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Nelfinavir Unbound Drug Interactions and Protein Binding Characteristics

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ABSTRACT

Background: Potential changes in free drug concentrations of antiretrovirals may alter the clinical effects of these drugs as it is the free drug fraction that is pharmacologically active. Therefore, we investigated the binding characteristics of nelfinavir and its active metabolite, M8, to human plasma proteins and the effects of concomitantly administered highly bound drugs on the protein binding of nelfinavir and M8.

Methods: Free fractions of nelfinavir and M8 were separated by equilibrium dialysis and the unbound concentrations of nelfinavir and M8 were determined by a validated LC/tandem MS method. Association constants were estimated using double reciprocal plots of the data. For interaction studies, various concentrations of drugs were added to α-1-acid glycoprotein (AAG) and serum albumin (HSA) solutions and plasma.

Results: Nelfinavir and M8 free fraction in plasma averaged 0.42 ± 0.08 and 0.64 ± 0.07%, respectively. Association constants of nelfinavir and M8 were 7.25 X 10^7 M^{-1} and 3.33 X 10^7 M^{-1} for AAG and 1.11 X 10^6 M^{-1} and 7.92 X 10^5 M^{-1} for HSA, respectively, indicating these drugs bind extensively to both proteins. When ritonavir or saquinavir, drugs that bind extensively to AAG, were added to an AAG solution, nelfinavir free fraction was significantly higher than control values [5.23 ± 1.27 and 1.18 ± 0.21% for ritonavir (10μg/ml) and control ($p<0.01$) and 2.55 ± 0.27 and 0.99 ± 0.16% for saquinavir (10μg/ml) and control ($p<0.01$)]. In contrast, free fraction was not altered when ritonavir or saquinavir were added to whole plasma ($p>0.05$). Similarly, when salicylic acid or valproic acid, drugs that bind extensively to HSA, were added to an HSA solution, free fraction changed significantly compared with controls, whereas there was no difference when these drugs were added to plasma.

Conclusions: In vitro results indicate nelfinavir binds extensively to both AAG and albumin in plasma, with binding unaffected by concomitantly administered drugs exhibiting high protein binding. This is attributed to compensatory binding by alternate proteins when nelfinavir is displaced from AAG or albumin.

INTRODUCTION

Nelfinavir, a selective, competitive inhibitor of HIV-1 protease, limits viral replication and improves immune function in HIV-infected individuals (1-3). It is highly bound to plasma proteins (4,5). The nelfinavir IC_{95} has been reported to be 59 nM (range, 7 to 130 nM), corresponding to 34 ng/ml (range, 4 to 74 ng/ml) (6) with in vitro sensitivity generally established in the absence of plasma proteins. The pharmacological effects of nelfinavir are more closely related to unbound rather than total drug concentration in plasma since only the free drug has the ability to diffuse through biological membranes and reach its intracellular site of action (7). Indeed, antiviral activity has been shown to be directly related to the intracellular concentration of the drug, which is decreased in the presence of increasing concentrations of AAG (8).

Previous work indicates nelfinavir binds extensively to plasma proteins [average free fractions of 0.41 to 0.43% (5) and 1.4 to 1.7% (4)] and primarily to AAG (9). Its major metabolite, M8, with comparable anti-HIV activity (4), binds to plasma proteins to a similar degree (4).

Characterizing the plasma protein binding of nelfinavir and M8 and evaluating the impact of concomitantly administered drugs exhibiting extensive binding is of clinical relevance. Changes in free fraction may occur with variable protein concentrations (10,11) or in the presence of drugs causing displacement from binding proteins (12,13). With changing free fraction, interpretation of concentrations for total drug (bound and unbound) needs modification. Total drug measurements are almost always used for TDM or drug interaction studies.

OBJECTIVES

1) Characterize the binding of nelfinavir and M8 to human plasma and purified plasma proteins.
2) Examine the effects of drugs known to displace compounds from AAG (ritonavir and saquinavir) and from albumin (valproic acid and salicylic acid) on nelfinavir free fraction measurements.

