Poster #9.2

Variability in Alpha-1 Acid Glycoprotein and Its Impact on Nelfinavir Free Fraction in HIV-Infected Subjects

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Abstract

Background: Currently, there is limited information regarding the effect of variable concentrations of the plasma binding protein alpha-1 acid glycoprotein (AAG) on the free concentration of protease inhibitors. Variation in the free fraction (free/total drug concentration) impacts the interpretation of total drug measurements used in Therapeutic Drug Monitoring (TDM). This prospective cohort study investigated the pattern of AAG variability in HIV-infected subjects and evaluated the effects of variations in AAG concentrations on the free fraction of the protease inhibitor nelfinavir (NFV). Twenty-three subjects have been enrolled in the ongoing study.

Methods: Subjects were enrolled into two groups: a) subjects with elevated AAG or conditions expected to result in elevated AAG levels, consisting of subjects with lymphoma and Kaposi sarcoma (KS) and b) controls without such conditions. AAG and NFV concentrations were obtained three times in each subject (monthly over a three month period). Free and total concentrations of NFV were determined using equilibrium dialysis for separation (free drug quantitation CV=6-9%) and LC-tandem MS for quantitation. The free fraction of NFV was calculated from the equation fu=Cu/Ct, with fu representing the free fraction, Cu the free concentration and Ct the total concentration. The relationship between AAG concentration and NFV free fraction was evaluated using linear regression analysis. Statistical significance was $p<0.05$.

Results: Subjects with Kaposi sarcoma or lymphoma, (n=4), had AAG values ranging from 84-214 mg/dl. Controls, (n=19), had AAG values ranging from 38-144 mg/dl. Free fraction results available to date indicate NFV free fraction in Kaposi sarcoma/lymphoma subjects (n=4) is 0.0021 compared to 0.0025 in controls (n=12). Regression analysis of all results to date (n=16) indicates NFV free fraction is not influenced by AAG concentration, with a correlation coefficient (r) of 0.14 (p=0.35). Separately, for controls, r=0.20 (p=0.25), and for Kaposi/lymphoma subjects, r=0.26 (p=0.41).

Conclusions: AAG concentrations vary among HIV-infected subjects and can be elevated in those with complications such as lymphoma and KS. The free fraction of NFV was <0.3 % for most study subjects. Preliminary results indicate that variations in AAG are minimally correlated with NFV free fraction. This may be attributed to compensatory binding of NFV to alternate plasma proteins, such as albumin.

Objectives

1) To determine the inter-subject variability of AAG concentrations in two groups:
   a) HIV-infected subjects with elevated AAG or conditions expected to result in elevated AAG levels (Kaposi sarcoma/lymphoma group)
   b) HIV-infected subjects (controls) without such conditions
2) To investigate the relationship between variations in AAG concentrations and the free fraction of nelfinavir.

Introduction

Protease inhibitors are highly bound to plasma proteins (> than 98%), except indinavir, and are predominately bound to alpha-1 acid glycoprotein (AAG) versus albumin (1). AAG is an acute phase reactant and is a high affinity, low capacity protein (2,3). AAG concentrations can become elevated significantly in response to inflammation and other conditions such as cancer and infections (3). Concentrations for AAG can range from 40 to 300 mg/dl with normal AAG levels approximately between 50 to 100 mg/dl (3). AAG levels have been reported to be increased up to 60% in HIV-infected subjects (4).

For most drugs, protein binding is not considered a clinically significant issue. However, for some drugs with all or some of the following characteristics, protein binding may be relevant:

a) highly protein bound, b) present in concentrations potentially resulting in saturated protein binding, c) high extraction ratio (5), and d) narrow therapeutic index (5).

Presently, there is limited information regarding the effect of variable concentrations of alpha-1 acid glycoprotein on the free, or active, concentration of protease inhibitors. Some researchers speculate that variability in AAG concentrations may change the free fraction (free/total drug concentration) and, therefore, the percentage of the total drug concentration that is pharmacologically active. Variation in the free fraction can affect the interpretation of total drug measurements used in Therapeutic Drug Monitoring (TDM). For HIV-infected subjects, elevations in AAG could potentially result in subtherapeutic plasma concentrations and contribute to treatment failure with highly active antiretroviral therapy (HAART).

In vitro results from the UCSF Drug Research Unit have concluded that nelfinavir binds to albumin and AAG. In AAG or albumin solution, nelfinavir (NFV) free fraction increased with increasing concentrations of a displacing drug (6). However, in plasma no change in nelfinavir free fraction was observed (6). Compensatory binding appeared to offset the potential of concurrently administered drugs to displace nelfinavir in plasma. Binding characteristics studies of nelfinavir and the active metabolite M8 determined that nelfinavir showed greater affinity in comparison to M8 to albumin and AAG and that both nelfinavir and M8 showed greater affinity to AAG (7).

Table 1. Nelfinavir and M8 Binding Characteristics in AAG and Albumin solution

	Albumin solution	AAG Solution
Nelfinavir	$1.11 \times 10^{6} M^{-1}$	$7.25 \times 10^{7} M^{-1}$
M8	$7.92 \times 10^{5} M^{-1}$	$3.33 \times 10^{7} M^{-1}$

This prospective cohort study investigated the pattern of variability in AAG concentrations in HIV-infected subjects and evaluated the effects of variations in AAG concentrations on the free fraction of the protease inhibitor nelfinavir.

