ANTIRETROVIRAL PROTEIN BINDING IN BLOOD AND SEMINAL PLASMAS

The most frequent method of human immunodeficiency virus (HIV) transmission is sex with an infected partner. We conducted two studies to determine the active concentration of antiretroviral drugs needed to help improve viral suppression. One study measured free drug levels in blood and seminal plasma. Although the antiretroviral drug can penetrate into semen, the drug that is protein bound is not available to suppress the virus. We also examined the ability of antiretroviral to interfere with HIV viral loads. Non-infected semen and blood plasma samples were used to determine protein bound and free concentrations of nelfinavir. Drug binding was measured at 3 µg/mL by dialysis. Drug concentrations were determined by HPLC. We determined the free fraction of the protease inhibitors in seminal plasma and found a lower degree of binding in the seminal plasma. This corresponded to a lower amount of total drug distributed into this compartment. HIV viral loads are used to determine the progress of therapy. These methods, however, use a type of reverse transcriptase, an enzyme that can be inhibited by some HIV drugs. To examine the possible effect of antiretroviral drugs on determining HIV load, nevirapine or efavirenz was added *in vitro* to the plasma of an HIV patient not receiving treatment. We found a high degree of viral load variation, making it difficult to determine whether antiretrovirals suppressed the results.

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BACKGROUND

Although HIV treatment cannot eradicate the infection, viral replication can be suppressed so that the virus does not continue to infect new immune cells that would cause disease progression. Suppression of virus replication can be achieved by using combinations of antiretroviral drugs. We conducted two studies to determine the active concentration of antiretroviral drugs needed to help improve viral suppression strategies. Viral loads, the total number of virions per mL of blood plasma, are used to monitor drug effectiveness. The method for determining viral loads, however, uses an avian viral reverse transcriptase and there was concern the drugs used to inhibit HIV could also affect the test results.

The most common means of HIV transmission is through semen via sex with an infected partner. The antiretroviral drug concentration is often much lower in semen than it is in blood. Several antiretroviral drugs are strongly bound to plasma proteins. Only, the unbound, free fraction of the drug is active. By determining the bound concentration of antiretrovirals in seminal plasma, the ability of the drug to inhibit the virus can be assessed.

METHODS

In our first study, non-infected semen and blood plasma samples were used to determine protein bound and free concentration of nelfinavir and lopinavir at concentrations of 3 ug/mL. Due to the viscosity of semen it was diluted in phosphate buffered saline (PBS) at different concentrations (1×, ½×, ¼×, ⅛×), making sure samples were at the same pH level. Samples for each drug concentration were run in duplicate. Dialysis membranes were soaked in PBS overnight. Semen samples (750 uL) were applied into one side of an equilibration dialysis chamber and in the other side PBS containing the drug. The chambers were in a shaking incubator for 4 hours at 37°C. The contents of each side of the chamber were analyzed for drug content using high performance liquid chromatography (HPLC). The results were determined as: amount of drug in PBS equals free drug, and amount of drug in semen equals free plus protein-bound drug. In a second study, we examined blood plasma from a therapy naïve HIV-infected patient to determine the effect of antiretroviral drugs on viral load measurements. The drugs nevirapine or efavirenz were added to the HIV-infected plasma. Because efavirenz is poorly soluble in water or buffer, a preparation of this drug was made using dimethylsulfoxide (DMSO). Three sets of duplicate samples were prepared by adding: either the 1, 5, and 10 ug/mL of nevirapine or efavirenz or PBS or DMSO alone.

RESULTS

Nevirapine did not suppress viral load measurements at any concentration. Efavirenz performed some suppression at a concentration of 5 ug/mL of drug. When 10 ug/ml was used, we observed an effect lower than expected. Viral loads of replicate samples had a high degree of variation. Nelfinavir was bound 70% to proteins in seminal plasma; in contrast, we found 98% protein binding in blood plasma. The concentration of nelfinavir in semen was only 4.0% of its concentration in blood plasma. The difference in binding can explain the lower concentration of nelfinavir in semen. Con-

trol values were normalized as 100% of viral load determined in the absence of drug. The concentrations 5 and 10 ug/mL decreased viral load determinations, but not consistently.

CONCLUSIONS

It is important to accurately determine the effective concentrations of antiretroviral drugs. The lower degree of binding of nelfinavir in seminal plasma can explain the low concentrations of the drug in this compartment. However, since only the free concentration is active, this low degree of binding does not prevent the drug from being effective. There was a high degree of variation in the results of the viral loads. Because of this, the role of these antiretrovirals in suppressing the viral load tests could not be determined.

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RESOURCES

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