

Differential brain tissue penetration of antiretrovirals and fluconazole

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ABSTRACT

Background: The central nervous system (CNS) is believed to be a significant reservoir for pathogens such as HIV and *Cryptococcus*; however, current understanding of drug penetration into the CNS is limited and largely based on cerebrospinal fluid (CSF) concentrations. However, CSF is not brain tissue. Herein we used tissues collected post-mortem from HIV-infected Ugandan subjects co-infected with *Cryptococcus* to characterize the relative distribution of antiretrovirals and antifungal agents across plasma and CNS compartments.

Methods: Following obtainment of written, informed consent from next of kin, post-mortems were performed on five subjects co-infected with HIV and cryptococcal meningitis. Tissues from cerebellum, pons, and CSF were snap frozen in liquid nitrogen. Whole blood was collected from femoral vein into EDTA tubes and stored on ice for 1 hour before separating plasma from cell pellets. All samples were transferred to -80° C for storage. Following tissue homogenization, drug quantification was performed using high performance (efavirenz) or ultrahigh performance (tenofovir, lamivudine, fluconazole) liquid chromatography coupled with triple quadrupole mass spectrometer. Calibrator standards and quality control (QC) samples were prepared in the matrix to match the sample tested; bovine brain homogenate was used for brain tissue, a solution of salt and proteins for CSF, and lithium heparin for plasma. Data are reported as median (range).

Results: Post-mortems were performed 5.2 (2.2-13.7) hours following death. Three individuals receiving daily tenofovir/lamivudine/efavirenz (300/300/600 mg) had detectable drug in all tissue compartments. Likewise, four individuals receiving fluconazole (400-1200 mg daily) had detectable drug in all compartments. CSF: plasma ratios were similar to values reported in the literature. Drug exposure in brain tissues was consistently lower than CSF for tenofovir, lamivudine, and fluconazole and higher than CSF for efavirenz.

Conclusions: Tenofovir, lamivudine, and fluconazole exposure in CSF over-predicted brain tissue penetration. In contrast, CSF exposure of efavirenz under-predicted brain exposure. These findings highlight the limitations of CSF as a surrogate for overall CNS drug exposure. Validation in larger cohorts is warranted. These data support the use of post-mortem tissues to assess drug distribution to and within the CNS, relevant for HIV reservoir eradication.

BACKGROUND

- HIV and related infections can lead to significant neurologic sequelae.

- HIV enters the brain within days of infection, leading to neuroinflammation and immune activation.
- Prevalence of HIV-associated neurocognitive disorder is estimated between 33-52% among HIV infected.
- Prevalence may be even higher in African countries.

- Knowledge of drug exposure in the CNS is largely limited to data in cerebrospinal fluid (CSF).

- CSF drug penetration does not correlate with neuro-cognitive outcomes.

- Due to inability to adequately sample brain tissue in living subjects, limited information is known about drug distribution to and between different CNS compartments.

