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Title Page

Nevirapine uptake into the CNS of the guinea-pig; an *in situ* brain perfusion study

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Running title page

Running title: Nevirapine transport across the blood-brain interfaces.

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Abbreviations: HAART, highly active anti-retroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; HIV, human immunodeficiency virus; HAD, HIV-1 associated dementia; NRTI, nucleoside reverse transcriptase inhibitor.

Neuropharmacology

Abstract

The presence of human immunodeficiency virus (HIV) in the 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 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, a non-nucleoside reverse transcriptase inhibitor (NNRTI), could effectively accumulate in the CNS. [^{3}H]nevirapine was co-perfused with [^{14}C]mannitol (a vascular/paracellular permeability marker) through the carotid arteries for up to 30 min, and accumulation in the brain, CSF and choroid plexus measured. [^{3}H]nevirapine uptake into the cerebrum was greater than uptake of [^{14}C]mannitol, indicating significant passage across the blood-brain barrier and accumulation into the brain (this was further confirmed with capillary depletion and HPLC analyses). Similarly [^{3}H]nevirapine showed a great ability to cross the blood-CSF barrier and accumulate in the CSF, when compared to [^{14}C]mannitol. The CNS accumulation of [^{3}H]nevirapine was unaffected by 100μM nevirapine suggesting that passage across the blood-brain barrier can occur by diffusion. Furthermore co-perfusion with 100μM efavirenz (another NNRTI) did not significantly alter CNS accumulation of [^{3}H]nevirapine, indicating that the efficacy of nevirapine in the CNS would not be altered by 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.

Introduction

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 utilised 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 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 which alter neural function (Williams 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-CSF barriers to reach the CNS. The blood-brain barrier is located at the level of the cerebral capillary endothelial cells and the blood-cerebrospinal (CSF) barrier is formed by the choroid plexuses and the arachnoid membrane. Indirect evidence that suggests 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 (NRTIs) significantly improves HIV-1 associated psychomotor slowing, compared with NRTIs alone (von Giesen et al., 2002). Direct clinical 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 15-40% (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). As human studies on the CNS are limited to CSF analysis and post-mortem examinations, experimental models in animals are necessary if we are to further our understanding on the potential efficacy of nevirapine. This study utilizes a well-established animal model to measure and compare the accumulation of nevirapine into both the brain and CSF simultaneously. Additionally, nevirapine uptake into the choroid plexus, a potential site of CNS entry and a known reservoir of productive viral infection (Petito, 2004;Chen et al., 2000), 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.

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 utilised to measure the uptake of radiolabelled nevirapine into the CNS. Guinea-pigs were anaesthetised (0.32 mg/kg fentanyl and 10mg/kg fluanisone (Hypnorm; Janssenn Animal Health, High Wycombe UK) and 5mg/kg midazolam (Hypnovel, Roche, Switzerland) intraperitoneally) and heparinised (25,000 units heparin sodium/ml, 1ml/kg intraperitoneally), before the carotid arteries were cannulated with fine tubing connected to a perfusion circuit. A warmed (37ºC) and filtered artificial plasma (Gibbs and Thomas, 2002) containing [^{3}H]nevirapine (65 nM) and [^{14}C]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. Perfusions 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 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 the samples were weighed and placed into scintillation vials and then 0.5ml tissue solubiliser (Solvable; Packard Berkshire, UK) was added and the samples were left for 48 h. Subsequently 3.5 ml 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 utilised for [^{3}H]/[^{14}C] dual counting and the cpm were converted to dpm by the use of internally stored quench curves. Uptake of [^{3}H]nevirapine and [^{14}C]mannitol into the brain and CSF samples (dpm/g) was determined as a ratio of levels detected in the artificial plasma (dpm/ml) and termed uptake % (ml/100g).

