Short Communication

EFFECTS OF A P-GLYCOPROTEIN INHIBITOR ON BRAIN AND PLASMA CONCENTRATIONS OF ANTI-HUMAN IMMUNODEFICIENCY VIRUS DRUGS ADMINISTERED IN COMBINATION IN RATS

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ABSTRACT:

Most of the existing anti-human immunodeficiency virus agents enter the central nervous system (CNS) inefficiently and thus may allow slow viral replication in the brain. This may provide a sanctuary for the virus in the CNS and contribute to the development of acquired immunodeficiency syndrome dementia complex. This study evaluates a prodrug approach to improve the CNS delivery of the reverse transcriptase inhibitor 2',3'-dideoxyinosine (ddI) in combination with inhibition of P-glycoprotein-mediated efflux to increase the CNS delivery of the protease inhibitor nevirapin and to determine whether any unanticipated drug interactions occur in this combination therapy. Three rats received either 6-chloro-2',3'-dideoxyxypurine (6-Cl-ddP), a prodrug of ddI activated by adenosine deaminase, nevirapin, nevirapin and 6-Cl-ddP, nevirapin and N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) (a P-glycoprotein inhibitor), 6-Cl-ddP and GF120918, or 6-Cl-ddP, nevirapin, and GF120918. Both 6-Cl-ddP and nevirapin were administered as i.v. infusions, whereas GF120918 was given as an i.v. bolus 2 h before sampling. Plasma and brain tissue concentrations of 6-Cl-ddP, ddI, and nevirapin were determined. Neither nevirapin nor GF120918 was shown to alter the brain/plasma ratios of 6-Cl-ddP or ddI. GF120918, however, increased the plasma concentrations of 6-Cl-ddP and ddI, resulting in increased brain concentrations. GF120918 increased the brain/plasma ratio of nevirapin significantly (−100-fold). The brain/plasma ratios of nevirapin were reduced nearly 2-fold in rats treated with nevirapin, 6-Cl-ddP, and GF120918 compared with rats receiving only nevirapin and GF120918, suggesting a modest inhibition of nevirapin uptake by 6-Cl-ddP. Overall, combined 6-Cl-ddP, nevirapin, and GF120918 administration enhances the brain/plasma ratios of both ddI and nevirapin.

Highly active antiretroviral therapy that includes both reverse transcriptase inhibitors (RTIs1) and protease inhibitors has improved the clinical management of HIV infection dramatically (Carpenter et al., 2000). A problem with current antiretroviral medication, however, is the limited entry of these drugs into the central nervous system (CNS) (Kaufmann and Cooper, 2000). HIV-1 can enter the CNS through infected monocytes that differentiate into macrophages and microglia in the brain (Rausch and Stover, 2001). Productive viral replication by infected macrophages and microglia in the CNS leads to loss of cognitive and motor function.

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inhibitor in humans (Witherspoon et al., 1996) and has been shown to inhibit P-gp in the blood-brain barrier, increasing the entry of P-gp substrates into the CNS in rats (Lettrent et al., 1999) and in mice (Polli et al., 1999). The observation that probenecid, an inhibitor of multidrug resistance-associated protein (MRP) in brain endothelial cells (Hua-Yun et al., 1998), also increases brain concentrations of certain dideoxynucleosides (Galinsky et al., 1991) implicates MRPs in the brain uptake of anti-HIV agents. Since MRP and P-gp have many substrates in common (Hollo et al., 1998), including small hydrophobic peptides (De Jong et al., 2001) and the HIV protease inhibitors (Srinivas et al., 1998), it is reasonable to speculate that there may be interactions between nelfinavir and 6-Cl-ddP/ddI when given in combination. Our goal is to introduce a different approach in the possible treatment of HIV infection in general and ADC in particular. Prodrug technology (6-Cl-ddP) is used to deliver an RTI (ddI) in sufficient amounts to the CNS, and a P-gp inhibitor (GF120918) is used to obtain higher concentrations of a protease inhibitor (nelfinavir) in the CNS. The aim of this study is to examine whether coadministration of 6-Cl-ddP, nelfinavir, and GF120918 enhances the brain delivery of ddI and nelfinavir in rats and whether significant interactions in CNS delivery occur in the combination therapy.

Materials and Methods

Chemicals. 2',3'-Dideoxyinosine was provided by the National Institute of Allergy and Infectious Diseases (Bethesda, MD). The preparation of 6-Cl-ddP has been described previously (Murakami et al., 1991). Nelfinavir was extracted from Viracept tablets (Agouron Pharmaceuticals, Inc., a Pfizer Company, Ann Arbor, MI), yielding a white solid having a purity of 98.6% by microtitration and exhibiting a single peak by HPLC. GF120918 was a gift from GlaxoSmithKline (Research Triangle Park, NC).

