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In vitro protein-binding characteristics of delavirdine and its *N*-dealkylated metabolite

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Abstract

This study was performed to determine delavirdine protein-binding characteristics as well as those of its *N*-dealkylated metabolite (*N*-DLV). Initial studies of 36 μM delavirdine and 30 μM *N*-DLV in solutions of plasma, albumin 4 g%, alpha-1-acid glycoprotein (AAG) 100 mg% or immune globulin (IVIG) 5 g% were conducted. Delavirdine (12, 36 and 73 μM) and *N*-DLV (10, 30 and 60 μM) were then studied alone and in combination in plasma and various concentrations of albumin. Studies were done in triplicate using equilibrium dialysis. The mean delavirdine fraction unbound (f_u) in plasma, albumin, IVIG and AAG was 0.013, 0.033, 0.752 and 0.912 while the mean f_u of *N*-DLV in these same protein solutions was 0.139, 0.195, 0.329 and 0.359. In plasma and albumin, a greater f_u was observed at higher delavirdine concentrations and no significant changes in f_u were noted with the addition of *N*-DLV. An increase in delavirdine f_u was noted as the albumin concentrations decreased. The f_u of *N*-DLV increased significantly as the concentration of albumin decreased as well as with decreasing *N*-DLV concentration. The potential implications of extensive delavirdine binding to plasma proteins, primarily albumin, are discussed.

Keywords: Delavirdine; Protein binding; HIV; Reverse transcriptase inhibitor; Pharmacokinetics; Bisheteroarylpiperazine

1. Introduction

Delavirdine mesylate is a bisheteroarylpiperazine (BHAP) non-nucleoside reverse transcriptase inhibitor (NNRTI) which has been shown in vitro to inhibit recombinant human immunodeficiency virus type 1 reverse transcriptase (HIV-1

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RT) at a 50% inhibitory concentration of 0.26 μM (range 0.001–0.69 μM in 25 HIV-1 clinical isolates). Similar activity has been seen against isolates with high-level resistance to zidovudine and didanosine (Dueweke et al., 1993). Its activity is highly specific for HIV-1 RT and inhibits both the DNA-directed and RNA-directed polymerase functions of HIV-1 RT, but not the RNase H activity of the enzyme. Delavirdine has low cellular toxicity, with less than an 8% decrease in peripheral blood lymphocyte viability at 100 μM (Dueweke et al., 1993). In vitro studies have shown this drug to be synergistic with both zidovudine and zalcitabine and ongoing clinical trials are therefore evaluating delavirdine 400 mg three times daily in combination with dideoxynucleosides (unpublished data).

Unlike nucleoside analog RTIs, delavirdine is administered in its active form, so that once in the intracellular environment, it is able to bind directly to the RT enzyme. However, like an earlier BHAP NNRTI, atevirdine, delavirdine is highly bound to plasma proteins. As with most agents, it is the unbound form of the drug which is thought to correlate with drug activity. As a result, the affinity and degree of protein binding may have a direct impact on the amount of delavirdine which is available to gain intracellular access and exert its antiviral effect.

The purpose of this study was to examine the in vitro protein-binding characteristics of delavirdine and its *N*-dealkylated metabolite (*N*-DLV) over the range of plasma concentrations currently attained in clinical trials (Para et al., 1996; Freimuth et al., 1996, 1994). Plasma concentrations in these studies range from 1 to 75 μM delavirdine, with most trough plasma levels falling into the 5–20 μM range on a regimen of 400 mg three times daily. The ratio of parent drug to metabolite (delavirdine/*N*-DLV) varies from 0.3 to 10.0, consistent with interpatient variation in capacity-limited metabolism of delavirdine (Morse et al., 1995).

