Nevirapine Uptake into the Central Nervous System of the Guinea Pig: An in Situ Brain Perfusion Study

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ABSTRACT
The presence of human immunodeficiency virus (HIV) in the central nervous system (CNS) is associated with the development of HIV-1-associated dementia (HAD), a major cause of HIV-related mortality. To eradicate HIV in the CNS, anti-HIV drugs need to reach the brain and cerebrospinal fluid (CSF) in therapeutic concentrations. This involves passage through the blood-brain and blood-CSF barriers. Using a well-established guinea pig in situ brain perfusion model, this study investigated whether nevirapine [6H-dipyrdo(3,2-b:2′,3′-e)(1,4)diazepin-6-one, 11-cyclopropyl-5,11-dihydro-4-methyl], a non-nucleoside reverse transcriptase inhibitor (NNRTI), could effectively accumulate in the CNS. [3H]Nevirapine was coperfused with [14C]mannitol (a vascular/paracellular permeability marker) through the carotid arteries for up to 30 min, and accumulation in the brain, CSF, and choroid plexus was measured. [3H]Nevirapine uptake into the cerebrum was greater than uptake of [14C]mannitol, indicating significant passage across the blood-brain barrier and accumulation into the brain (this was further confirmed with capillary depletion and high-performance liquid chromatography analyses). Likewise, [3H]Nevirapine showed a great ability to cross the blood-CSF barrier and accumulate in the CSF, compared with [14C]mannitol. The CNS accumulation of [3H]Nevirapine was unaffected by 100 μM nevirapine, suggesting that passage across the blood-brain barrier can occur by diffusion. Furthermore, coperfusion with 100 μM efavirenz (2H-3,1-benzoxazin-2-one, 6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-, (4S)-; another NNRTI] did not significantly alter CNS accumulation of [3H]Nevirapine, indicating that the efficacy of nevirapine in the CNS would not be altered by the addition of this drug to a combination therapy. Together, these data indicate that this anti-HIV drug should be beneficial in the eradication of HIV within the CNS and the subsequent treatment of HAD.

Nevirapine belongs to the class of anti-HIV drugs called the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and was the first of these drugs to be approved for clinical use. Although early clinical trials with nevirapine found that its use as a monotherapy resulted in the rapid onset of resistance and hypersensitivity reactions (Carr and Cooper, 1996; Hammer, 2005), it is now evident that nevirapine is effective when used as part of highly active antiretroviral therapy (HAART) (Hartmann et al., 2005) and may offer an alternative to the inclusion of protease inhibitors (which have been associated with toxicity during long term use) in such treatment (Barreiro et al., 2000). Furthermore, compared with the protease inhibitors, nevirapine offers more convenient administration regimens (once daily dosing is possible, and there are no food restrictions), which has meant that it is a particularly successful anti-HIV treatment for young children (Verweel et al., 2003). Single-dose nevirapine treatment can also significantly reduce perinatal transmission of HIV (Guay et al., 1999; Jackson et al., 2003; Hammer, 2005). The introduction of HAART to the field of HIV treatment has had a dramatic impact on the clinical consequences of HIV infection, successfully reducing patients’ viral loads, leading to decreases in mortality and morbidity (Sabin, 2002). Although the incidence of opportunistic CNS infections has reduced in the era of HAART (Collazos, 2003), the occurrence of HIV-1-associated dementia (HAD) is still on the increase with the improved survival of AIDS patients (McArthur et al., 2003). Consequently, HAD continues to be a major cause of HIV-related morbidity and mortality (Sacktor et al., 2002; Albright et al., 2003; McArthur et al., 2003). The neuronal damage associated with HAD is, in part, a result of HIV-infected macrophages/microglia producing neurotoxic factors that alter neural function (Williams

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ABBREVIATIONS: nevirapine, 6H-dipyrdo(3,2-b:2′,3′-e)(1,4)diazepin-6-one, 11-cyclopropyl-5,11-dihydro-4-methyl-; HIV, human immunodeficiency virus; NNRTI, non-nucleoside reverse transcriptase inhibitor; HAART, highly active antiretroviral therapy; CNS, central nervous system; HAD, HIV-1-associated dementia; CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; DMSO, dimethyl sulfoxide; efavirenz, 2H-3,1-benzoxazin-2-one, 6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-, (4S)-; ANOVA, analysis of variance.
and Hickey, 2002). It is believed that if therapeutic levels of anti-HIV drugs are achieved in the CNS, this will help to eradicate HIV within this region and thus reduce the occurrence of HAD.