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 homogenised in a glass homogeniser with 1.5ml capillary depletion solution (HEPES 100mM; NaCl 141 mM; KCl 4 mM; $CaCl_2.2H_2O$ 2.8mM; $MgSO_4.3H_2O$ 1mM; $NaH_2PO_4.2H_2O$ 1mM; D-glucose 10 mM) before the addition of 2 ml dextran solution (26% w/v in water) and further homogenisation. Duplicate samples of this homogenate were taken, and the remainder was separated into two microcentrifuge tubes and centrifuged for 15 min (5400 g, 4ºC). 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 radiolabelled nevirapine during perfusion through the cerebral circulation, samples of the arterial inflow, venous outflow and perfused brain were taken during 30 min [^{3}H]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 x 3.9 mm, Bondclone C18 column (Phenomenex, Macclesfield, Cheshire, UK) using an isocratic gradient of 76% 0.025M $KHPO_4$:24% MeOH with 0.6%$TFA_{(aq)}$ over 20 min. The flow rate was set at 1 ml/min and the UV absorbance 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 unlabelled nevirapine on [^{3}H]nevirapine uptake into the CNS were established by means of self-inhibition studies. Due to the limited solubility of unlabelled nevirapine, it was first dissolved in dimethyl sulfoxide (DMSO) to a 100mM concentration. This was then added to artificial plasma to achieve a nevirapine concentration of 100μM (final DMSO concentration 0.1%). This artificial plasma was then utilised in 20 min [^{3}H]nevirapine/[^{14}C]mannitol perfusions, as described before.

Cross competition study

Additionally, the affect of 100μM efavirenz on [^{3}H]nevirapine CNS uptake over 20 min was assessed. Unlabelled efavirenz was added to the artificial plasma, which was then perfused into the carotid arteries with [^{3}H]nevirapine and [^{14}C]mannitol. A 100mM 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 [^{3}H]nevirapine was determined. 0.75ml phosphate buffered saline (pH 7.4) containing [^{3}H]nevirapine was added to a microcentrifuge tube with 0.75ml octanol and vortexed. This was then centrifuged for 5 min (1000 g 4ºC) and triplicate 100μl 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 (dpm)/mean

radioactivity in saline samples (dpm)) of [^{3}H]nevirapine was determined in triplicate and reported as the mean ± the standard error of the mean.

Data Analysis

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

Materials

D-[^{14}C]mannitol (specific activity: 53 mCi/mmol) was purchased from Moravek Biochemicals Inc (California, USA). Nevirapine (6H-Dipyrido(3,2-b:2',3'-e)(1,4)diazepin-6-one, 11-cyclopropyl-5,11-dihydro-4-methyl-; MW 266.3) was provided by Boehringer-Ingelheim Pharmaceuticals, Inc. (Connecticut, USA) and was custom radiolabelled with [^{3}H] by Moravek Biochemicals Inc (specific activity: 2 Ci/mmol: Figure 1). Efavirenz (2H-3,1-Benzoxazin-2-one, 6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-, (4S)-; MW 315.7) was provided by Bristol-Myers Squibb Company (New York, USA). Unless specified, all other materials were purchased from Sigma (Dorset, UK).

Results

Multiple-time uptake studies

Uptake of [^{3}H]nevirapine and [^{14}C]mannitol into the guinea-pig cerebrum is shown on Figure 2a. As expected, [^{14}C]mannitol uptake, which represents cerebral vascular space, ranged between 0.5±0.1% at 2.5 min to 3.7±0.2% at 30 min. Levels of [^{3}H]nevirapine detected in the cerebrum were significantly greater at each individual time point (paired T test, $P<0.01$) and peaked at 55.7±9.2% after 30 min (vascular space corrected).

Figure 2b shows uptake of the two radiolabelled substances into the cerebellum over time. [^{14}C]mannitol uptake into this brain region was 0.51±0.07 ml/100g at 2.5 min to 4.3±0.5 ml/100g at 30 min. [^{3}H]nevirapine uptake into the cerebellum was significantly greater than [^{14}C]mannitol at each time point (paired T test, $P<0.05$) and reached 41.3 ± 7.2 ml/100g at 30 min (vascular space corrected).