METHODS

Materials

Nelfinavir mesylate and M8 mesylate were supplied by Agouron Pharmaceuticals, Inc and methyl indinavir sulfate (internal standard) was supplied by Merck Research Laboratory.

Drug free human plasma was obtained from the Blood Centers of the Pacific (San Francisco, CA, USA). Human AAG (G-9885), HSA (A-3782) and Sigmacote® were obtained from Sigma Chemical Co.

Characterization of Nelfinavir Binding

Nelfinavir and M8 free fractions in plasma and their respective association constants were determined using nelfinavir or M8 concentrations in plasma or protein solutions ranging from 1 to 32 μg/ml and 1 to 24 μg/ml, respectively.

Concentrations of AAG and HSA were 75 mg/dl and 4.0 or 1.0 g/dl, respectively with AAG of 75 mg/dl and HSA of 4.0 g/dl representing physiological concentrations.

To evaluate the impact of varying concentrations of AAG or HSA on binding, concentrations of AAG and HSA ranged from 25 to 200 mg/dl and 1.0 to 8.0 g/dl, respectively with physiological concentrations of nelfinavir and M8 fixed at 4.0 and 2.0 μg/ml respectively.

Samples were pre-incubated at 37°C for 30 minutes prior to equilibrium dialysis.

Impact of Displacing Drugs

Aliquots of nelfinavir and the potential competitive drug (saquinavir, ritonavir, salicylic acid or valproic acid) were added to plasma or protein solutions and pre-incubated as above.

Nelfinavir, AAG and HSA concentrations were 4.0 μg/ml, 75 mg/dl and 4.0 g/dl, representing a typical nelfinavir "peak" and physiological concentrations of proteins.

Saquinavir and ritonavir concentrations were 0, 1, 10 and 100 μg/ml and the salicylic acid and valproic acid concentrations were 0, 10, 100, 300 and 1,000 μg/ml.

Equilibrium Dialysis

Spectrum equilibrium dialysis system, consisting of two-chambered Teflon® dialysis cells, separated by a Cellu Sep F3 membrane (molecular weight cut-off of 12,000 to 14,000 Daltons).

After pre-incubation, 0.9 ml of each solution was dialyzed against ammonium formate buffer. Dialysis was carried out for 4 hours at 37°C in a rotating water bath.

Plasma or protein solution and buffer dialysates were removed, and drug concentrations determined by LC/tandem MS.

To minimize nelfinavir adherence, polypropylene tubes were silianized.

Quantitation of Nelfinavir and M8

LC/tandem (API 2000 LC/MS/MS System, Perkin Elmer SCIEX, Foster City, CA, USA) was used for quantitation (5).

Mass scanning mode was set to positive ion multiple reaction monitoring (MRM) mode with a parent/daughter ion pair of 568.4/330.0 for nelfinavir, 584.2/330.2 for M8 and 628.6/421.2 for methyl-indinavir.

Two separation calibration curves were prepared for quantitation of total drug in plasma or protein solution dialysate and unbound drug in buffer dialysate. Calibration curves for unbound drug ranged from 1.0 to 50 ng/ml and for total drug from 100 to 5,000 ng/ml.

CV of quality controls (3, 30, 750 and 2500 ng/ml) were 8.6, 8.8, 4.3 and 4.5 percent for nelfinavir and 8.5, 7.9, 5.0 and 6.6 percent for M8, respectively.

Calculations

Fraction of unbound drug (fu) was determined using:

$$fu = Cu/Ct$$

where Cu (unbound concentration) is the concentration of drug in the buffer reservoir after dialysis and Ct (total concentration) is the drug concentration in the plasma or protein solution reservoir after dialysis.

Association constants (k) were estimated by linear least-squares regression analysis of double-reciprocal plots expressed as:

$$1/r = (1/kn)(1/[Df]) + 1/n$$

where r is the molar concentration of bound drug ([Db]) per molar concentration of protein ([Pt]), n is the number of binding sites, [Df] is the molar concentration of free (unbound) drug.