2. Methods

Preliminary equilibration and recovery studies were performed with 25 μM delavirdine mesylate and 30 μM *N*-DLV (Pharmacia and Upjohn, Kalamazoo, MI) in plasma to determine the time required for the dialysis system to equilibrate. Degradation studies were then performed in the same media over a similar time period to determine if delavirdine or *N*-DLV loss occurred during the period of equilibrium dialysis. Equilibration and recovery studies were also performed for delavirdine and *N*-DLV in phosphate buffered saline (PBS) to verify the equilibration time and to examine drug recovery from the dialysis system.

Delavirdine and *N*-DLV solutions were prepared by first adding the required amount of stock solution (delavirdine or *N*-DLV dissolved in methanol) to a volumetric flask. A propylene glycol volume equivalent to 1% of the final volume of the volumetric flask was then added and the resultant mixture was dried using nitrogen gas. The diluent was then added to yield the desired final volume.

Protein binding was determined by equilibrium dialysis, using plexiglass dialysis cells with a 400 μl maximum capacity. The Spectrapor membrane (Spectrum Medical Industries, Los Angeles, CA) used for all analyses had a molecular mass cut-off of 12 000–14 000 Da. Screening studies were performed using 36 μM delavirdine, 30 μM *N*-DLV and a combination of these in albumin 4 g% (Sigma Chemical, St. Louis, MO), human plasma (normal volunteer), immune globulin 5% (Gamimune N, Cutter/Miles, Elkhart, IN) and alpha-1-acid glycoprotein (AAG) 100 mg% (Sigma Chemical, St. Louis, MO). Delavirdine and *N*-DLV in the protein solutions were dialyzed against a 0.1 M PBS solution at 37°C and pH 7.4. Prior to dialysis, the pH of the protein solutions was adjusted to 7.4 using microliter quantities of 0.3 M HCl or 0.3 M NaOH. If the average fraction unbound (f_u) for any protein solution was determined to be $\leqslant 20\%$ (see calculation below), further studies were carried out in which the concentration of drug and protein were varied. Combinations of different concentrations of delavirdine and *N*-DLV were also examined in the protein solutions to evaluate significant displacement interactions. The PBS quality controls (delavirdine 0.364 μM, 4.26 μM and 15.0 μM; *N*-DLV 0.327 μM, 3.54 μM and 12.9 μM) were

dialyzed with PBS in the opposite chamber to study any possible delavirdine and *N*-DLV binding to the dialysis system components as well as their solubility in aqueous media in the absence of protein.

Each possible combination of protein, delavirdine and *N*-DLV at the selected concentrations were tested during equilibrium dialysis three times. All protein-containing samples were analyzed for delavirdine and *N*-DLV by high-performance liquid chromatography (HPLC) (Howard and Schwende, 1995). The procedure utilized a protein precipitation step by adding 200 μl of sample to 400 μl of acetonitrile which also contained internal standard (U-88822, 10 μg/ml, Pharmacia and Upjohn, Kalamazoo, MI). After centrifugation, the supernatant was diluted 1 : 2 with 10 mM potassium phosphate buffer (pH 6.0), then directly injected onto the chromatographic system and eluted with a mobile phase consisting of 10 mM potassium phosphate (pH 6.0) and acetonitrile in a ratio of 2 : 1 at a flow rate of 1.5 ml/min. The HPLC column was a Zorbax SB-CN (MAC-MOD Analytical, Chadds Ford, PA) with a 5-μm particle size. The compounds were measured with fluorescence detection (Hitachi F1080 Fluorescence Spectrophotometer) with excitation at 302 nm and emission filtered at 425 nm. Quantitation of the compounds was performed by the Millenium 2000 Chromatography Manager (Waters Assoc., Milford, MA) a software program which calculates peak height ratios relative to the internal standard for unknown specimens, and determines unknown values by comparing these ratios to a standard curve determined from plasma calibration standards. The standard curve is formed by a linear regression of peak height ratio versus concentration (c) with a weighting of $1/c^2$. The measurable concentration ranges were 0.210–91.8 μM for delavirdine and 0.174–71.7 μM for *N*-DLV. The interday percent coefficient of variation for the delavirdine quality controls were 6.5% (0.366 μM), 2.6% (3.85 μM) and 5.3% (35.9 μM), while those for *N*-DLV were 7.3% (0.359 μM), 2.7% (3.79 μM) and 5.8% (35.9 μM).