[^{3}H]nevirapine (MW 266.3) and [^{14}C]mannitol (MW 182.0) uptake into the CSF is plotted on Figure 2c. [^{14}C]mannitol uptake into the CSF, which signifies the rate of paracellular diffusion of a low molecular weight molecule across the blood-CSF barrier, reached 1.7 ± 0.4 ml/100g after 30 min perfusions. [^{3}H]nevirapine uptake into the CSF was greater than [^{14}C]mannitol uptake and reached 31.7 ± 6.0 ml/100g at the longest perfusion time. Uptake of [^{3}H]nevirapine into the CSF was significantly greater than [^{14}C]mannitol uptake at 10, 15, 20 and 30 min (paired T tests, $P<0.05$).

[^{3}H]nevirapine uptake into the choroid plexus is plotted on Figure 2d. [^{14}C]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 ± 1.4 ml/100g at 2.5 min to 11.4 ± 1.0 ml/100g at 30 min. Uptake of [^{3}H]nevirapine into

the choroid plexus also increased over time from 2.6 ± 1.0 ml/100g at 2.5 min to 25.2 ± 7.8 ml/100g at 30 min ([^{14}C]mannitol corrected).

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

HPLC analysis

Figure 3 illustrates the HPLC/radiodetector analysis obtained from arterial inflow samples containing the [^{3}H]-labelled nevirapine. In all the samples tested the presence of intact radiolabelled nevirapine could be seen eluting with a retention time of approximately 10 and a half minutes. 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 radiolabelled nevirapine. In addition, pooled whole brain samples taken from animals which had undergone 30 min perfusions also contained intact radiolabelled nevirapine.

Capillary depletion analysis

Results from capillary depletion analysis performed after 30 min brain perfusions are shown on Figure 4. Uptake levels of [^{3}H]nevirapine (corrected for [^{14}C]mannitol) into the homogenized cerebrum (44.9 ± 8.3 ml/100g) and uptake into the cerebrum after 30 min (55.7 ± 9.2ml/100g, Figure 2a) were not statistically different (One way ANOVA followed by Bonferroni t test). Similar levels of [^{3}H]nevirapine were detected in the

whole brain (homogenate) and the brain parenchyma (supernatant) (one way ANOVA followed by Bonferroni t test). However, uptake of [^{3}H]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 unlabelled nevirapine and unlabelled efavirenz on [^{3}H]nevirapine uptake into the CNS is summarised in Figure 5, values are corrected for [^{14}C]mannitol. The presence of 100μM unlabelled nevirapine in the artificial plasma did not have a significant influence on uptake of radiolabelled nevirapine into any region of the CNS. Likewise, co-perfusion of 100μM efavirenz with [^{3}H]nevirapine caused no significant change in [^{3}H]nevirapine CNS uptake. The presence of 0.1% DMSO in the artificial plasma had no significant effect on [^{14}C]mannitol uptake in any of the tissue compartments (One way ANOVA).

Octanol-saline partition coefficient

Following three experiments, the octanol-saline partition coefficient of [^{3}H]nevirapine was determined to be 10.9 ± 0.2.