Surgical Procedure and Preparation of Infusion Solution. Male Sprague-Dawley rats were obtained from Harlan, Inc. (Prattville, AL) and housed and cared for at the Division of Laboratory Animal Research facilities at the University of Kentucky. All animal procedures conformed to the guidelines for the care and use of laboratory animals set by the University of Kentucky. Animals were anesthetized with 100 mg/kg ketamine and 8 mg/kg xylazine i.p. Using aseptic technique, catheters were implanted into the jugular and femoral veins, as described by Waynforth and Flecnell (1994). The animals were allowed a minimum recovery period of 24 h after surgery, and the catheters were flushed daily with 0.3 ml of normal saline containing 0.9% heparin. 6-Cl-ddP, nelfinavir, and GF120918 solutions were prepared in normal saline, deionized water adjusted to pH 2.4 with HCl, and dimethyl sulfoxide, respectively.

Experimental Designs. Intravenous infusions were performed in three rats per group receiving either 6-Cl-ddP (target dose, 134.8 mg/kg/h), nelfinavir (target dose, 10.5 mg/kg/h), nelfinavir and 6-Cl-ddP, nelfinavir and GF120918 (10 mg/kg), 6-Cl-ddP and GF120918, or 6-Cl-ddP, nelfinavir, and GF120918. Body weights on the day of the experiment were 292 ± 41 g (mean ± S.D.; n = 18). Nelfinavir was infused for 8 h and 6-Cl-ddP for 0.5 h beginning at 7.5 h. The time of administration of GF120918 was chosen based on its plasma distribution kinetics and elimination half-life (2.7 h) (Hyafil et al., 1993) and was given at 6 h as an i.v. bolus. At the end of 8 h, the samples were collected for 6-Cl-ddP and ddI analyses, as described previously by Morgan et al. (1992). Blood samples for analysis of nelfinavir were centrifuged, and plasma was collected and frozen before extraction. Brain tissue samples were quick-frozen until the time of sample preparation.

Sample Preparation. Plasma samples for 6-Cl-ddP and ddI analysis. The plasma samples were treated as described previously by Morgan et al. (1992). Dried extracts were stored at −20°C before analysis.

Plasma samples for nelfinavir analysis. Samples were thawed, and 0.5 ml of acetonitrile was added to 0.1 ml of plasma. Then the samples were vortexed for 4 min and centrifuged at 3500 rpm for 5 min. Supernatants were removed and dried under a nitrogen stream. Dried extracts were resuspended in mobile phase and analyzed by HPLC.

Brain samples for 6-Cl-ddP and ddI analysis. Brain tissue samples were treated as described previously by Morgan et al. (1992). Dried extracts were stored at −20°C before analysis.

Brain samples for nelfinavir analysis. Brain homogenates were prepared in the same manner as above (Morgan et al., 1992). The prepared homogenates were vortexed with 12.5 ml of acetonitrile and centrifuged (400g for 10 min). The supernatants were collected in 20-ml glass scintillation vials and evaporated to dryness under a nitrogen stream. Dried extracts were stored at −20°C before analysis. Frozen spiked plasma and brain controls that were prepared and analyzed simultaneously with actual samples indicated no degradation of analytes over the time period of storage in the freezer.

HPLC Analyses. Dry frozen samples were thawed and redissolved either in phosphate buffer (6-Cl-ddP and ddI) or in mobile phase (nelfinavir). Plasma and brain concentrations were determined by reversed-phase HPLC with UV detection at 254 nm. The separations were achieved on a Supelcosil LC-18S column (Bellefonte, PA; 6-Cl-ddP and ddI) and Supelcosil ABZ+ Plus column (nelfinavir). Assays for the spiked tissue samples showed good linearity (r² between 0.9953 and 0.9999) over the concentration ranges studied. Recoveries (mean ± S.D.) of 6-Cl-ddP, ddI, and nelfinavir from spiked plasma samples were 83.0 ± 4.3% (n = 10), 94.3 ± 3.3% (n = 10), and 81.3 ± 7.3% (n = 5), respectively. Recoveries of 6-Cl-ddP, ddI, and nelfinavir from spiked brain samples were 96.8 ± 2.9% (n = 10), 105.5 ± 6.0% (n = 11), and 86.3 ± 2.8% (n = 8), respectively.

Statistical Analysis. Two-way analysis of variance with Tukey's post hoc test was used to determine the statistical difference between the dose combinations, and p < 0.05 was considered to be significant.