Samples from the PBS side of the equilibrium dialysis cell were also analyzed by HPLC with fluorescence detection. Acetonitrile containing internal standard was added to the sample in a 2 : 1 ratio and then directly injected onto the HPLC column and analyzed by the methods described above. The measurable concentration ranges were 0.068–21.9 μM for delavirdine and 0.056–17.1 μM for *N*-DLV. The interday percent coefficient of variation for the delavirdine quality controls were 5.2% (0.263 μM), 4.8% (2.63 μM) and 4.7% (8.76 μM), while those for *N*-DLV were 2.7% (0.217 μM), 2.6% (2.17 μM) and 2.8% (7.24 μM).

Fraction bound (f_b) was determined using a previously described formula (Boudinot and Jusko, 1984), which corrects for volume shifts that occur during the equilibrium dialysis period:

$$f_b = \frac{(D_T - D_F)\cdot(V_{pe}/V_{pi})}{(D_T - D_F)\cdot(V_{pe}/V_{pi}) + D_F}$$

where D_T = total drug concentration (protein side of dialysis cell), D_F = free drug concentration (buffer side of dialysis cell), V_{pe} = volume after equilibrium established (protein side of cell), V_{pi} = initial volume (protein side of cell) and

fraction unbound (f_u) = $1 - f_b$

One-way analysis of variance (ANOVA) was performed on the protein-binding data from the screening phase using 36 μM delavirdine and 30 μM *N*-DLV to compare the mean f_u of each of these compounds in albumin, AAG, immune globulin and plasma. A Tukey's test was used for individual comparisons. Two-way ANOVA was used to compare changes in the f_u of delavirdine and *N*-DLV resulting from alterations in protein, delavirdine and *N*-DLV concentrations. A *P*-value < 0.05 was considered to be statistically significant.

Using the PCNONLIN 4.0 program (SCI Software, Lexington, KY) with non-linear least squares regression, the number of binding sites and the association constant for binding were determined for delavirdine in those pure protein solutions in which delavirdine was studied at multiple concentrations. The fraction bound (f_b) was plotted versus the log total drug concentration (D_T) to ascertain whether saturation was occurring over the range of concentrations being stud-

ied. If notable saturation was observed, both the number of binding sites and the binding affinity constants can be determined by using the following formulae:

$$\frac{D_B}{P_T} = \frac{n \cdot k \cdot D_F}{1 + k \cdot D_F}$$

(assuming a single class of binding sites)

$$D_B = D_T - D_F$$

where D_B = bound drug concentration, D_T = total drug concentration (protein side of dialysis cell), D_F = free drug concentration (buffer side of dialysis cell), n = number of binding sites, k = binding affinity constant and P_T = protein concentration.In the case where a lack of adequate saturation was observed in the plot of f_b versus log D_T, only the product of the number of binding sites and affinity constant was determined by the following formula:

$$\frac{D_B}{D_F} = n \cdot k \cdot P_T$$

Note that in the above calculation, it would be ideal to obtain an estimate of $n \cdot k$ from data obtained over a range of protein concentrations. Since it was not possible to alter the protein concentration of plasma, this formula was only applied to pure protein solutions which were studied.

When both delavirdine and *N*-DLV were examined in combination, the average f_u at each level was calculated and comparisons were made by two-way ANOVA. Displacement occurred if the average f_u for one compound was altered significantly by the presence of the other.