Discussion

Through the use of an *in situ* brain perfusion method we measured [^{3}H]nevirapine uptake into the guinea-pig CNS, compared [^{3}H]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. [^{3}H]Nevirapine uptake in the cerebrum and cerebellum after 30 min was high being 59.4±9.4 % and 45.6±7.4%, respectively and greater than the corresponding levels of the vascular marker, [^{14}C]mannitol. Nevirapines' ability to cross the blood-brain barrier has previously been observed using an *in vitro* bovine model (Glynn and Yazdanian, 1998). HPLC and capillary depletion analyses (Figures 3 and 4) confirmed that intact [^{3}H]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'-dideoxyinosine, 2'3'-didehydro-3'deoxythymidine, 2'3'-dideoxycytidine, abacavir, 9-[9(R)-2-(phosphonomethoxy)propyl]adenine, ritonavir and amprenavir (Anthonypillai et al., 2006;Gibbs and Thomas, 2005). Nevirapine also crossed the *in vitro* blood-brain barrier at a higher rate than amprenavir, 2'3'-dideoxyinosine, 2'3'-didehydro-3'deoxythymidine, 2'3'-dideoxycytidine, 3'-azido-3'-deoxythymidine, indinavir and saquinavir (Glynn and Yazdanian, 1998). Our nevirapine results, together with the reported IC_{50} values for nevirapine against HIV replication (10-100nM or 2.5-25ng/ml) (Veldkamp et al., 2001b), and the maximum plasma concentration of nevirapine being 2516 –9455ng/ml (9.4-35.5pM) in HIV-infected individuals (Van Praag et al., 2002), indicates that nevirapine is a promising therapeutic to tackle HIV residing in the brain. Previous studies indicate that the nucleoside analogues (except abacavir) exhibit a limited ability to pass from blood to brain (Thomas,

2004;Sawchuk and Yang, 1999;Glynn and Yazdanian, 1998). Furthermore, the protease inhibitors are highly bound to plasma proteins, although this may not restrict brain access (Thomas, 2004;Anthonypillai et al., 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 (Almond et al., 2005;Glynn and Yazdanian, 1998)), as lipophilicity is a key determinate of a drug's ability to cross the blood-brain barrier.

The role of the blood-brain barrier in regulating [^{3}H]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 and 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 (Gross, 1992). Whilst [^{3}H]nevirapine uptake into the pituitary was greater than uptake into the cerebrum and cerebellum (Figure 2), the difference was not as dramatic as could be expected based on the paracellular permeability marker data, indicating that [^{3}H]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 if 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% ([^{14}C]mannitol corrected)). This would equate to a unidirectional transfer constant (K_{in}), determined by single-time uptake analysis (i.e. uptake divided by perfusion time), of 24.4µl/min/g, which is lower than substances that are essentially flow dependent such as bromo-benzodiazepine (830µl/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.

[^{3}H]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 200mg BID) of 219–1837ng/ml (0.8-6.9pM) (Van Praag et al., 2002). When this is compared to plasma C_{max} measured in the same study (2516–9455ng/ml; 9.4-35.5pM) this gives a lower CSF/plasma ratio of 15%. As shown in Figure 2, [^{3}H]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 which may facilitate [^{3}H]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 figure 2, is suggestive of a CNS-to-blood transport system, which is saturated after 15 min of [^{3}H]nevirapine perfusion, when cerebrum/pituitary gland uptake is >20% (i.e.>13nM) and the choroid plexus uptake is equivalent to >3.9% (i.e.>2.5nM). However, our self-inhibition and cross-competition studies revealed that an excess of nevirapine or efavirenz in the plasma did not affect [^{3}H]nevirapine uptake into the brain, CSF, or choroid plexus (Figure 5). This suggests that nevirapine passage into or out of the CNS is not assisted by saturable transporters. However, this data was from 20 min brain perfusion experiments, so after the CNS-to-blood transporter indicated in the cerebrum, pituitary gland and choroid plexus graphs in figure 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 30μM nevirapine induces P-glycoprotein expression in the intestinal cell line LS 180 (Stormer et al., 2002), this was not observed with 10μM 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 multi-drug resistance protein (MRP-1) (Almond et al., 2005). In agreement with our cross-competition studies with efavirenz, a study of HIV-1 infected patients found nevirapine pharmacokinetics were unaffected by efavirenz co-administration (Veldkamp et al., 2001a).

