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 CNS is limited and largely based on cerebrospinal fluid (CSF) concentrations. Hence, 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 obtaining of written, informed consent from next of kin, post-mortems were performed on five subjects co-infected with HIV and cryptococcal meningitis. Brain 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 liquid chromatography (HPLC) or ultrahigh performance 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. Samples are described as median (range).

Results: Post-mortems were performed 5.2 (2.2-13.7) hours following death. These individuals received the following: tenofovir/tenofovir disoproxil fumarate (300/300 mg) and fluconazole (100 mg). HIV-1 RNA was detected in all tissue compartments. Likewise, four individuals receiving tenofovir disoproxil fumarate had detectable drug in all compartments. CSF: plasma ratios were similar to values reported in the literature. Drug exposure was consistent across subjects, with a higher CSF pharmacokinetic profile for tenofovir, lamivudine, fluconazole, and fluconazole and higher than CSF for efavirenz.

Conclusions: Tenofovir, lamivudine, and fluconazole exposure in CSF is not consistent with blood-brain barriers. In all tissues, CSF and tissue levels of fluconazole were consistent across subjects, with a higher CSF pharmacokinetic profile for tenofovir, lamivudine, fluconazole, and fluconazole and higher than CSF for efavirenz.

PHARMACOKINETIC RESULTS

Individual PK

CONCLUSIONS

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

• 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 UNM Clinical Pharmacology and Analytical Services Laboratory using high performance liquid chromatography (HPLC) or ultrahigh performance 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. Samples are described as median (range).

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• With post-mortem samples collected within 14 hours of death, Cryptococcus CSF levels were similar to those previously reported in the literature, suggesting minimal impact of post-mortem redistribution.

• Tenofovir, lamivudine, and fluconazole exposure in CSF was not consistent with blood-brain barriers. In all tissues, CSF and tissue levels of fluconazole were consistent across subjects, with a higher CSF pharmacokinetic profile for tenofovir, lamivudine, fluconazole, and fluconazole and higher than CSF for efavirenz.

• Medical (range) time between HIV diagnosis and death was 14.2 (1.7-75.5) months.

• Median (range) time between Cryptococcus diagnosis and death was 42 (3-157) days.

• Autopsy revealed Cryptococcus disease in 9 of 11 cases. 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 regions for CSF, plasma, and tissues.

• Compare results to antemortem 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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