients

Of the 65 patients in the ADAM study, 12 patients were willing to participate in the sub-study. In one patient the time of assessment was not according to protocol, he had a lumbar puncture at week 12 for a medical reason and did not supply any semen. Four patients did not succeed in obtaining semen on the requested day. Two patients refused the lumbar puncture for logistic or personal reasons, but were able to collect semen.

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<th>CD8+ cells/mm³</th>
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Median: 43 181 81 78000 380 1260  
Range: 30-50 168-200 62-92 4200-270000 180-560 320-2840

Patient characteristics

Characteristics of all participating patients at baseline and at time of sampling are tabulated in Tables 1 and 2. None of the patients was diagnosed as having AIDS. The median CD4+ and CD8+ cell count at baseline was 380 and 1260 cells/mm³, respectively. All but one patient (12) were using saquinavir – SGC at time of sampling. In all patients the CD4+ cell count increased during therapy. The median plasma HIV-1 RNA concentration at baseline was 4.89 log₁₀ copies/mL. In one patient the plasma HIV-1 RNA concentration was >400 copies/mL at the time of sampling. In all other patients, the plasma HIV-1 RNA concentration had declined below 400 copies per mL, although in four of these patients the plasma HIV-1 RNA was detectable (Table 1).
Drug concentrations in plasma, CSF, and seminal plasma

None of the patients had a neurologic disease or genital infection at the time of sampling. The median total protein concentration in CSF was 0.45 g/L (IQR 0.28-0.47 g/L), and the total cell count in CSF did not exceed 14 cells/μL (data not shown).

Table 2 Parameters at the time of sampling

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<th>Patient</th>
<th>week</th>
<th>CD4⁺ cells/mm³</th>
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Median Range 560-900 240-810 230-1980 16-3890 4-1514

*NA: not assessed; - : not sampled

HIV-1 RNA

The HIV-1 RNA concentrations in plasma and CSF were assessed in 12 and 10 samples respectively (Table 2). HIV-1 RNA concentrations in CSF were in the same order of magnitude than those in plasma. Two patients had a detectable HIV-1 RNA concentration in CSF. One of these patients also had a high HIV-1 RNA in plasma. This patient might not have been compliant with the study medication, since drug levels of this patient were in the lower range (See Table 3). Unfortunately, HIV-1 RNA assessment in semen failed due to processing errors.

Drug concentrations of the NRTIs and PIs in plasma, CSF and seminal plasma are listed in Table 3. The median plasma concentration of stavudine and lamivudine was 84 ng/mL (range <10-321 ng/mL) and 359 ng/mL (range 135-1187 ng/mL), respectively. Nelfinavir and saquinavir were present in plasma at a median concentration of 1190 ng/mL (range 870-2860 ng/mL) and 387 ng/mL (range 57-1062 ng/mL), respectively.
Table 3  Drug concentrations in plasma, CSF, and semen

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<td>1273</td>
<td>319</td>
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Drug concentrations

The penetration of the CSF by NRTIs was limited. The median concentration of stavudine and lamivudine in CSF was 49 ng/mL (range 32-80) and 100 ng/mL (range 38-409) respectively. These concentrations of stavudine and lamivudine in CSF were usually above the in vitro IC50 concentrations for most reported wild-type HIV-1 strains.\textsuperscript{16,17} Neither saquinavir nor nelfinavir was detected in the CSF.

Both stavudine and lamivudine were present in semen (median 104 ng/mL (range 30-1273), and median 1329 ng/mL (range 454-6642 ng/mL), respectively). Of the PIs only saquinavir was present at a low concentration in three of eight semen samples.

In some patients the time interval between drug intake and time of sampling was not known. CSF and plasma were drawn as a paired sample at a median time interval of 3.5h (range 0.33-7h, n=9)). Semen was usually sampled close to the time-point of medication intake (median 0.1h (range -0.5-1.75h, n=5)). Figure 1 illustrates the CSF, semen and plasma concentrations of stavudine and lamivudine in relation to the time interval between drug intake and time of sampling.

The CSF/blood plasma and seminal plasma/blood plasma concentration ratios were used as a measure for penetration of the NRTI into the CSF and semen. Using these ratios, the penetration of stavudine into the CSF and semen appeared comparable (median CSF/blood plasma ratio 0.87, IQR 0.23-1.69; median seminal plasma/blood plasma ratio 1.41, IQR 0.06-3.18).
The penetration of lamivudine was, however, significantly higher in semen than in CSF (Figure 2; median CSF/blood plasma ratio 0.33, IQR 0.12-0.38; median seminal plasma/blood plasma ratio 2.57, IQR 1.14-4.04).

Upon comparing absolute concentrations of plasma, CSF and semen, the lamivudine concentrations in semen were higher than the concentrations in both CSF and plasma (Figure 3; p=0.006 and p=0.02, respectively).
Figure 1  The concentrations of NRTIs and the time interval between medication intake and sampling. The white dots represent blood plasma concentrations and the black dots and stars represent CSF and seminal plasma concentrations, respectively.
Panel A: Stavudine.
Panel B: Lamivudine.
Discussion

To our knowledge, these are the first data regarding the concentrations of stavudine, lamivudine, nelfinavir, and saquinavir in both CSF and semen of HIV-1-infected patients. In most patients, the concentration of stavudine and lamivudine that entered the semen was higher than the CSF. In most cases, nelfinavir and saquinavir were not detectable in CSF and semen. These findings confirm that the male genital tract and the central nervous system should be considered as different biological compartments (as compared to plasma) from a pharmacokinetic point of view. Different penetration of antiretroviral agents may therefore attribute to distinct viral dynamics in the different body compartments.