Drugs must cross the blood-brain and blood-cerebrospinal fluid (CSF) barriers to reach the CNS. The blood-brain barrier is located at the level of the cerebral capillary endothelial cells, and the blood-CSF barrier is formed by the choroid plexuses and the arachnoid membrane. Indirect evidence that nevirapine reaches the CNS comes from the headaches and neuropsychiatric complications sometimes associated with its use (Wise et al., 2002). Furthermore, nevirapine in combination with nucleoside reverse transcriptase inhibitors significantly improves HIV-1-associated psychomotor slowing, compared with nucleoside reverse transcriptase inhibitors alone (von Giesen et al., 2002). Direct evidence indicates that, as a group, the NNRTIs are able to penetrate the CSF, and for nevirapine, the CSF/plasma ratio has been reported to range from 15 to 40% (Van Praag et al., 2002; von Giesen et al., 2002). However, drug concentrations in the CSF do not necessarily indicate drug levels in the brain and may prove not to be the best indicator of treatment efficacy in the CNS (Thomas and Segal, 1998). Because human studies on the CNS are limited to CSF analysis and postmortem examinations, experimental models in animals are necessary if we are to further our understanding of the potential efficacy of nevirapine. This study uses a well-established animal model to measure and compare the accumulation of nevirapine into both the brain and CSF simultaneously. In addition, nevirapine uptake into the choroid plexus, a potential site of CNS entry and a known reservoir of productive viral infection (Chen et al., 2000; Petito, 2004), was evaluated. Furthermore, with the knowledge that certain anti-HIV drugs interact with influx and efflux transporters present at the blood-brain and blood-CSF barriers (Taylor, 2002; Thomas, 2004), the possibility that the passage of nevirapine across the brain barriers is influenced by transporters was also investigated.

**Materials and Methods**

All experiments were performed within the guidelines of the Scientific Procedures Act 1986 UK. An in situ brain perfusion method (Gibbs and Thomas, 2002) was used to measure the uptake of radiolabeled nevirapine into the CNS. Guinea pigs were anesthetized [0.32 mg/kg fentanyl and 10 mg/kg flumisone (Hypnorm; Janssen Animal Health, High Wycombe, UK) and 5 mg/kg midazolam (Hynovil; Roche, Basel, Switzerland)] i.p. and hepianized (25,000 units heparin sodium/ml, 1 ml/kg i.p.) before the carotid arteries were cannulated with fine tubing connected to a perfusion circuit. A warmed (37°C) and filtered artificial plasma (Gibbs and Thomas, 2002) containing [3H]nevirapine (65 nM) and [14C]mannitol (1.3 μM) was perfused through the circuit into both left and right carotid arteries at a total rate of 8.4 ml/min. The jugular veins were sectioned on initiation of the perfusion. Perfusion were terminated after 2.5, 10, 15, 20, or 30 min, when a cisterna magna CSF sample was taken using a fine-glass cannula. The animal was then decapitated, and the brain was removed for sampling. Both lateral ventricle choroid plexuses were extracted, and then triplicate samples of the left and right cerebrum were taken along with samples of the cerebellum and the pituitary gland. Samples (100 μl) of the inflowing artificial plasma were also taken in triplicate. All of the samples were weighed and placed into scintillation vials, and then 0.5 ml of tissue solubilizer (Solvable; Packard, Berkshire, UK) was added, and the samples were left for 48 h. Subsequently, 3.5 ml of scintillation fluid (Lumasafe; Packard) was added to the samples, and they were vortexed in preparation for scintillation counting. A Packard Tri-Carb 1900TR liquid scintillation counter was used for [3H]/[14C] dual counting, and the counts per minute were converted to disintegrations per minute by the use of internally stored quench curves. Uptake of [3H]nevirapine and [14C]mannitol into the brain and CSF samples (disintegrations per minute per gram) was determined as a ratio of levels detected in the artificial plasma (disintegrations per minute per milliliter) and termed percentage uptake (milliliters per 100 grams).

