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Low atazanavir concentrations in cerebrospinal fluid

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Abstract

Objective—Protease inhibitors may not penetrate into the central nervous system in therapeutic concentrations, which may allow ongoing HIV replication and injury. The objective of this study was to determine atazanavir penetration into cerebrospinal fluid (CSF).

Design—Single random plasma or paired plasma and CSF samples were drawn from participants enrolled in a multicenter, observational cohort study and taking atazanavir with or without ritonavir between October 2003 and October 2005.

Methods—Plasma samples were assayed by high performance liquid chromatography and immunoassay; lower limit of detection was 45 ng/ml. CSF samples were assayed by immunoassay (ARK ATV-test); lower limit of detection was 5 ng/ml.

Results—One hundred and seventeen participants (43 ± 7.7 years, 79% men, 81 ± 15 kg) had plasma or plasma and CSF paired samples drawn a median (interquartile range) of 10 (5–17) h postdose. Median (interquartile range) plasma atazanavir concentrations with or without ritonavir were 1278 (525–2265) and 523 (283–1344) ng/ml. The median (interquartile range) CSF concentrations with or without ritonavir were 10.3 (<5–21.1) and 7.9 (6.6–22) ng/ml. Nineteen of 79 (24%) CSF samples were less than 5 ng/ml. CSF concentrations were less than 1% of plasma concentrations and near the atazanavir wild-type IC_{50} of 1–11 ng/ml.

Conclusion—Atazanavir CSF concentrations are highly variable and 100-fold lower than plasma concentrations, even with ritonavir boosting. CSF concentrations of atazanavir do not consistently exceed the wild-type IC_{50} of atazanavir and may not protect against HIV replication in the CSF.

Keywords

atazanavir; central nervous system; cerebrospinal fluid; pharmacology; protease inhibitors

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Author contributions: All coauthors reviewed, revised for content, and approved this article. Best, Letendre, and Capparelli participated in conception and design of study, with revision and approval by Clifford, Collier, Gelman, McArthur, McCutchan, Simpson, Grant, and Ellis. Data were acquired by Clifford, Collier, Gelman, McArthur, McCutchan, and Simpson. Brigid, Best, Letendre, and Capparelli analyzed and interpreted the data, with review by Clifford, Collier, Gelman, McArthur, McCutchan, Simpson, Grant, and Ellis.

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Background

Combining protease inhibitors or nonnucleoside reverse transcriptase inhibitors with nucleoside analogue reverse transcriptase inhibitors can dramatically reduce HIV replication, preserve immune function, and prolong survival [1–3]. Combination therapies also may reduce HIV replication in the central nervous system (CNS), contributing to the declines noted in the incidence of neurological complications of HIV infection [4]. However, HIV-associated neurocognitive impairment remains prevalent for reasons that are unclear [5–7].

In some advanced AIDS patients, HIV replicates in the CNS independent of the systemic sources of HIV found in the blood as shown by genetic differences between HIV from brain/cerebrospinal fluid (CSF) and from blood [8,9]. As some antiretrovirals penetrate into the CNS poorly, it could act as a compartment in which low drug levels allow ongoing HIV replication, local tissue injury, development of resistant virus, and treatment failure in spite of suppression of HIV systemically [10]. Multiple protease inhibitors have been found in much lower concentrations in the CSF than in blood [11–15]. Most protease inhibitors are substrates of the efflux pump, P-glycoprotein; are highly protein-bound; and are large molecules. All of these characteristics may inhibit penetration across the blood–brain barrier in therapeutic concentrations. Thus, the contribution of protease inhibitors to antiviral efficacy in the CNS is unclear.

Atazanavir is one of the most frequently prescribed antiretrovirals. This protease inhibitor is 86% bound to plasma proteins, leaving 14% free to penetrate into the CNS. Randall *et al.* [16] found that CSF atazanavir concentrations were approximately 1% of plasma concentrations in seven HIV-infected patients. In the Atazanavir-Ritonavir Monomaintenance (ATARITMO) study [17], atazanavir CSF concentrations averaged 0.9% of plasma concentrations and three of 20 (15%) patients whose HIV levels were immeasurable in plasma had detectable levels of HIV in CSF after 24 weeks on atazanavir/ritonavir maintenance therapy. The objective of this study was to expand the limited observations of atazanavir penetration into the CSF of HIV-infected individuals.