Methods

Enrollment

Subjects were enrolled into two groups:

1) HIV-infected subjects with elevated AAG or conditions expected to result in elevated AAG levels, such as lymphoma and Kaposi sarcoma (KS). HIV-infected subjects could either have an active disease condition or be in remission.
2) HIV-infected subjects (controls) without such conditions.

Inclusion criteria

1) HIV-infected subjects receiving nelfinavir 1250 mg BID or 750 mg TID as part of HAART

Exclusion criteria

1) HIV-infected subjects receiving medications that could cause drug displacement from the plasma proteins AAG and albumin. Criteria for excluding medications are:
   a) Highly protein bound: >95%
   b) Present in concentrations that could cause saturated protein binding

Study procedures

Enrolled subjects were scheduled for monthly (approximately every four weeks) study visits over a three month period at the General Clinical Research Center (GCRC) at San Francisco General Hospital. Subjects were seen within one to six hours of self administration of nelfinavir. Blood samples were collected to quantify albumin, AAG, and nelfinavir total and free concentrations.

Analytical Methods

Equilibrium dialysis was used to separate total and free nelfinavir drug concentrations, using a previously published method (8). Liquid chromatography-tandem mass spectrometry (LC-tandem MS) was used to quantitate total and free nelfinavir concentrations.

1) Free nelfinavir lower and upper limit of quantitation: 1 and 50 ng/ml. (CV for 1 ng/ml: 13%)
2) Total nelfinavir lower and upper limit of quantitation: 25 to 4000 ng/ml

Table 2. Method Validation

A) Equilibrium Dialysis Validation

Initial concentration (ng/ml)	Mean Free Concentration (ng/ml)	CV %
1000	2.2	9.4
2000	6.5	8.1
3000	8.6	6

B) Total Concentration Validation

Concentration (ng/ml)	CV%
25	16.5
4000	7.1

Regression Analysis

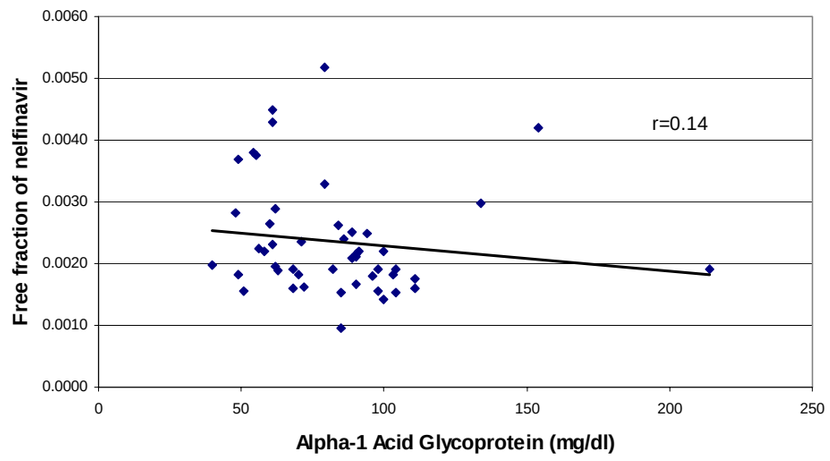
The free fraction of NFV was calculated from the equation fu=Cu/Ct, with fu representing the free fraction, Cu the free concentration and Ct the total concentration. AAG was plotted as the independent (X) variable and nelfinavir free fraction was plotted as the dependent (Y) variable. Linear regression analysis was utilized to obtain correlation coefficient (r) values. P values were also calculated using a two-tailed t-test. Statistical significance was set at $p < .05$.

Results

Table 3. Alpha-1 Acid Glycoprotein (AAG) and Nelfinavir Free Fraction

	Combined Cohort Data	Control Group	Kaposi sarcoma/lymphoma Group
AAG (mg/dl)	n=23	n=19	n=4
Range	38-214	38-144	84-214
Mean	82.9	73.6	110.8
Nelfinavir Free Fraction	n=16	n=12	n=4
Mean	0.0024	0.0025	0.0021
SD	0.0009	0.0009	0.0008
Correlation coefficient (r)	0.14	0.2	0.26
P value	0.35	0.25	0.41

Figure 1. Alpha-1 Acid Glycoprotein vs.Nelfinavir Free Fraction



Conclusions

AAG concentrations in HIV-infected subjects are widely variable, but in most subjects concentrations are within the normal range of 50-100 mg/dl. AAG concentrations can be elevated in those with complications such as lymphoma and KS. The mean AAG concentration was higher in HIV-infected subjects with Kaposi sarcoma or lymphoma versus control subjects.

The free fraction of NFV was <0.3 % for most study subjects and was similar for both study groups. Preliminary results indicate that differences in AAG concentrations are minimally correlated with nelfinavir free fraction and are not statistically significant. This may be due to compensatory binding of nelfinavir to other plasma proteins as AAG concentrations change.

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