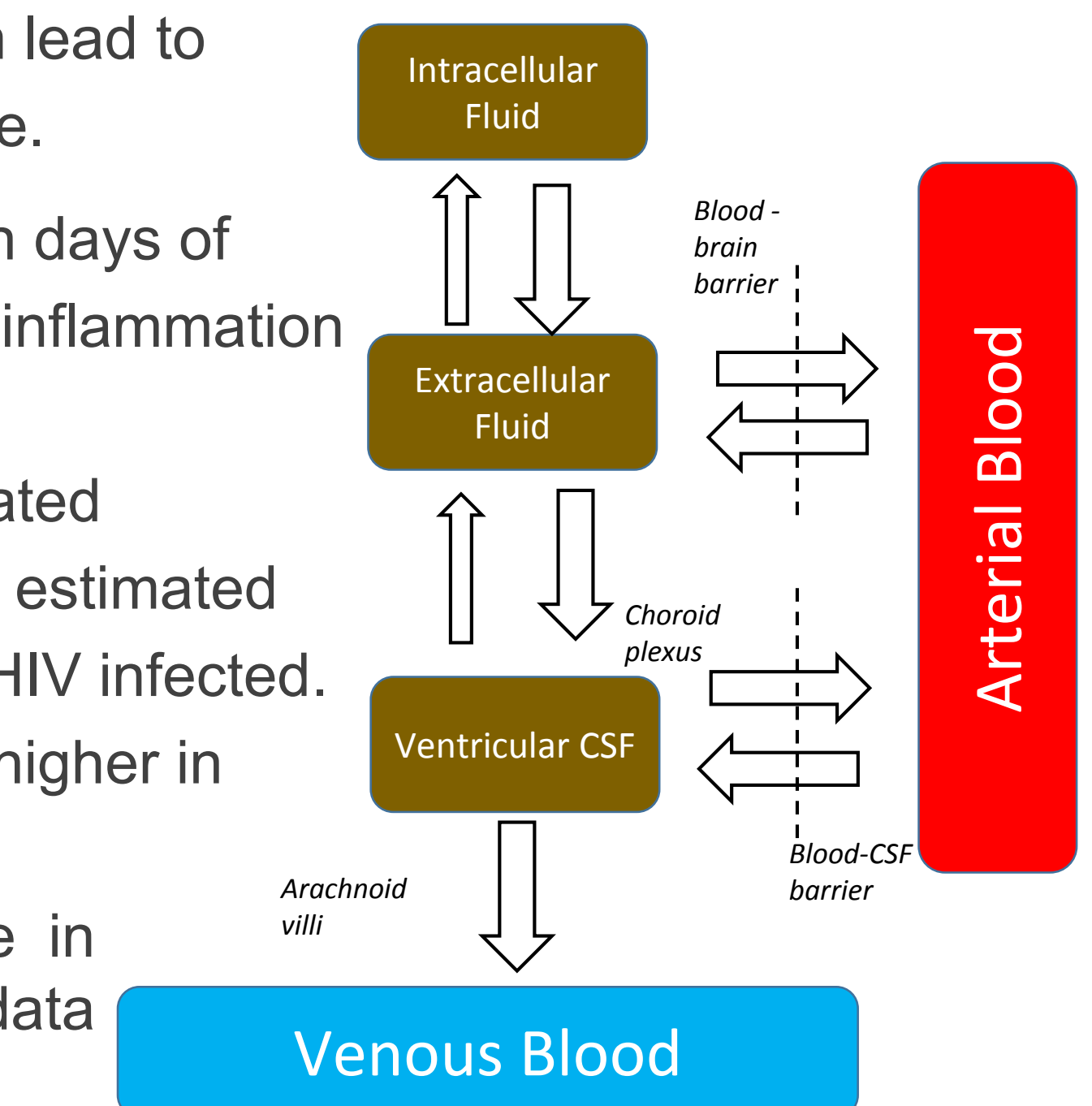


Figure 1: Schematic of solute trafficking between CNS and systemic circulation. CSF is generated in the choroid plexus and can be secreted passively into venous blood or actively transported into arterial blood. Drug transporters at the blood-brain and blood-CSF barriers limit movement between compartments. Adapted from Shen *et al* 2004 and Shannon *et al* 2013.

METHODS

- Upon death, study staff obtained written, informed consent from next of kin to perform post-mortem and collect tissues.

Table 1: CNS Tissues Collected for Analysis	
Blood plasma	Occipital Lobe
Cerebrospinal fluid	Globus pallidus
Cerebellum	Hippocampus
Pons	Midbrain/substantia nigra
Frontal Lobe	Medulla oblongata
Corpus Callosum	Cervical spinal cord/meninges
Parietal lobe	Choroid plexus/arachnoid granulations

- Tissues from discrete regions of the brain (see Table 1) as well as other organs were snap frozen in liquid nitrogen. Whole blood was collected from femoral vein into EDTA tubes before being separated into plasma and cell pellets.

- Drug quantification was performed at the UMN Clinical Pharmacology and Analytical Services Laboratory using high performance (efavirenz) or ultrahigh performance (tenofovir, lamivudine, fluconazole) liquid chromatography coupled with triple quadrupole mass spectrometer.

- Calibrator standards and quality control (QC) samples were prepared in the matrix to match the sample tested; bovine brain homogenate was used for brain tissue, a solution of salt and proteins for CSF, and lithium heparin for plasma.