Methods

Participants

Participants were enrolled in a six-center, observational cohort study, CNS HIV Antiretroviral Therapy Effects Research (CHARTER), to determine the effects of potent antiretroviral therapy on HIV-associated neurological disease. Single plasma and CSF samples were drawn at biannual study visits between October 2003 and October 2005. Data from one to three study visits were included for each participant in this analysis. Demographic and clinical characteristics were summarized from the first visit included in this analysis for each participant. Plasma/CSF sample pairs were drawn within an hour of each other [median (interquartile range, IQR), 23 (17–34) min]. The 117 participants included in this analysis were taking atazanavir with or without ritonavir for a median (IQR) of 6.6 (2.2–12.2) months at the time of first sampling. Eighty pairs of CSF and plasma samples and an additional 80 plasma samples from participants taking atazanavir were randomly selected from the sample repository. Doses included 300 or 400 mg of atazanavir daily, with or without concomitant 100 mg of ritonavir daily.

Measurements

Samples were assayed by rapid, automated enzyme immunoassays (ARK ATV-tests, ARK Diagnostics, Inc. Sunnyvale, California, USA). Plasma validation interassay precision was less than 9.2% coefficient of variation and accuracy was within 11% deviation. Calibration

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standards ranged from 0.25 to 8 μg/ml with a sensitivity of 0.128 μg/ml. CSF validation, interassay precision, and accuracy were within 18% at 5 ng/ml (the CSF atazanavir sensitivity limit) and within 15% for other controls. Concentrations from the ARK method strongly correlated with those from a validated high performance liquid chromatography (HPLC) method ($r^2 = 0.96$).

Analyses

Population pharmacokinetic parameters were estimated for participants using nonlinear mixed effects modeling (NONMEM version V; ICON Development Solutions, Ellicott City, Maryland, USA), with the FOCE (first-order conditional estimation) subroutine with interaction. A one-compartment model with first-order absorption and elimination (ADVAN2 TRANS1) provided parameter estimates of plasma elimination rate and apparent volume of distribution, with absorption rate (k_a) fixed to the value reported in the Reyataz Capsules (atazanavir sulfate; Bristol-Myers Squibb Company, Princeton, New Jersey, USA) prescribing information (0.9 h^{-1}) [18]. A two-compartment physiologic model (ADVAN4 TRANS1) with first-order absorption and elimination provided estimates of atazanavir penetration into the CSF. For concentrations below the assay limit of quantitation, a value of one-half the quantitation limit was used for modeling. Concentrations drawn more than 48 h after a reported atazanavir dose were excluded from the analysis (one CSF and 12 plasma samples).

Pearson's correlation measured the association between plasma and CSF atazanavir concentrations. Wilcoxon rank-sum tests compared atazanavir concentrations with concomitant ritonavir to those without ritonavir. The χ^2 test was used to compare the proportion of participants with detectable atazanavir in the CSF to the proportion of participants with detectable CSF viral loads (>50 copies/ml).

Results

Participants were mostly men [92/117 (79%)], averaged 43 ± 7.7 years of age, and weighed a mean of 81 ± 15 kg. The median (IQR) plasma HIV RNA and CD4 cell counts were less than 50 (<50–648) copies/ml and 376 (215–537) cells/μl, respectively. CSF HIV RNA levels were suppressed to less than 50 copies/ml in 53 of 76 persons (70%) and plasma levels were similarly suppressed in 58 of 114 (51%). These participant characteristics were similar to those in the overall CHARTER cohort [19].

Ritonavir more than doubled plasma atazanavir concentrations, but elevated CSF concentrations less dramatically. From 148 plasma and 79 CSF samples, median (IQR) plasma atazanavir concentrations were 1278 (525–2265) ng/ml in patients taking concomitant ritonavir and 523 (283–1344) ng/ml in patients on atazanavir alone (Table 1). Median (IQR) CSF concentrations of atazanavir with and without concomitant ritonavir were 10.3 (<5–38) and 7.9 (<5–40) ng/ml, respectively.

Eighteen plasma samples from participants taking atazanavir without concomitant ritonavir were too few to estimate with confidence the pharmacokinetic parameters in this group; therefore, the population model was restricted to those participants taking atazanavir with ritonavir. Table 1 summarizes the estimated population pharmacokinetic parameters. The elimination rate (k_e) and apparent volume of distribution (V_d/F) correspond to a plasma half-life of 15 h, and an oral clearance of 9.4 l/h, similar to other published estimates [20,21]. The variation in plasma concentrations was 49%, whereas variation in CSF concentrations was less, at 26%. The modeled estimate of atazanavir penetration into the CSF was low, at 0.74%, meaning that CSF atazanavir concentrations and area-under-the time-concentration-curves (AUCs) are less than 1% of the corresponding plasma concentrations and AUCs (Fig. 1a and b).