**Capillary Depletion Analysis.** Capillary depletion analysis was also carried out on the perfused brains, as described by Triguero et al. (1990). Approximately 500 mg of cerebrum was homogenized in a glass homogenizer with 1.5 ml of capillary depletion solution (100 m M HEPES, 141 mM NaCl, 2.8 mM CaCl2, 2H2O, 1 mM MgSO4.3H2O, 1 mM NaH2PO4, 2H2O, and 10 mM d-glucose) before the addition of 2 ml of dextran solution (26% w/v in water) and further homogenization. Duplicate samples of this homogenate were taken, and the remainder was separated into two microcentrifuge tubes and centrifuged for 15 min (5400 g, 4°C). The resulting supernatant (consisting of the brain parenchyma) and the pellet (rich in cerebral capillaries) were separated and taken together with the homogenate samples for liquid scintillation counting as described before.

**HPLC Analysis.** To ensure the integrity of the radiolabeled nevirapine during perfusion through the cerebral circulation, samples of the arterial inflow, venous outflow, and perfused brain were taken during 30-min [3H]nevirapine perfusions and prepared for HPLC and radiodetector analysis as described previously (Thomas et al., 2001). A Jasco HPLC system was used (Jasco Great Dunmow, Essex, UK) linked to a Packard Radioactive detector (Packard, Pangbourne, UK). All samples were eluted from a 300 × 3.9-mm Bondclone C18 column (Phenomenex, Macclesfield, Cheshire, UK) using an isocratic gradient of 76% 0.025 M KH2PO4/24% MeOH with 0.6% trifluoroacetic acid (v/v) over 20 min. The flow rate was set at 1 ml/min, and the UV absorbance was monitored at 244 nm. After HPLC analysis, the column outflow continued on to the radioactive detector, where it was mixed with a scintillation fluid (Ultima Flow M; Packard) and passed through a 0.5-ml flow cell for real-time radioactive analysis.

**Self-Inhibition Studies.** The effects of excess unlabeled nevirapine on [3H]nevirapine uptake into the CNS were established by means of self-inhibition studies. Due to the limited solubility of unlabeled nevirapine, it was first dissolved in dimethyl sulfoxide (DMSO) to a 100 mM concentration. This was then added to artificial plasma to achieve a nevirapine concentration of 100 μM (final DMSO concentration of 0.1%). This artificial plasma was then used in 20-min [3H]nevirapine/[14C]mannitol perfusions, as described before.

**Cross Competition Study.** In addition, the affect of 100 μM efavirenz on [3H]nevirapine CNS uptake over 20 min was assessed. Unlabeled efavirenz was added to the artificial plasma, which was then perfused into the carotid arteries with [3H]nevirapine and [14C]mannitol. A 100 mM stock solution of efavirenz was made up in DMSO, which was then added to the artificial plasma to achieve the required concentration (final DMSO concentration was 0.1%).

**Nevirapine Lipophilicity.** As a measure of lipophilicity, the octanol-saline partition coefficient of [3H]nevirapine was determined. Phosphate-buffered saline, pH 7.4 (0.75 ml), containing [3H]nevirapine was added to a microcentrifuge tube with 0.75 ml of octanol and vortexed. This was then centrifuged for 5 min (1000g, 4°C), and triplicate 100-μl samples of the upper phase (octanol) and lower phase (saline) were taken for radioactive scintillation counting. The octanol-saline partition coefficient (mean radioactivity in octanol samples (disintegrations per minute)/mean radioactivity in saline samples (disintegrations per minute) of [3H]nevirapine was determined in triplicate and reported as the mean ± S.E.M.