The molar concentration of bound drug ([Db]) was equal to the molar concentration of total drug ([Dt]) minus unbound drug ([Df]).

Statistical Analysis

SYSTAT Version 8.0 was used including ANOVA and post hoc Bonferroni tests. Differences are significant at $p<0.05$.

RESULTS

Characterization of Nelfinavir Binding

Free fractions of nelfinavir in plasma, AAG (75 mg/dl) and HSA (4.0 gm/dl) solutions were 0.42 ± 0.08%, 1.48 ± 0.17% and 1.55 ± 0.17%, and free fractions of M8 in these solutions were 0.64 ± 0.07%, 0.84 ± 0.14% and 3.19 ± 0.37%, respectively.

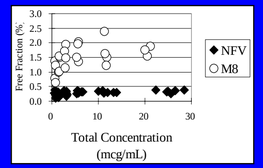


Figure 1: Free fraction of nelfinavir and M8 tended to increase with increasing concentrations beyond the range of concentrations observed clinically.

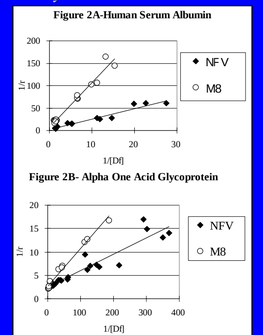


Figure 2: Association constants for nelfinavir and M8 are 7.25 X 10^7 M^{-1} and 3.33 X 10^7 M^{-1}, respectively, for AAG and 1.11 X 10^6 M^{-1} and 7.92 X 10^5 M^{-1} for HSA.

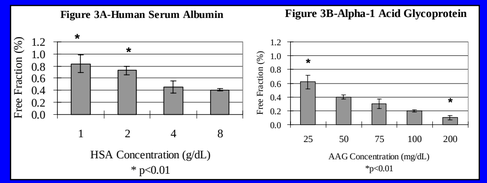


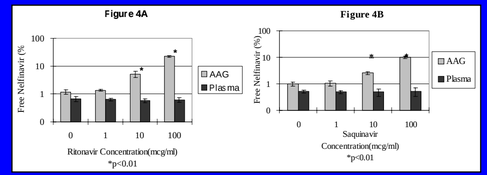
Figure 3: Change in nelfinavir free fraction when 4 g/dl HSA or 75 mg/dl AAG solutions are supplemented with varying concentrations of the alternate protein.

Free fraction estimates for AAG 25 and 200 mg/dl were different from values for 75 mg/dl ($p<0.01$). Free fractions for HSA 1 and 2 g/dl were higher than for 4 g/dl ($p<0.01$).

Impact of Displacing Drugs

Figure 4: Nelfinavir free fraction increased with increasing concentrations of ritonavir and saquinavir in AAG solution.

This increase was not observed with increasing concentrations of the displacing drug in plasma.



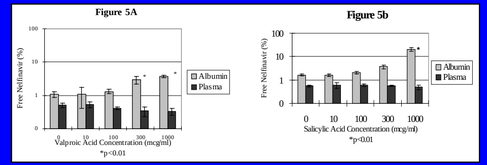


Figure 5: Nelfinavir free fraction increased with increasing concentrations of salicylic acid and valproic acid in HSA solution.

This change was not observed with increasing concentrations of the displacing drugs in plasma.

DISCUSSION

1. Free fractions of nelfinavir and M8 in plasma average 0.42 and 0.64%, respectively.
2. The affinity of nelfinavir for human plasma proteins is higher than that of M8.
3. Both nelfinavir and M8 show higher affinity for AAG than for HSA, although both drugs bind extensively to both proteins.
4. Displacement from AAG has been confirmed with addition of ritonavir or saquinavir and from HSA with addition of valproic acid and salicylic acid.
5. Displacement does not impact nelfinavir free fraction in plasma. This is attributed to compensatory binding by alternative plasma proteins.
6. Ongoing work in patients is evaluating the impact of varying AAG and HSA concentrations on free fraction. Further in vitro work is evaluating the impact of displacement from AAG and HSA simultaneously.

Acknowledgments

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