Atazanavir concentrations in 17 of 149 (11%) plasma and 19 of 79 (24%) CSF samples were undetectable (<0.13 **μ**g/ml and <5 ng/ml, respectively). Eight pairs of plasma and CSF samples were undetectable in two participants taking atazanavir alone and six taking atazanavir with ritonavir, suggesting recent poor adherence to atazanavir. Nine participants had undetectable plasma concentrations without a corresponding CSF sample from that study visit. The remaining 11 CSF samples that had no detectable atazanavir had measurable plasma atazanavir, with a median (IQR) plasma concentration of 315 (280–432) ng/ml. The 60 CSF samples with detectable atazanavir had a corresponding median (IQR) plasma concentration of 1743 (925–2919) ng/ml. Consistent with this finding, higher plasma concentrations correlated with higher CSF concentrations ($r^2 = 0.35$).

Fifty-four percentage (43/79) of CSF atazanavir concentrations were below the approximate IC_{50} for wild-type virus (~11 ng/ml [18,22]) measured in human serum containing drug-binding proteins and 24% (19/79) were near (<5 ng/ml) the wild-type IC_{50} of 1 ng/ml [18, 22] estimated with no protein. If no protein binding occurs in CSF, then the IC_{50} estimated under experimental conditions that exclude protein would be an appropriate comparator. After excluding specimens from patients with probable nonadherence (samples that were below detection in both plasma and CSF), 11/67 (16%) of CSF specimens had concentrations near the protein-free IC_{50} (<5 ng/ml). Seven of 19 (37%) participants with no measurable atazanavir in the CSF had detectable (>50 copies/ml) HIV RNA in the CSF, whereas 12/60 (20%) participants with more than 5 ng/ml of atazanavir in the CSF had measurable CSF HIV RNA ($P = 0.13$).

Discussion

In this large study of patients taking atazanavir in six academic clinics in the United States, modeled pharmacokinetic parameter estimates were similar to data published from two smaller studies, including substantial intersubject variability. Atazanavir concentrations in the CSF are about 100-fold lower than plasma concentrations, even with ritonavir boosting. Our modeled and observed value of less than 1% penetration of atazanavir into the CSF from the plasma confirmed the previous reports in smaller studies [16,17]. The estimated free concentration of atazanavir in plasma (14% of 1510 ng/ml) is approximately 210 ng/ml. If unbound atazanavir freely distributed into the CSF by passive diffusion, then CSF concentrations should be approximately 210 ng/ml. The observed CSF concentrations of 10 ng/ml support highly effective transport of atazanavir from the CSF. Other potential explanations for this finding include inaccurate protein-binding estimates or interference with free plasma atazanavir from entering the CSF. Our observation of a positive correlation between plasma and CSF atazanavir concentrations suggests that increasing plasma atazanavir exposure may increase CSF penetration.

The exact 50% inhibitory concentration of atazanavir against wild-type HIV in the CSF has not been directly measured. Normal CSF has only low levels of binding proteins, like albumin, and, in a protein-free medium, the IC_{50} of 1 ng/ml is ten-fold less than that with plasma in the system (IC_{50} of 11 ng/ml) [18,22]. As CSF does contain some protein, the true IC_{50} is probably between 1 and 11 ng/ml. More than half of the CSF specimen concentrations in our study were below 11 ng/ml and a quarter were less than 5 ng/ml. This suggests that atazanavir CSF concentrations may exceed the wild-type IC_{50} in some, but not all patients, and atazanavir-containing regimens may not fully suppress HIV replication in CSF. This conclusion is supported by the ATARITMO study in which 15% of participants with undetectable plasma viral loads had detectable HIV RNA in the CSF while on ritonavir-boosted atazanavir monotherapy [17]. Notably, this marginally effective penetration contrasts with that of another widely used protease inhibitor, lopinavir/ritonavir, which had CSF concentrations that exceeded the IC_{50} by an average of five-fold in one study [23], and which produced substantial declines in CSF HIV RNA levels over just 3 weeks in all 13 participants receiving monotherapy [24]. Of course, CSF concentrations of antiretrovirals may not be a true reflection of concentrations at the site of action in the brain tissues. However, assuming that the CSF concentrations are a reasonable surrogate marker for brain antiviral activity, we recommend that when atazanavir is part of an antiretroviral regimen, additional CNS-penetrating antiretrovirals should be included to assure adequate treatment of the CNS in patients on atazanavir-based regimens.