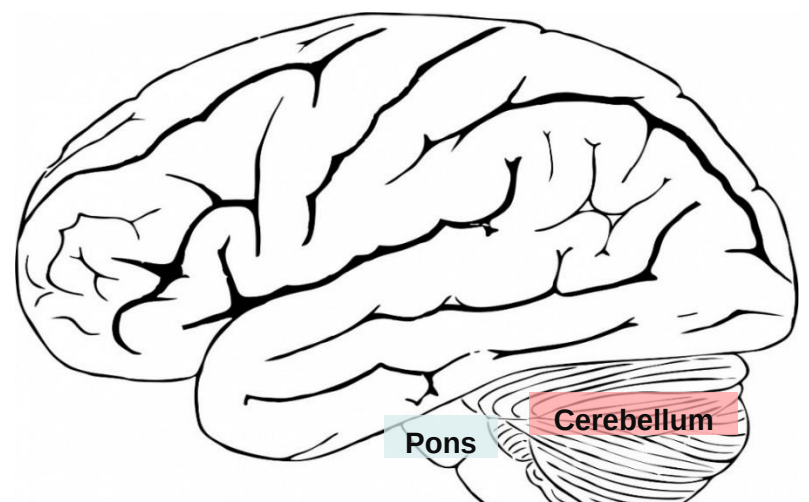


Figure 2: Location of cerebellum and pons, the tissues described in this analysis.

STUDY SUBJECTS

Table 2: Subject demographics and dosing history

Subject	Sex	Age yrs	Weight kg	Post-mortem interval hours	ARV regimen (mg)	Months on ARV regimen	Fluconazole mg/day	Days on fluconazole	Hours since last fluconazole dose
007	M	43	Unk	13.7	TDF/3TC/EFV (300/300/600)	26+	--	---	--
009	M	32	53	9.5	None	--	800	42	26
010	M	47	50	2.5	None	--	1200	3*	13
011	F	28	30	5.2	TDF/3TC/EFV (300/300/600)	2	400	22	30
013	F	30	55	2.2	TDF/3TC/EFV (300/300/600)	4	800	3*	6

*010 had received 2 doses of fluconazole prior to death; 013 had received 4 doses
TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; EFV= fluconazole; Unk= unknown

- Median (range) time between HIV diagnosis and death was 14.2 (1.4-75.5) months.
- Median (range) time between Cryptococcosis diagnosis and death was 42 (3-157) days.
- Autopsy results for all subjects were consistent with infectious cause of death.
- Interstitial inflammation in kidneys of 009 and 011 was noted, consistent with either *Cryptococcus* or HIV-associated nephropathy.