Results and Discussion

The infusion times for nelfinavir and 6-Cl-ddP were sufficient to reach steady state in plasma. Previous pharmacokinetic studies have shown that plasma and brain steady-state levels for 6-Cl-ddP are reached within 30 min at the dose used in this study (Anderson et al., 1990; Morgan et al., 1992). Nelfinavir’s elimination half-life is ~1.3 h in rats (Shetty et al., 1996), and plasma steady state is obtained with an 8 h infusion (data not shown). The time necessary for nelfinavir concentrations to reach steady state in the brain during an i.v. infusion has not been established. Parenchymal brain concentrations were obtained by correcting the measured brain sample concentrations for the vascular space component, which was set at 2% based on our previous findings (Anderson et al., 1990; Morgan et al., 1992). The concentrations in brain were calculated according to the equation: C = C(0) - Vp Cpp, where C(0) is the brain concentration (extracellular + intracellular), C(0) is the drug concentration in the brain sample, Vp is the fraction of brain tissue space occupied by plasma, and Cpp is the drug concentration in plasma. The specific gravity of brain tissue was assumed to be 1.0. The brain parenchyma to plasma ratio (mean ± S.D.) of nelfinavir was 0.022 ± 0.015 and 0.011 ± 0.013 in the absence and presence of 6-Cl-ddP, respectively (Fig. 1A). Similar low brain penetration of nelfinavir has been reported in mice (Choo et al., 2000). GF120918 increased the brain/plasma ratio of nelfinavir significantly (~100-fold; Fig. 1A). This increase exceeds those reported in the literature when using other P-gp inhibitors (Choo et al., 2000). However, Choo et al. (2000) used mice, and nelfinavir was given as an injection rather than as an infusion to steady-state concentrations in plasma, making it difficult to interpret the differences in apparent P-gp inhibitor potencies observed.

The possibility that part of the increase in brain tissue concentration of nelfinavir following GF120918 treatment may be due to displacement of protein-bound nelfinavir merits further consideration because nelfinavir has been shown to be ~98.5% protein bound in human serum (Zhang et al., 2001). However, there are several observations suggesting that protein-binding effects were not the source of the increased brain tissue concentrations caused by GF120918. First, as shown in Table 1, the nelfinavir concentration in plasma was 12.5 μg/ml in the absence of GF120918 and 12.3 μg/ml 2 h after a dose of GF120918. Significant displacement of protein-bound nelfinavir might
be expected to have altered the steady-state plasma concentration. This may not have occurred, however, due to the likelihood that the plasma concentration of GF120918 was much lower than the steady-state concentration of nelfinavir. Although the steady-state nelfinavir concentration in plasma was found to be $H_{11011}^{12}_{9262}$ g/ml, Hyafil et al. (1993) determined the pharmacokinetics of GF120918 in mice following a 10 mg/kg i.v. bolus (the same dose administered in this study) and found a blood concentration at 2 h after i.v. administration of $H_{11011}^{0.1}_{9262}$ g/ml. Although we did not monitor GF120918 concentrations in this study, it is likely that they were substantially below the nelfinavir concentrations in plasma. Additional evidence for a minimal effect of GF120918 on protein binding comes from a study of the tissue distribution of amprenavir, another HIV protease inhibitor that is also extensively protein bound (Polli et al., 1999). These authors demonstrated that the distribution of amprenavir in blood, brain, cerebrospinal fluid, testes, and muscle was similar in mdr 1a/1b genetic double-knockout mice and after GF120918 pretreatment.

FIG. 1. Brain parenchyma/plasma ratios (mean ± S.D.; n = 3) of nelfinavir (A) and ddI (B) after different drug combinations.

The ddI brain parenchyma/plasma ratio after ddI treatment in 1B is from Morgan et al. (1992). a, values are significantly different from 6-Cl-ddP/Nelf; b, values are significantly different from Nelf; c, values are significantly different from Nelf + 918 ($p < 0.05$; two-way analysis of variance and Tukey’s post hoc test). 918, GF120918; Nelf, nelfinavir.