The percent mass recovered (% Rec) from the dialysis system following equilibration was calculated as:

$$\%\text{Rec} = \frac{(D_T \cdot V_{pe} + D_F \cdot V_{be}) \cdot 100}{D_{ti} \cdot V_{pi}}$$

where D_T = total drug concentration (protein side of cell), D_F = free drug concentration (buffer side of cell), V_{pe} = volume after equilibrium established (protein side of cell), V_{be} = volume after equilibrium established (buffer side of cell), D_{ti} = initial drug concentration (protein side of cell) and V_{pi} = initial volume (protein side of cell).

3. Results

From the preliminary experiments in both plasma and PBS it was determined that a 6-h dwell time was adequate to allow equilibration of both compounds. From the initial screening studies, it was determined that the average f_u for delavirdine and *N*-DLV in plasma, albumin 4 g%, immune globulin 5% and AAG 100 mg% were 0.013, 0.033, 0.752, 0.912 and 0.139, 0.195, 0.329 and 0.359, respectively. The recovery of delavirdine (12–73 μM) in albumin solution (2, 3, and 4 g%) was 85.7 $\pm$ 2.3%. Plasma recovery was higher at 92.1 $\pm$ 2.1%. Recovery in immune globulin and AAG was in the range 79–81%. Delavirdine and *N*-DLV differed significantly in binding interactions with these protein solutions (Table 1). *N*-DLV binding to immune globulin and AAG were similar.

As a result of the above screening studies, further studies of 12, 36 and 73 μM of delavirdine and 10, 30 and 60 μM *N*-DLV, alone and in all possible combinations, were then carried out in plasma as well as in albumin 2, 3 and 4 g solutions %. The plasma used which was obtained from a normal, healthy, male volunteer who was not on any chronic medications, was analyzed and determined to contain 8.0 g% of total protein and 5.0 g% of albumin.

In the studies of delavirdine in plasma, the f_u was determined to be dependent upon the concentration of delavirdine present in the solution, with higher concentrations resulting in an elevated f_u ($P < 0.005$). The f_u was not influenced by the presence of *N*-DLV at concentrations up to 60 μM ($0.05 < P < 0.10$) (Table 2).

The studies of delavirdine in albumin solution confirmed the above results. Addition of *N*-DLV had very little impact on the delavirdine f_u at all three concentrations of albumin studied. Once again, increases in the delavirdine concentration resulted in increases in the delavirdine f_u ($P < 0.005$). Decreases in the albumin concentration from 4 to 2 g% resulted in an approximate doubling of the f_u of delavirdine at each of the three concentrations studied (Table 3).

When *N*-DLV was studied in plasma, its f_u was related to both the *N*-DLV ($P < 0.005$) and

Table 1
Fraction unbound of delavirdine (36 μM) and *N*-DLV (30 μM) in various protein solutions

Drug	Plasma	Albumin 4 g%	Immune globulin 5%	AAG 100 mg%
DLV (36 μM)				
n	6	6	6	4
Mean	0.0127	0.0326	0.752	0.913
S.D.	0.0021	0.0007	0.072	0.047
CV (%)	16.5	2.1	9.6	5.1
ANOVA	$P<0.05$			
N-DLV (30 μM)				
n	6	6	5	6
Mean	0.141	0.198	0.335	0.336
S.D.	0.004	0.003	0.004	0.056
CV (%)	2.8	1.5	1.2	15.3
ANOVA	$P<0.05$			

delavirdine ($P < 0.005$) concentration. An increased f_u was noted when concentrations of delavirdine were increased. Interestingly, the *N*-DLV f_u was found to decrease as *N*-DLV concentrations were increased in plasma.

The metabolite was also studied at various albumin concentrations and, in a similar fashion, the f_u of *N*-DLV increased as the concentration of delavirdine in the solution was increased. The finding that the *N*-DLV f_u actually decreased as *N*-DLV concentration was raised was confirmed at all albumin concentrations studied ($P < 0.005$). A decrease in the albumin concentration resulted in a significant increase in the f_u of *N*-DLV.