PHARMACOKINETIC RESULTS

Individual PK

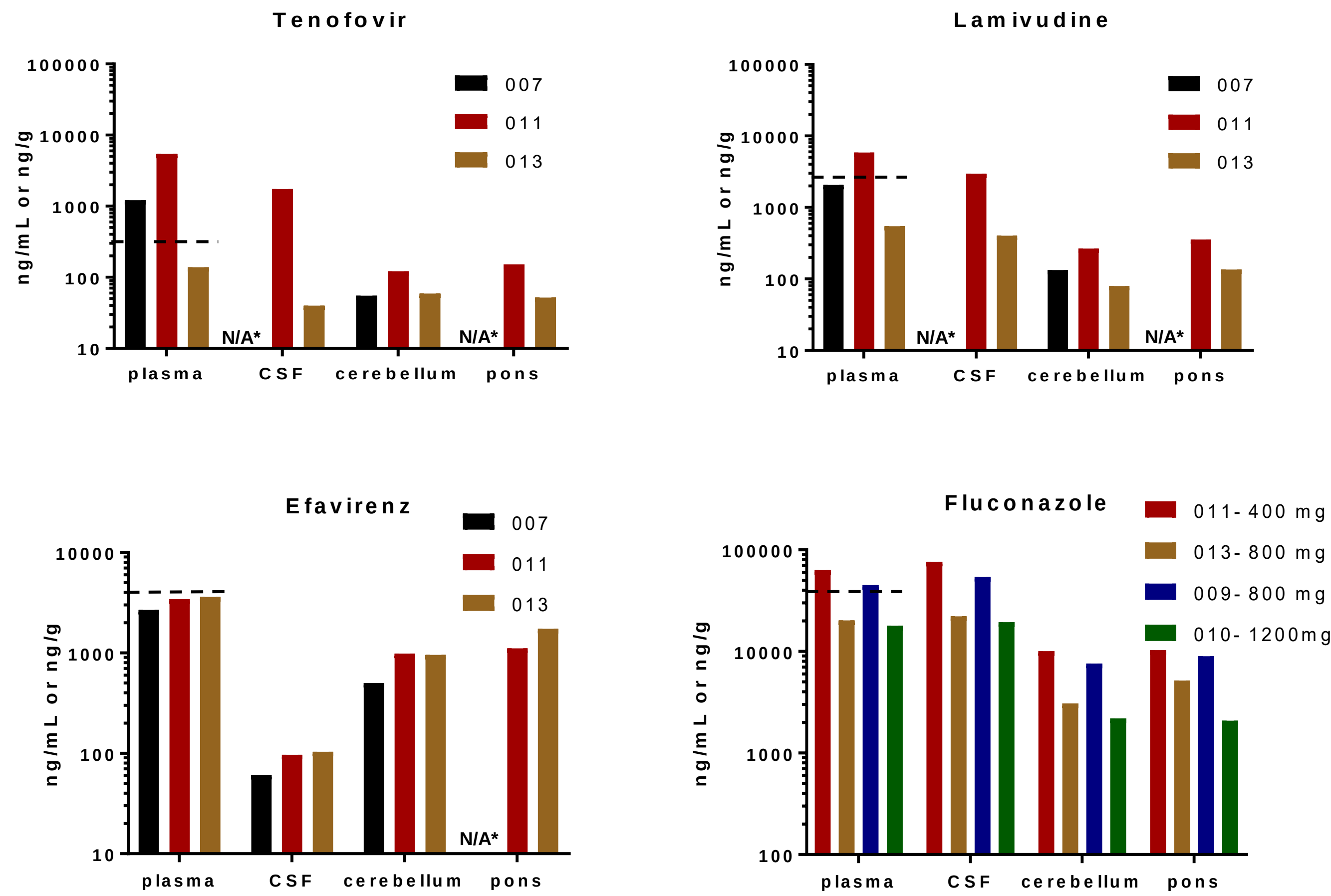


Figure 3: Drug quantification in plasma and CNS compartments. Dashed lines represent literature reported steady-state concentrations. * 007 pons tissue was not collected; 007 tenofovir and lamivudine values for CSF were excluded from analysis due to abnormal internal standard

- Higher than historical plasma values for tenofovir and lamivudine, but not efavirenz, in some subjects, may be due to differences in post-mortem redistribution, or to kidney damage as observed at autopsy.
- Inverse relationship between dose and concentration of fluconazole may be in part explained by time on therapy and time since last dose.