**Data Analysis.** Data from all of the experiments are presented as mean ± S.E.M. Statistical analysis was carried out using Sigma
Stat software (Jandel Scientific, San Rafael, CA) and significant at \( P < 0.05 \).

**Materials.** \( \Delta^-[14C] \)Mannitol (specific activity, 53 mCi/mmol) was purchased from Moravek Biochemicals (Brea, CA). Nevirapine (mol. wt. 266.3) was provided by Boehringer Ingleheim USA (Ridgefield, CT) and was custom-radiolabeled with \( ^3H \) by Moravek Biochemicals (specific activity, 2 Ci/mmol; Fig. 1). Efavirenz (mol. wt. 315.7) was provided by Bristol-Myers Squibb Co. (Stamford, CT). Unless specified, all other materials were purchased from Sigma (Dorset, UK).

**Results**

**Multiple-Time Uptake Studies.** Uptake of \( ^3H \)nevirapine and \( ^14C \)mannitol into the guinea pig cerebrum is shown in Fig. 2A. As expected, \( ^14C \)mannitol uptake, which represents cerebral vascular space, ranged from 0.5 \( \pm \) 0.1% at 2.5 min to 3.7 \( \pm \) 0.2% at 30 min. Levels of \( ^3H \)nevirapine detected in the cerebrum were significantly greater at each individual time point (paired Student’s \( t \) test, \( P < 0.01 \)) and peaked at 55.7 \( \pm \) 9.2% after 30 min (vascular space corrected). Figure 2B shows uptake of the two radiolabeled substances into the cerebellum over time. \( ^14C \)Mannitol uptake into this brain region was 0.51 \( \pm \) 0.07 ml/100 g at 2.5 min to 4.3 \( \pm \) 0.5 ml/100 g at 30 min. \( ^3H \)Nevirapine uptake into the cerebellum was significantly greater than \( ^14C \)mannitol at each time point (paired Student’s \( t \) test, \( P < 0.05 \)) and reached 41.3 \( \pm \) 7.2 ml/100 g at 30 min (vascular space corrected).

\( ^3H \)Nevirapine (mol. wt. 266.3) and \( ^14C \)mannitol (mol. wt. 182.0) uptake into the CSF is plotted in Fig. 2C. \( ^14C \)Mannitol uptake into the CSF, which signifies the rate of paracellular diffusion of a low-mol. wt. molecule across the blood-CSF barrier, reached 1.7 \( \pm \) 0.4 ml/100 g after 30-min perfusions. \( ^3H \)Nevirapine uptake into the CSF was greater than \( ^14C \)mannitol uptake and reached 31.7 \( \pm \) 6.0 ml/100 g at the longest perfusion time. Uptake of \( ^3H \)nevirapine into the CSF was significantly greater than \( ^14C \)mannitol uptake at 10, 15, 20, and 30 min (paired Student’s \( t \) tests, \( P < 0.05 \)).

\( ^3H \)Nevirapine uptake into the choroid plexus is plotted in Fig. 2D. \( ^14C \)Mannitol uptake into the choroid plexus was monitored as a measure of vascular and extracellular space within this tissue. Choroid plexus levels of this marker molecule ranged from 3.0 \( \pm \) 1.4 ml/100 g at 2.5 min to 11.4 \( \pm \) 1.0 ml/100 g at 30 min. Uptake of \( ^3H \)nevirapine into the choroid plexus also increased over time from 2.6 \( \pm \) 1.0 ml/100 g at 2.5 min to 25.2 \( \pm \) 7.8 ml/100 g at 30 min (\( ^14C \)mannitol corrected).

Figure 2E shows uptake of \( ^3H \)nevirapine and \( ^14C \)mannitol into the pituitary gland. \( ^14C \)Mannitol uptake into this CNS region reached 30.5 \( \pm \) 2.0 ml/100 g after 30 min (notably greater than \( ^14C \)mannitol uptake into the cerebrum and cerebellum, which are brain regions protected by the blood-brain barrier). Likewise, \( ^3H \)nevirapine uptake into the pi-
tuitary was at its highest in the pituitary gland and was measured as 160.7 ± 26.4 ml/100 g at 30 min.