Table 2
Mean fraction unbound of delavirdine in plasma[a]

N-DLV (μM)	Delavirdine (μm)		
	12.1	36.3	72.6
0	0.014 ± 0.001	0.013 ± 0.002	0.015 ± 0.001
10.0	0.013 ± 0.000	0.015 ± 0.000	0.017 ± 0.000
30.0	0.014 ± 0.001	0.013 ± 0.002	0.017 ± 0.001
60.0	0.013 ± 0.001	0.016 ± 0.001	0.017 ± 0.001
Two-way ANOVA	Tukey test		
DLV: P< 0.005	0.0314 (12.1 μm), 0.0138 (36.3 μm), *0.0161 (72.6 μm)*		
N-DLV: *P*> 0.10			

Italics indicate significance

[a] $n = 3$ for each cell.

Delavirdine in PBS at concentrations of 0.364, 4.26 and 15.0 μM when dialyzed against PBS yielded mass recoveries of 110, 88 and 88%, respectively. *N*-DLV in PBS at concentrations of 0.327, 3.54 and 12.9 μM when dialyzed against PBS yielded mass recoveries of 113, 97 and 94%, respectively. Volume recoveries from both dialysis cells for all of the above experiments were > 96%.

The degree of binding saturation observed was insufficient to characterize either the number of binding sites or the binding affinity constant for delavirdine. The data did allow for the determination of the product of these for delavirdine which was calculated to be 54 000/M.

4. Discussion

Delavirdine demonstrates saturable metabolism via the hepatic cytochrome *P*-450IIIa enzyme system. Small changes in daily dosage increments (particularly among patients receiving > 1200 mg daily) of delavirdine have been noted to dramatically increase plasma concentrations (Morse et al., 1995). As a result of the non-linear pharmacokinetic properties of this drug, clinical trials of

Table 3
Mean fraction unbound of delavirdine in albumin[a]

Albumin (g%)	*N*-DLV (μm)	Delavirdine (μm)		
		12.1	36.3	72.6
2	0	0.060 ± 0.001	0.059 ± 0.003	0.045 ± 0.011
3	0	0.033 ± 0.003	0.038 ± 0.001	0.038 ± 0.006
4	0	0.026 ± 0.001	0.033 ± 0.001	0.031 ± 0.002
2	10.0	0.053 ± 0.001	0.060 ± 0.003	0.071 ± 0.005
3	10.0	0.037 ± 0.003	0.038 ± 0.001	0.050 ± 0.005
4	10.0	0.028 ± 0.002	0.027 ± 0.001	0.032 ± 0.001
2	30.0	0.054 ± 0.001	0.061 ± 0.003	0.072 ± 0.004
3	30.0	0.037 ± 0.001	0.043 ± 0.002	0.049 ± 0.001
4	30.0	0.026 ± 0.002	0.032 ± 0.000	0.032 ± 0.001
2	60.0	0.044 ± 0.012	0.057 ± 0.007	0.077 ± 0.001
3	60.0	0.027 ± 0.003	0.041 ± 0.002	0.049 ± 0.001
4	60.0	0.026 ± 0.001	0.031 ± 0.001	0.034 ± 0.003

Two-way ANOVA
Albumin concentration: $P < 0.005$
DLV concentration: $0.01 < 0.025$
N-DLV concentration: $P > 0.01$

Tukey test
0.0598 (2 g%), 0.0393 (3 g%), 0.0291 (4 g%)

Italics indicate significance

[a] $n = 3$ for each cell.

delavirdine have included an extensive therapeutic drug monitoring program in order to maintain targeted trough steady-state plasma concentration ranges (e.g. < 75 μM at 6-12 after the prior dose).