Relative Distribution

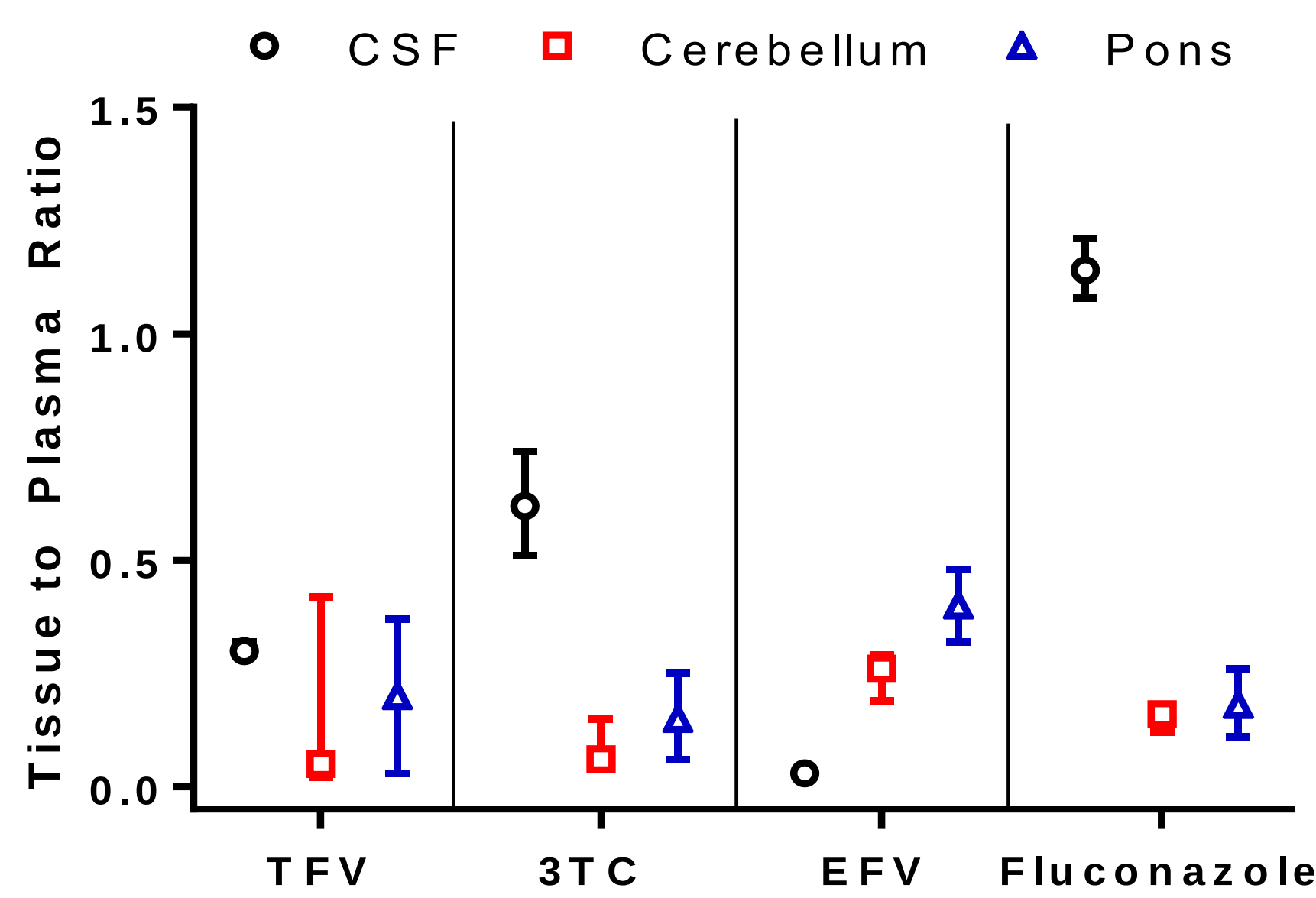


Figure 4: Tissue to plasma ratio for tenofovir (TFV), lamivudine (3TC), efavirenz (EFV) and fluconazole. Median and range of values are reported. Corresponding data are summarized in Table 3 below.

Table 3: Relative tissue distribution of antiretrovirals and fluconazole (median/range)

	N	CSF: Plasma Ratio	Cerebellum: Plasma Ratio	Pons: Plasma Ratio	Cerebellum: CSF Ratio	Pons: CSF Ratio
Tenofovir	3	0.30 (0.29-0.32)	0.05 (0.02-0.42)	0.20 (0.03-0.37)	0.77 (0.7-1.48)	0.69 (0.09-1.30)
Lamivudine	3	0.62 (0.51-0.74)	0.06 (0.05-0.15)	0.15 (0.06-0.25)	0.14 (0.09-0.20)	0.23 (0.12-0.34)
Efavirenz	3	0.03 (0.02-0.03)	0.26 (0.19-0.29)	0.40 (0.32-0.48)	9.20 (8.25-10.16)	14.13 (11.50-16.77)
Fluconazole	4	1.14 (1.08-1.21)	0.16 (0.12-0.17)	0.18 (0.11-0.26)	0.14 (0.11-0.14)	0.15 (0.10-0.23)

CONCLUSIONS

- With post-mortem samples collected within 14 hours of death, CSF: plasma ratios were similar to those previously reported in the literature, suggesting minimal impact of post-mortem redistribution.
- Tenofovir, lamivudine, and fluconazole exposure in CSF over-predicted brain tissue penetration ~7-fold.
- Efavirenz exposure in CSF under-predicted brain tissue penetration between 9- and 14-fold.
- Despite good penetration into the CSF, fluconazole penetration in actual brain tissue was low.
- CSF is not a good surrogate for overall drug exposure in CNS tissues.

NEXT STEPS

- Expand cohort to validate findings in these and additional brain compartments.
- Compare results to ante-mortem plasma and CSF values to quantify post-mortem effects on drug metabolism and distribution.
- Correlate drug distribution to pathogen distribution.
- Compare to non-meningitis patients to determine role of inflammation.
- These data can be used with advanced pharmacokinetic modeling to develop CNS-targeted therapeutic approaches.

ACKNOWLEDGEMENTS

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