**HPLC Analysis.** Figure 3 illustrates the HPLC/radiodetector analysis obtained from arterial inflow samples containing the 3H-labeled nevirapine. In all of the samples tested, the presence of intact radiolabeled nevirapine could be seen eluting with a retention time of approximately 10.5 min. Further studies found that the samples taken from the plasma after it had passed through the cerebral circulation (termed venous outflow) also contained intact and radiolabeled nevirapine. In addition, pooled whole-brain samples taken from animals that had undergone 30-min perfusions also contained intact radiolabeled nevirapine.

**Capillary Depletion Analysis.** Results from capillary depletion analysis performed after 30-min brain perfusions are shown in Fig. 4. Uptake levels of [3H]nevirapine (corrected for [14C]mannitol) into the homogenized cerebrum (44.9 ± 8.3 ml/100 g) and uptake into the cerebrum after 30 min (55.7 ± 9.2 ml/100 g; Fig. 2A) were not statistically different (one-way ANOVA followed by Bonferroni t test). Similar levels of [3H]nevirapine were detected in the whole brain (homogenate) and the brain parenchyma (supernatant) (one-way ANOVA followed by Bonferroni t test). However, uptake of [3H]nevirapine into the cerebral capillary-rich pellet was significantly lower than uptake into the whole-brain homogenate (one-way ANOVA and Bonferroni t test, P < 0.05).

**Cross-Competition Studies.** The effect of unlabeled nevirapine and unlabeled efavirenz on [3H]nevirapine uptake into the CNS is summarized in Fig. 5 (values are corrected for [14C]mannitol). The presence of 100 μM unlabeled nevirapine in the artificial plasma did not have a significant influence on uptake of radiolabeled nevirapine into any region of the CNS. Likewise, coperfusion of 100 μM efavirenz with [3H]nevirapine caused no significant change in [3H]nevirapine CNS uptake. The presence of 0.1% DMSO in the artificial plasma had no significant effect on [14C]mannitol uptake in any of the tissue compartments (one-way ANOVA).

**Octanol-Saline Partition Coefficient.** Following three experiments, the octanol-saline partition coefficient of [3H]nevirapine was determined to be 10.9 ± 0.2.

**Discussion**

Through the use of an in situ brain perfusion method, we measured [3H]nevirapine uptake into the guinea pig CNS, compared [3H]nevirapine passage across the blood-brain and blood-CSF barriers, and assessed whether the CNS accumulation of this drug is influenced by transporters or by the presence of a further NNRTI. Results indicated that nevirapine accumulates in the brain over time. [3H]Nevirapine uptake in the cerebrum and cerebellum after 30 min was high, 59.4 ± 9.4 and 45.6 ± 7.4%, respectively, and greater than the corresponding levels of the vascular marker [14C]mannitol. The ability of nevirapine to cross the blood-brain barrier has been observed previously using an in vitro bovine model (Glynn and Yazdanian, 1998). HPLC and capillary depletion analyses (Figs. 3 and 4) confirmed that intact [3H]nevirapine crossed the blood-brain barrier to accumulate in the brain. The degree of nevirapine brain uptake was high for an anti-
HIV drug. In fact, nevirapine has the highest brain accumulation of any of the anti-HIV drugs we have tested using this animal model, including 3′-azido-3′-deoxythymidine, (−)-β-L-2′,3′-dideoxy-3′-thiacytidine, 2′,3′-dideoxyninosine, 2′,3′-dideoxy-hydro-3′-deoxythymidine, 2′,3′-dideoxycytidine, abacavir, 9-[9(R)-2-(phosphonomethoxy)propyl]adenine, ritonavir, and amprenavir (Gibbs and Thomas, 2005; Anthonypillai et al., 2006). Nevirapine also crossed the in vitro blood-brain barrier at a higher rate than amprenavir, 2′,3′-dideoxynosine, 2′,3′-dideoxyhydro-3′-deoxythymidine, 2′,3′-dideoxycytidine, indinavir, and saquinavir (Glynn and Yazdanian, 1998). Our nevirapine results, together with the reported IC50 values for nevirapine against HIV replication (10–100 nM or 2.5–25 ng/ml) (Veldkamp et al., 2001b) and the maximum plasma concentration of nevirapine being 2516 to 9455 ng/ml (9.4–35.5 pM) in HIV-infected individuals (Van Praag et al., 2002), indicate that nevirapine is a promising therapeutic to tackle HIV residing in the brain. Previous studies indicate that the nucleoside analogs (except abacavir) exhibit a limited ability to pass from blood to brain (Glynn and Yazdanian, 1998; Sawchuk and Yang, 1999; Thomas, 2004). Furthermore, the protease inhibitors are highly bound to plasma proteins, although this may not restrict brain access (Anthonypillai et al., 2004; Thomas, 2004).