[^{3}H]Nevirapine accumulated in the choroid plexus to levels above that of the vascular/extracellular space marker (Figure 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 (Groothuis 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 (Ghersi-Egea et al., 1996;Rennels et al., 1985). 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. Secondly, 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 supra-epithelial area of post-mortem 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 [^{3}H]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 necessarily indicative of drug levels in the brain.

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Footnotes

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b) Reprint requests

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Legends for Figures

Figure 1: The structure of nevirapine, a dipyridodiaqepinone.

Figure 2: Uptake of [^{3}H]nevirapine and [^{14}C]mannitol into 5 regions of the guinea-pig CNS over 30 min, values are mean ± S.E.M, n=3-5.

Figure 3: HPLC linked to radiodetection analysis of a [^{3}H]nevirapine standard (represented by arterial inflow), compared with venous outflow and pooled brain samples (n=2) taken after a 30 min carotid artery perfusion.

Figure 4: Capillary depletion analysis. Uptake of [^{3}H]nevirapine (corrected for [^{14}C]mannitol) into the whole brain, brain parenchyma and cerebral capillaries following 30 min carotid perfusion. Values are mean ± S.E.M, n=4. Uptake levels in the 3 compartments were compared using ANOVA followed by Bonferroni's t-test *P>0.05.

Figure 5: CNS uptake (%) of [^{3}H]nevirapine (corrected for [^{14}C]mannitol) following 20 min carotid artery perfusion in the absence and presence of 100μM unlabelled nevirapine or 100μM unlabelled efavirenz. Values are mean ± S. E. M, n=3-4. There were no differences in uptake of the radiolabelled test drug under any of the conditions (One way ANOVA or Kruskal Wallis one way ANOVA as applicable).

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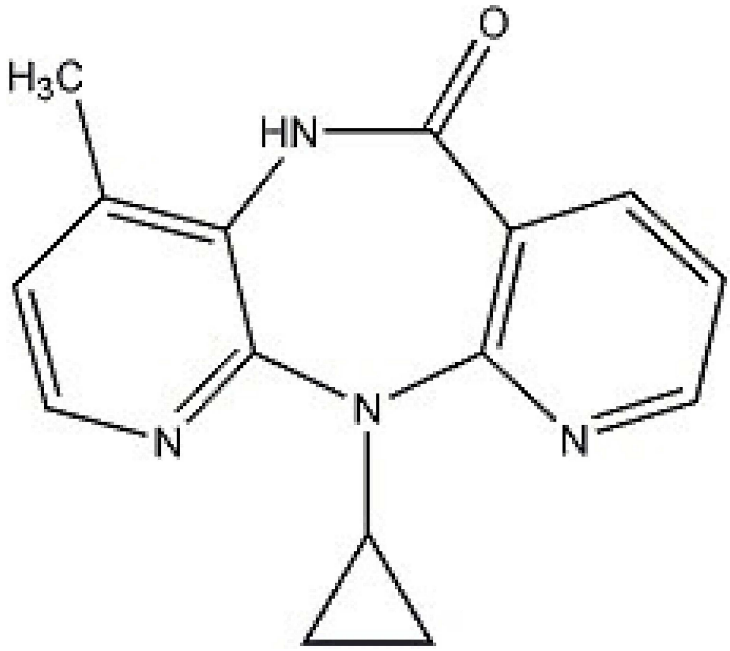