Although data are limited, the blood tissue barrier of the male genital tract is assumed to behave like a lipid barrier.18 Passive diffusion from blood to semen is therefore hypothesised to become facilitated if an agent has high lipid solubility, a low plasma protein-binding, and a favourable dissociation constant.18 This dissociation constant indicates the pH at which equimolar concentrations of non-ionised and ionised forms of the drug exist. Non-ionised compounds diffuse more easily across lipid barriers. In addition, ion-trapping mechanisms can attribute to the accumulation of acid compounds in alkaline compartments and of basic compounds in acidic compartments. In our study, the concentration of stavudine and lamivudine that entered the genital tract was higher than the concentration present in plasma. The seminal plasma/blood plasma concentration ratios of the NRTIs in our study were not as high as the seminal plasma/blood plasma concentration ratios of zidovudine (6) and lamivudine (13) as recently reported by Pereira et al.12 However, the comparison is difficult to make, since both studies have low numbers of patients and the two studies differ for their design, especially regarding the time interval between sampling of semen and blood. These studies indicate that, in addition to passive diffusion, active transportation or accumulation might contribute to the relatively high concentrations of NRTIs in semen. Stavudine and lamivudine differ for their proteinbinding (negligible vs. 10-50%) and dissociation constant (pKa: 10 vs. 4.3), thus providing a possible explanation for the observed differences in the seminal plasma/blood plasma concentration ratios of stavudine and lamivudine. In addition to differences in passive diffusion, the physico-chemical characteristics of lamivudine may be more favourable for entrapment in the protein-loaded, alkaline semen.18
Figure 2  The seminal plasma/blood plasma ratios and CSF/blood plasma ratios of stavudine and lamivudine. The black dots represent the CSF/blood plasma ratios. The white dots represent the seminal plasma/blood plasma ratios. Panel A: Stavudine. Panel B: Lamivudine.
Figure 3  The drug concentrations of stavudine and lamivudine in plasma, CSF, and semen. Data are median and interquartile range; the bars represent the complete range.
Panel A: Stavudine.
Panel B: Lamivudine.
A more trivial explanation for the observed differences between the semen/plasma ratios is the difference in time to \( C_{\text{max}} \) of stavudine (3.8 h) and lamivudine (1.0-1.5 h).

Since the sampling time of plasma was often closer to the time to \( C_{\text{max}} \) of stavudine than of lamivudine, and semen concentrations were suggested to be rather stable over time [13,20], seminal plasma/blood plasma concentration ratios of stavudine may have been underestimated as compared to lamivudine.\(^\text{18}\)

The concentrations of stavudine and lamivudine in CSF were low compared to the concentrations in semen. Differences in time of sampling are probably not significant for this comparison, since both the concentrations in semen and in CSF are considered to be rather stable over time.\(^\text{6,19}\) There are however several differences in the physiology of CSF and semen that might influence the penetration of these antiretroviral drugs. The blood tissue barrier of the central nervous system is probably more impermeable and more extensive than that of the male genital tract.\(^\text{20}\) The choroid plexuses actively produce CSF. The renewal of CSF occurs 4-5 times daily.\(^\text{6}\) Semen, however, consists of a composition of secretions from the testes, the seminal vesicles and the prostate, all of which have their own physiological characteristics.\(^\text{18}\) The renewal of semen is partly dependent on the frequency of ejaculation. The impact of these fluid characteristics on antiretroviral drug concentrations in both CSF and semen needs further exploration.

In contrast to the substantial penetration of the NRTIs into CSF and semen, neither nelfinavir nor saquinavir was present in the semen or the CSF at significant concentrations. The lack of penetration of both nelfinavir and saquinavir into the CSF confirms earlier observations of poor penetration of highly protein-bound PIs with a high molecular weight into the CSF.\(^\text{6}\) Even if protein-binding capacity, lipophilicity and molecular weight would not prevent the influx into these compartments, it is possible that an active efflux by, for example, P-glycoprotein interfered with the achievement of detectable levels of these agents in the central nervous system and the male genital tract.\(^\text{20-22}\)

Our data support the hypothesis that poor penetration of the central nervous system and the male genital tract by antiretroviral drugs can partly explain the distinct viral dynamics in these tissues as compared to plasma.\(^\text{4,5}\) We were unable to assess the efficacy of the quadruple regimen in these compartments, since no samples for the assessment of baseline HIV-1 RNA concentrations in CSF or semen were obtained. The HIV-1 RNA concentrations in plasma and CSF were however in the same order of magnitude after 26 or 50 weeks of quadruple drug therapy.
Drug concentrations in plasma, CSF, and seminal plasma

Future studies might shed more light on the consequences of varying levels of antiretrovirals in CSF and semen, not only for the durability of viral suppression, but also on HIV-1 transmission within the population.

Appendix

In the ADAM Study Team participated:
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References


Drug concentrations in plasma, CSF, and seminal plasma