The ability of nevirapine to accumulate in the cerebrum is a reflection of this drug’s high octanol-saline partition coefficient (which was 10.9 ± 0.2, similar to previously reported values; Glynn and Yazdanian, 1998; Almond et al., 2005), because lipophilicity is a key determinant of the ability of the drug to cross the blood-brain barrier.

The role of the blood-brain barrier in regulating [1H]nevirapine entry into the brain was explored by measuring drug uptake into the pituitary gland. The neural lobe of the pituitary (part of the posterior pituitary) lies outside the blood-brain barrier; thus, the capillaries in this region are more permeable than the blood-brain barrier capillaries and allow the free exchange of substances between the blood and pituitary gland (Groas, 1992). Although [1H]nevirapine uptake into the pituitary was greater than uptake into the cerebrum and cerebellum (Fig. 2), the difference was not as dramatic as could be expected based on the paracellular permeability marker data, indicating that [1H]nevirapine is efficient at crossing the blood-brain barrier. Again, this appears to be a reflection of this drug’s lipophilicity. Movement of highly lipid-soluble compounds across the barriers can be so fast that uptake is limited by blood flow rather than permeability. Although we did not investigate whether nevirapine uptake was affected by cerebral blood flow, nevirapine was not completely cleared from the plasma after a 2.5-min perfusion [brain uptake being 6.1 ± 1.2% ([14C]mannitol corrected)]. This would equate to a unidirectional transfer constant (Km), determined by single-time uptake analysis (i.e., uptake divided by perfusion time), of 24.4 μl/min/g, which is lower than substances that are essentially flow-dependent, such as bromo-benzodiazepine (830 μl/min/g) (Drewes et al., 1987).

Overall, the lipophilic nature of nevirapine and its incomplete plasma clearance suggest that the brain entry of nevirapine is determined by both cerebral blood flow and its permeability across the blood-brain barrier.