Figure 1

Figure 2

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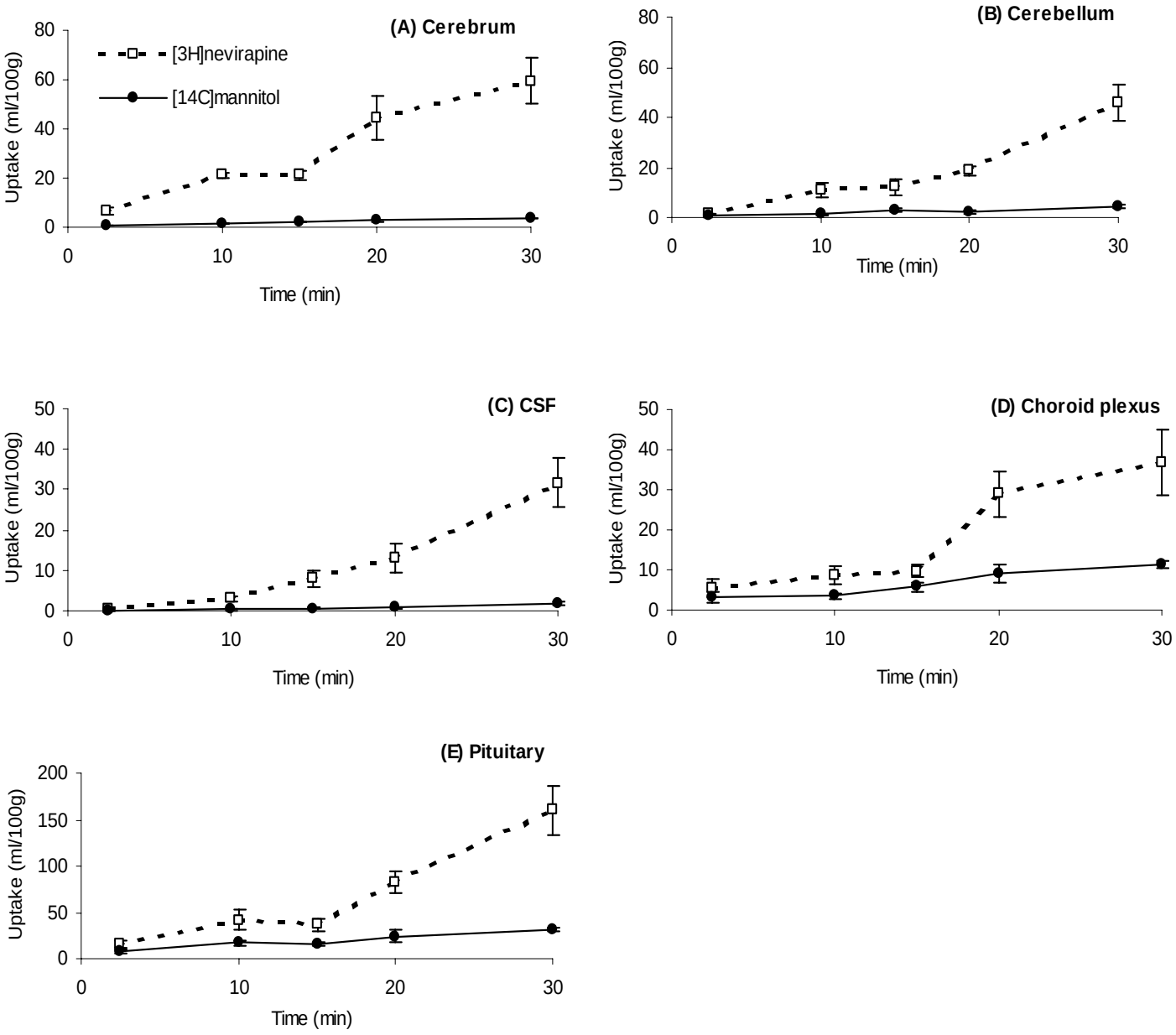