It is noteworthy that the current delavirdine therapeutic monitoring program has been employing total drug concentrations for clinical monitoring and dosage adjustments. However, not all drug contained in plasma may be available for eliciting antiviral activity. According to the free drug hypothesis, only that fraction of drug which is circulating in the unbound form is available to exert therapeutic activity. However, these theoretical issues need to be considered in light of recent data which describe the beneficial effect of delavirdine on HIV load (as measured by plasma HIV RNA) and surrogate markers (Para et al., 1996; Freimuth et al., 1996, 1994). Hopefully, greater insight into the influence of protein binding on antiviral activity will be gained when the results from these trials are further analyzed.

The present study examined the protein binding characteristics of delavirdine, as well as its *N*-dealkylated metabolite, over the range of concentrations currently being studied in clinical trials. Atevirdine (Pharmacia and Upjohn, Kalamazoo, MI), a structurally related BHAP, was previously shown to exhibit a high degree of protein binding in serum, plasma and albumin with the f_u in the range 1–2% (Rosser et al., 1994). Based on the similarities between delavirdine and atevirdine, we hypothesized that delavirdine would exhibit similar binding properties. We selected an equilibrium dialysis methodology over ultrafiltration and ultracentrifugation because it has been considered the reference method for such studies as these.

This study was designed such that the binding characteristics of delavirdine and *N*-DLV were examined in physiologic concentrations of the major protein which could be expected to be present in the HIV-infected patient. As plasma contains many different proteins potentially contributing to drug-protein binding, it was one of the protein

solutions used. Additionally, since albumin and AAG are the most important binding proteins in plasma, these were studied separately. Their normal concentrations in plasma average 4 g% and 50–100 mg%, respectively (MacKichan, 1992). Albumin is generally considered to bind acidic drugs with low affinity but high capacity, while AAG binds predominantly basic and neutral drugs with high affinity but low capacity (MacKichan, 1992). These proteins are especially important to study in the HIV-infected individual. AAG is an acute phase reactant which can show large fluctuations in both physiological and pathological conditions (Kremer et al., 1988). Albumin can be significantly lowered in these individuals as a result of malnutrition (Singer et al., 1992), gastrointestinal disease (Chlebowski et al., 1989), microalbuminuria (Luke et al., 1992; Bourgoignie et al., 1988) and nephrotic syndrome (Ingulli et al., 1991; Korbet and Schwartz, 1992; Kaplan et al., 1987). Immune globulins were also among the protein solutions studied, because there is evidence to suggest that patients with AIDS have a strong likelihood of developing hypergammaglobulinemia secondary to B-cell hyperactivity (Lane et al., 1983). Additionally, intravenous immune globulin has been used therapeutically in the prevention of bacterial infections (National Institute of Child Health and Human Development Intravenous Immunoglobulin Study Group, 1991) and HIV-associated immune thrombocytopenic purpura (Bussel and Haimi, 1988; Tertian et al., 1987).

One interesting finding in the initial screening phase was the high recovery of drug noted for albumin and plasma, and the lower overall recovery from the immune globulin and AAG solutions. Volume recoveries from the dialysis system were similar for all protein solutions studied and exceeded 95%, decreasing the likelihood that dialysis cell leakage contributed to the low recovery. Another possible reason is that in samples where significantly less drug is bound to protein, the free drug concentration exceeded the solubility in PBS resulting in precipitation. However, this was not noted visually during any of the experiments. A third explanation could be that the free drug binds significantly to the dialysis system. The latter two theories were tested experimentally by dialyzing three low concentrations of delavirdine and *N*-DLV in PBS with PBS in the opposite chamber. Recoveries ranged from 88 to 113% for each compound, suggesting minimal binding to dialysis equipment, as well as good solubility in PBS at low concentrations in the absence of protein. The range of recovery reflected the variance seen within the assay methodology at 95% confidence.