[1H]Nevirapine levels in the guinea pig CSF were 31.7 ± 6.0% of plasma levels at 30 min, similar to the reported human CSF/plasma ratio of 40% (von Giesen et al., 2002). Another study reported nevirapine concentrations in the CSF of HIV-1-infected individuals (measured 1 h after administration of 200 mg b.i.d.) of 219 to 1837 ng/ml (0.8–6.9 pM) (Van Praag et al., 2002). When this is compared with plasma Cmax measured in the same study (2516–9455 ng/ml; 9.4–35.5 pM), this gives a lower CSF/plasma ratio of 15%. As shown in Fig. 2, [1H]nevirapine CSF uptake was lower than brain uptake, indicating that where clinical trials of nevirapine have measured CSF uptake alone, brain uptake may be significantly greater. The higher brain uptake of this drug may be due to the influence of transporters at these barriers that may facilitate [1H]nevirapine entry into the brain or impede the entry of this drug into the CSF. Earlier studies by our research group found evidence for nevirapine interaction with a transporter for the protease inhibitor, ritonavir, at the basolateral and apical membranes of the choroid plexus (Anthonypillai et al., 2004). Furthermore, the biphasic nature of the cerebrum, pituitary gland, and choroid plexus graphs in Fig. 2 is suggestive of a CNS-to-blood transport system, which is saturated after 15 min of [1H]nevirapine perfusion when cerebrum/pituitary gland uptake is >20% (i.e., >13 nM) and the choroid plexus uptake is equivalent to >3.9% (i.e., >2.5 nM). However, our self-inhibition and cross-competition studies revealed that an excess of nevirapine or efavirenz in the plasma did not affect [1H]nevirapine uptake into the brain, CSF, or choroid plexus (Fig. 5). This suggests that nevirapine passage into or out of the CNS is not assisted by saturable transporters. However, these data were from 20-min brain perfusion experiments, thus after the CNS-to-blood transporter indicated in the cerebrum, pituitary gland, and choroid plexus graphs in Fig. 2 had been saturated. Previous studies indicated that nevirapine is not a substrate for the P-glycoprotein transporter (Glynn and Yazdanian, 1998; Stormer et al., 2002), which is expressed at the blood-brain barrier. Although 30 μM nevirapine induces P-glycoprotein expression in the intestinal cell line LS 180 (Stormer et al., 2002), this was not observed with 10 μM nevirapine in peripheral blood mononuclear cells in vitro (Chandler et al., 2003). Interestingly, another study suggested that nevirapine does up-regulate P-glycoprotein expression in circulating lymphocytes and that nevirapine is a substrate for a lymphocyte efflux transporter, possibly P-glycoprotein or multidrug resistance protein-1 (Almond et al., 2005). In agreement with our cross-competition studies with efavirenz, a study of HIV-1-infected patients found that nevirapine pharmacokinetics were unaffected by efavirenz coadministration (Veldkamp et al., 2001a).

[1H]Nevirapine accumulated in the choroid plexus to levels above that of the vascular/extracellular space marker (Fig. 2). Interestingly, the choroid plexus levels were similar to those in the CSF, which may be expected since the presence of a drug in the CSF relates to blood-CSF barrier permeability and does not necessarily reflect blood-brain barrier permeability or brain drug concentrations (Grootwuis and Levy, 1997; Thomas and Segal, 1998). The ability of drugs to cross the choroid plexuses and reach the CSF is of interest in HIV treatment because drugs in the ventricular CSF will have rapid access to the infected perivascular and meningeal macrophages (Rennels et al., 1985; Ghersi-Egea et al., 1996). Certain characteristics of the choroid plexus also make it a potential site for HIV to gain entry into the CNS. Firstly, the permeable nature of the choroid plexus capillaries and the exclusion of this tissue from the protection of the blood-brain barrier make it a potential route of virus entry into the CNS.
Additionally, the choroid plexus stroma contains T-lymphocytes and monocytes derived from circulation; hence, it is a prospective site for infected lymphocytes and monocytes to enter the CSF from the blood and gain access to the brain parenchyma. HIV in the choroid plexus is a mixture of systemic and brain viral sequences (Chen et al., 2000), which suggests that the choroid plexus is a site of viral entry into the CNS. Furthermore, HIV-infected cells have been found in the stroma and supraepithelial area of postmortem choroid plexus tissue from patients who died with AIDS (Petito et al., 1999). Earlier studies have also suggested that the choroid plexus epithelial cells become infected with HIV (Bagasra et al., 1996). Thus, the choroid plexus is implicated in the entry of HIV into the CNS and also is a possible reservoir for the virus; as such, it can be considered as one of the principal targets for HIV treatment in the CNS.

In summary, this animal study shows that [H]n Nevirapine accumulates in the brain, CSF, and choroid plexus. Thus, nevirapine shows great potential as an effective treatment for HIV within the CNS. In concordance, nevirapine has a beneficial effect on HIV-1-associated psychomotor slowing in patients (von Giesen et al., 2002). This positive effect was suggested to be a consequence of the drug's CSF availability. Here, we demonstrate that, in addition to accumulating in the CSF, nevirapine has a great ability to enter the brain parenchyma, and it is likely that any improvement in psychomotor function associated with nevirapine is not only consequential of its presence in the CSF but also its presence in the brain. This study also highlights another issue of clinical importance, that drug levels in the CSF are not consequential of its presence in the CSF but also its presence in the brain from the subarachnoid space. Brain Res 326:47–63.