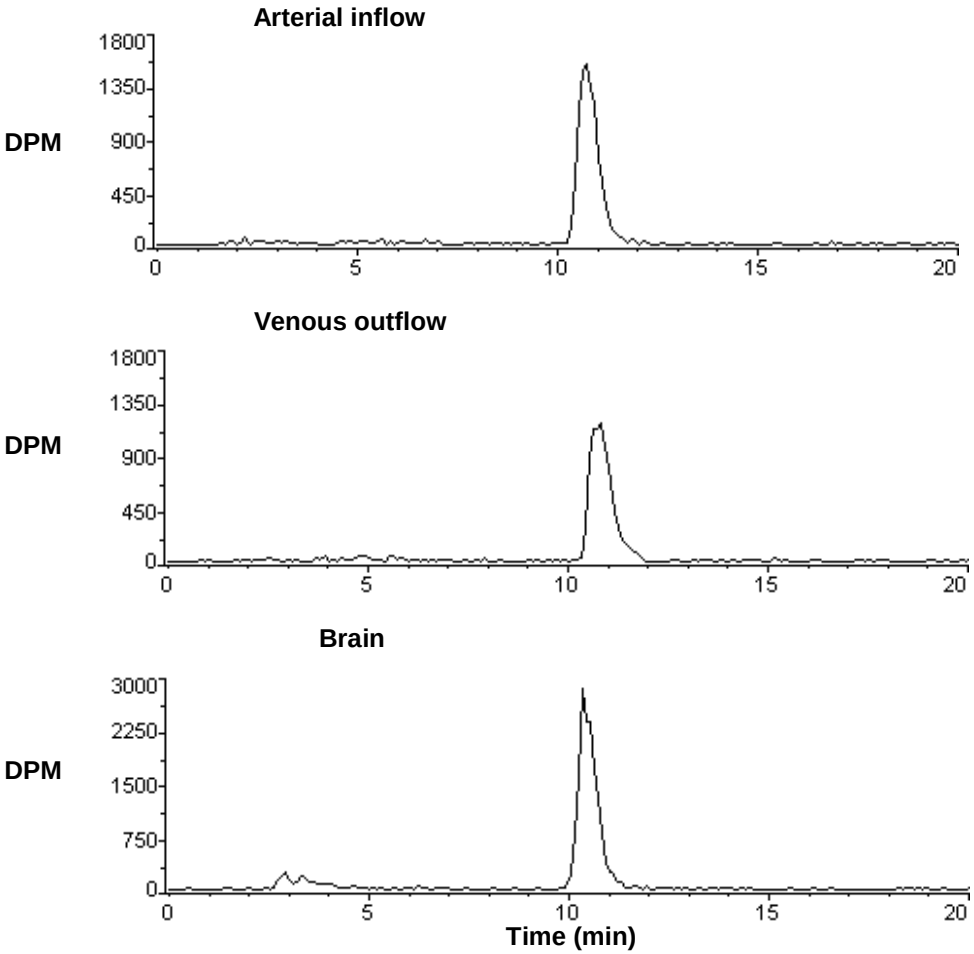

Figure 3

Figure 4

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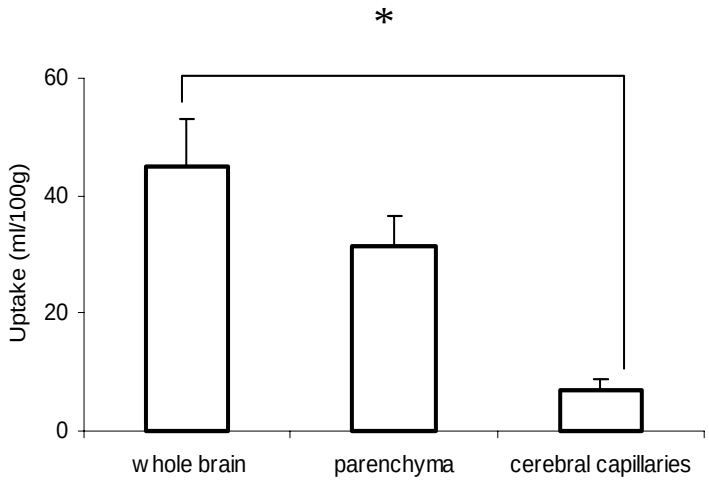

Figure 5

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