<table>
<thead>
<tr>
<th>Dose</th>
<th>6-Cl in Plasma</th>
<th>6-Cl in Brain Parenchyma</th>
<th>ddI in Plasma</th>
<th>ddI in Brain Parenchyma</th>
<th>Nelf in Plasma</th>
<th>Nelf in Brain Parenchyma</th>
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<tbody>
<tr>
<td></td>
<td>$\mu g/ml$</td>
<td>$\mu g/g$</td>
<td>$\mu g/ml$</td>
<td>$\mu g/g$</td>
<td>$\mu g/ml$</td>
<td>$\mu g/g$</td>
</tr>
<tr>
<td>6-Cl</td>
<td>14.7 ± 6.0</td>
<td>5.5 ± 2.3</td>
<td>11.6 ± 0.8</td>
<td>6.2 ± 0.2</td>
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<tr>
<td></td>
<td>(17.2 ± 3.6)</td>
<td>(11.6 ± 4.6)</td>
<td>(7.9 ± 1.8)</td>
<td>(6.4 ± 1.7)</td>
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<tr>
<td>6-Cl + Nelf</td>
<td>18.8 ± 10.4</td>
<td>7.3 ± 2.2</td>
<td>12.3 ± 1.2</td>
<td>5.3 ± 0.8</td>
<td>15.9 ± 4.4</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>6-Cl + 918</td>
<td>22.6 ± 9.8</td>
<td>10.9 ± 0.8</td>
<td>13.9 ± 3.1</td>
<td>7.9 ± 0.2$^	ext{a,b}$</td>
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<tr>
<td>Nelf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5 ± 1.2</td>
<td>0.3 ± 0.2</td>
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<tr>
<td>Nelf + 918</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.3 ± 2.5</td>
<td>23.2 ± 2.6$^	ext{a,d}$</td>
</tr>
<tr>
<td>6-Cl + Nelf + 918</td>
<td>25.4 ± 10.3</td>
<td>11.0 ± 4.5</td>
<td>20.2 ± 3.0$^	ext{a,b,c}$</td>
<td>12.2 ± 0.3$^	ext{a,b,c}$</td>
<td>14.4 ± 5.8</td>
<td>16.0 ± 4.9$^	ext{a,b,c}$</td>
</tr>
</tbody>
</table>

$6\text{-Cl}, 6\text{-Cl-ddP}; Nelf, nelfinavir; 918, GF120918.$

$^a$ Values are significantly different from 6-Cl.

$^b$ Values are significantly different from 6-Cl + Nelf.

$^c$ Values are significantly different from 6-Cl + 918.

$^d$ Values are significantly different from Nelf.

$^e$ Values are significantly different from Nelf + 918 ($p < 0.05$, Two-way analysis of variance and Tukey’s post hoc test).
producing “chemical knockouts”, a highly unlikely outcome if protein binding of nelfinavir had been substantially altered after GF120918 pretreatment.

In rats administered GF120918, the brain/plasma ratios of nelfinavir were significantly reduced (nearly 2-fold) with 6-Cl-ddP treatment (1.95 ± 0.48 versus 1.14 ± 0.13) (Table 1; Fig. 1A), suggesting an effect of 6-Cl-ddP on nelfinavir uptake into the brain. A reduction upon 6-Cl-ddP treatment was also seen in brain/plasma ratios of nelfinavir in the absence of GF120918, but the significance of this difference was not established, perhaps due to the limited number of animals used in each group. Although ddI and other reverse transcriptase inhibitors do not affect the transport of nelfinavir in an LLC-PK1 cell line (Shiraki et al., 2000), the reduced uptake of nelfinavir upon 6-Cl-ddP treatment is reminiscent of the observed reduction of nelfinavir brain/plasma ratios in mdrla(−/−) mice caused by valspador, another P-gp inhibitor, which was assumed to reflect inhibition of one or more drug uptake transport systems (Choo et al., 2000). Co-administration of nelfinavir with 6-Cl-ddP did not change the distribution of 6-Cl-ddP and ddI between plasma and brain (Table 1; Fig. 1B). The actual brain and plasma concentrations are similar to those found earlier after 6-Cl-ddP infusion alone (Morgan et al., 1992). After 6-Cl-ddP infusion, the brain/plasma ratio of ddI increases significantly compared with an infusion of ddI (Morgan et al., 1992; Fig. 1B). GF120918 increased the brain concentrations of both 6-Cl-ddP and ddI (up to 2-fold). The magnitude of the increase, however, parallels that in plasma; thus, GF120918 did not seem to affect the brain/plasma ratios of 6-Cl-ddP or ddI (Table 1). The elevated brain and plasma levels of 6-Cl-ddP and ddI in the presence of GF120918 may be due to a reduction in their systemic elimination. These results indicate that coadministration of 6-Cl-ddP, nelfinavir, and GF120918 significantly enhances the brain concentrations of ddI and nelfinavir in comparison with the administration of ddI and nelfinavir alone. Thus, the combination of a prodrug-approach and P-gp inhibition may provide a new alternative to treat HIV infection in general and ADC in particular.

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**References**