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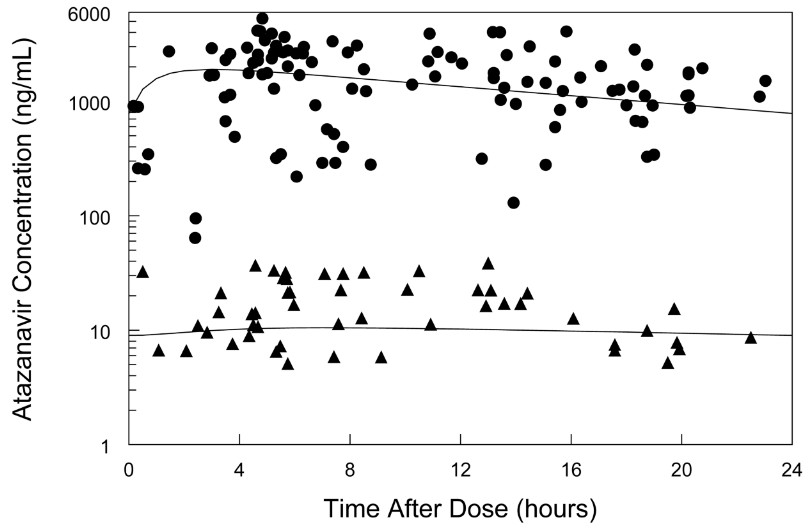
1a.



1b.

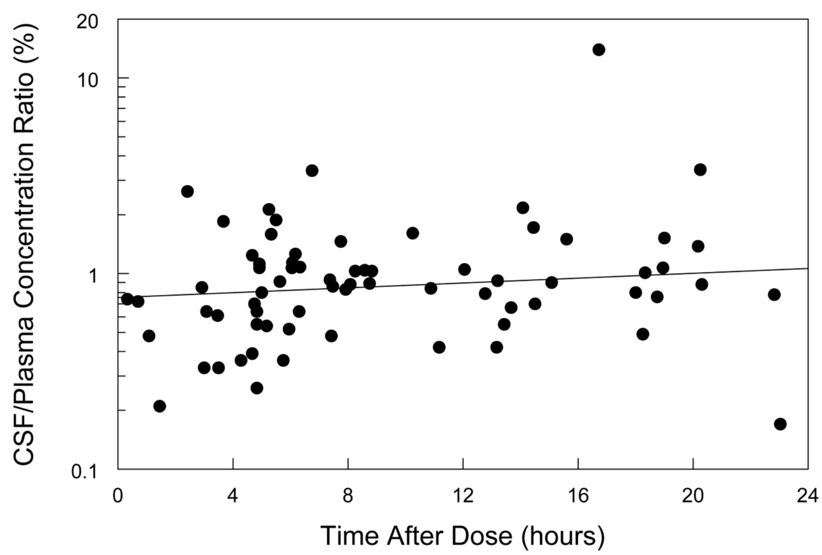


Fig. 1. Cerebrospinal fluid and plasma concentrations of atazanavir over time

(a) It depicts measurable atazanavir concentrations on a log scale as a function of time after dose. Closed circles show plasma concentrations measured from participants taking atazanavir with ritonavir. Closed triangles show cerebrospinal fluid (CSF) concentrations measured from participants taking atazanavir with ritonavir. Lines show the model-predicted plasma and CSF concentrations over time in the population. (b) It shows the CSF/plasma atazanavir concentration ratio over time (closed circles), with a linear regression line.

Table 1

Atazanavir concentrations and pharmacokinetic parameter estimates.

Concentrations	Without ritonavir	With ritonavir
Number of plasma samples	18	130
Median (range) plasma concentration (ng/ml)	523 (<128–6200)	1278 (<128–5295)[*]
Median (range) time postdose (h)	9.1 (1.1–48)	10.5 (0.2–38)
Number of CSF samples	11	68
Median (range) CSF concentration (ng/ml)	7.9 (<5–40)	10.3 (<5–38)[†]
Median (range) time postdose (h)	8.5 (1.4–18)	7.7 (0.5–27)
Number of paired CSF/plasma samples	9[a]	62[a]
Median (range) CSF/plasma ratio	0.0112 (0.005–0.139)	0.009 (0.002–0.034)

Population parameter estimates[b]	Atazanavir with ritonavir
N	100
V_d/F: apparent volume of distribution (l)	209 (40)
k_e: elimination rate (h^{-1})	0.045 (0.007)
CSF penetration (% of plasma concentration)	0.0074 (0.74%)
Intersubject variability of k_e	17%
Residual variability of plasma concentrations (%)	49%
Residual variability of CSF concentrations (%)	26%

CSF, cerebrospinal fluid.

[a] Participants with undetectable concentrations in plasma and CSF were excluded.

[b] Expressed as population estimate (standard error of the estimate).

[*] $P = 0.07$ by Wilcoxon rank-sum test for median plasma atazanavir without versus with ritonavir.

[†] $P = 0.8$ by Wilcoxon rank-sum test for median CSF atazanavir without versus with ritonavir.