Given the high degree of binding, further studies to examine the protein binding properties of three different concentrations of delavirdine and *N*-DLV, alone and in combination, in plasma and a range of albumin concentrations were performed. When delavirdine was studied in plasma in concentrations ranging from 12 to 73 μM, a significant increase in the fraction unbound was noted as the delavirdine concentration was increased, although this increase is unlikely to be clinically significant as the change in the f_u was only a fraction of a percent. The addition of *N*-DLV to the system did not result in any significant changes in the degree of delavirdine protein binding in plasma, indicating that the metabolite does not displace the parent drug to any significant extent. Similarly, the study of delavirdine in albumin solution revealed an increased f_u as the concentration of delavirdine increased, but again this increase is unlikely to be clinically significant. As expected, when the concentration of albumin is increased, the f_u of delavirdine decreased. Additionally, the studies of delavirdine in albumin solution did not reveal any significant delavirdine displacement by *N*-DLV.

Studies of *N*-DLV in plasma and albumin revealed a higher f_u than that seen with delavirdine. Fraction bound was noted to increase with increasing protein concentration. Interestingly, it was noted that at any given protein concentration, increasing the drug concentration resulted in an increase in the f_u rather than a decrease as might be expected in a system where saturation is expected to occur. These results were duplicated in independent studies of albumin and plasma suggesting they were not laboratory artifact. One possible explanation for this observation could be that as *N*-DLV binds to a primary site on albu-

min, it induces a conformational change in the protein structure, thereby leading to the formation of additional binding sites. Although an interesting observation, the clinical significance is probably unimportant. The addition of delavirdine to plasma and albumin solutions containing *N*-DLV resulted in a small but significant increase in the f_u of *N*-DLV indicating that displacement did occur.

Although an attempt was made to examine the number of binding sites and binding affinity constant for delavirdine, the concentrations studied did not allow for adequate saturation to occur to fully elucidate each of these. For this reason, only the product of these two constants is reported, the significance of which is academic. Ideally, one would want to know the values of the individual constants, but this would only be possible by evaluating higher concentrations of delavirdine than those examined in our study.

From the above information, one can conclude that delavirdine is significantly bound to the proteins present in plasma, primarily to albumin. Over the range of delavirdine concentrations being targeted in clinical trials, there appears to be saturation of the binding proteins, but probably not to any clinically significant extent as exhibited by very small changes in the f_u as the concentration is increased from 12 to 73 μM. Of note is the fact that delavirdine is not displaced significantly by *N*-DLV.

Although patients with HIV may have alterations in circulating albumin, immune globulins and AAGs, delavirdine demonstrates significant binding only to albumin, hence this is the primary protein of clinical relevance when considering the plasma protein binding of this new compound. In the present in vitro study, delavirdine protein binding was studied at albumin concentrations as low as 2 g% and as high as 4 g%. Over this range of concentrations, a statistically significant change in the fraction unbound was demonstrated. The question remains as to whether this increase is clinically significant. Delavirdine is known to have a very large therapeutic window and increases in the free concentration would be unlikely to lead to severe toxicity if unmonitored. However, it is theoretically possible that this increase in free concentration could result in increased antiretroviral efficacy as more drug would be available to interact with RT. Additionally, the increasing fraction unbound at higher concentrations taken together with the saturable metabolism may enhance free drug penetration into peripheral distribution compartments such as the central nervous system. Therefore, the question remains as to whether it is most appropriate in concentration-controlled trials or during a therapeutic drug monitoring program to monitor total or free concentrations as a correlate to clinical efficacy, as the f_u will differ between patients based on their plasma concentration of delavirdine and the level of plasma proteins. The interpatient variability in delavirdine pharmacokinetics may also be complicated by certain comorbid conditions such as renal failure with uremia, hepatic failure and hyperbilirubinemia which may all result in a higher delavirdine fraction unbound.

One must also consider, as with any drug which is so highly protein bound, the possibility for drug interactions via drug displacement from competitive interactions for a similar protein-binding site. Based on the nature of HIV disease, patients could be on a number of different medications which may displace delavirdine; these studies are ongoing in our laboratory.

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