CNS effects of a CCR5 inhibitor in HIV-infected subjects: a pharmacokinetic and cerebral metabolite study

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Background: We conducted a pharmacokinetic and in vivo cerebral 1H magnetic resonance spectroscopy (1H-MRS) study to assess CSF exposure and cerebral metabolite ratios (CMRs) following maraviroc intensification.

Methods: HIV-infected neurologically asymptomatic adults receiving tenofovir, emtricitabine and lopinavir/ritonavir with plasma HIV RNA <50 copies/mL were eligible and received intensified therapy with 150 mg of maraviroc twice daily. 1H-MRS was performed in several cerebral locations, including the right basal ganglia (RBG), to assess CMRs, including N-acetyl aspartate/creatine (NAA/Cr), at baseline and after 14 days. Subsequently, on day 15, blood samples were obtained to determine plasma concentrations of maraviroc pre-dose (C_trough) and then paired blood and CSF samples were collected at 4 or 6 h post-dose. Associations between maraviroc exposure, clinical parameters and changes to CMRs were evaluated. Trial registry: ClinicalTrials.gov (http://clinicaltrials.gov/ct2/show/NCT00982878).

Results: Twelve subjects (75% male) participated with a mean (SD) CD4+ cell count of 503 (199) cells/μL. Mean (SD) maraviroc plasma concentrations at pre-dose, 4 h post-dose and 6 h post-dose were 337 (74), 842 (174) and 485 (100) ng/mL and CSF concentrations at 4 h post-dose and 6 h post-dose were 7.5 (1.3) and 5.1 (1.2) ng/mL. The mean maraviroc CSF:plasma ratio (range) was 1.01% (0.57%–1.61%). An increase of 14.8% was observed for the RBG NAA/Cr ratio, which was significantly associated with higher maraviroc plasma C_trough (P=0.05, r=0.61), but not CSF concentration (P=0.16, r=0.46).

Conclusions: After 14 days of maraviroc intensification, small increases in cerebral metabolite markers of neuronal integrity (NAA/Cr ratios) were observed and are associated with maraviroc plasma C_trough.

Keywords: maraviroc, CSF, magnetic resonance spectroscopy

Introduction

The CNS exposure of antiretroviral agents may aid protection against the development of HIV-associated cerebral impairment.1,2 CSF exposure of antiretroviral agents provides an estimation of penetration into the CNS and varies widely between different drugs, due to factors including drug size, lipophilicity and protein binding.3–7 Maraviroc is a recently licensed antiretroviral with a novel mechanism of action.8 It selectively blocks the CCR5 chemokine receptor, thus preventing HIV cell entry. In clinical trials to date, virological efficacy has been described when maraviroc is administered as part of combination antiretroviral therapy (cART) in both therapy-naive HIV-infected subjects9 and in subjects harbouring HIV strains with mutations associated with drug resistance.10

Several factors suggest maraviroc may have CNS antiviral activity. First, due to pharmacological properties, such as a relatively low degree of plasma protein binding (~76%),8 maraviroc...
may theoretically cross the blood–brain barrier (BBB) and gain exposure in the CSF at concentrations great enough to suppress HIV viral replication. Second, as a predominance of CCR5-tropic HIV has been described within the CNS, CCR5 inhibitors such as maraviroc may have profound antiviral activity within this compartment.

Estimations of maraviroc CNS exposure, via CSF exposure in HIV-infected subjects, have been reported. Yilmaz et al. sampled CSF and plasma from seven neurologically asymptomatic HIV-infected subjects and reported a median maraviroc CSF:plasma ratio of 3% (range 1%–10%). Similarly, Tiraboschi et al. studied 12 subjects without neurological symptoms, but with advanced HIV disease (median CD4+ cell count 281/µL) and observed variable CSF:plasma ratios (0.4%–17%). Finally, in antiretroviral-naïve subjects with neurocognitive impairment, greater CSF:plasma ratios (up to 29%) have recently been reported. Crucially, in all these reported series, background cART regimens and clinical indications for undertaking lumbar puncture (LP) examinations varied, which may confound findings. Also, the direct cerebral effects of maraviroc therapy were not evaluated.

The aim of this study was to assess CSF exposure and corresponding cerebral effects via changes in cerebral metabolite ratios (CMRs) following maraviroc intensification in a population of neurologically asymptomatic HIV-infected subjects receiving a standardized cART regimen.

### Methods

#### Study design and subject selection

This Phase I pharmacokinetic and in vivo cerebral 1H magnetic resonance spectroscopy (1H-MRS) study was conducted at St Mary’s Hospital, London, UK, between August 2009 and August 2010. The study was registered on the European Clinical Trials Database (EudraCT number 2008-008437-10), an international clinical trials database (http://clinicaltrials.gov/ct2/show/NCT00982878), and prior local ethical approval was obtained (Imperial College Healthcare NHS Trust local ethics approval). All subjects provided written informed consent.

Eligible subjects were neurologically asymptomatic adults with chronic HIV infection receiving tenofovir (245 mg once daily), emtricitabine (200 mg once daily) and lopinavir/ritonavir (400/100 mg twice daily). All had a plasma HIV RNA level of <50 copies/mL (Bayer Quanti-plex Assay®) for at least 3 months prior to study entry. Exclusion criteria included any neurological disease or dementia, viral hepatitis C co-infection or prohibited concomitant medication that might have pharmacokinetic interactions with the study medication, such as agents with hepatic enzyme-inducing or -inhibiting potential with specific medications defined in the study protocol. As all eligible subjects had a plasma HIV RNA level of 50 copies/mL at study entry, HIV strain co-receptor usage was not determined.

#### Clinical procedures

At baseline, 150 mg of maraviroc twice daily was introduced. Drug intake was witnessed after a standard breakfast containing 20 g of fat (600 kcal) on days 1, 7, 14 and 15. At each visit subjects were questioned about adverse events, tolerability and concomitant medications. Adherence was assessed using a validated questionnaire and urine was screened for recreational drug use (Williams Medical Supplies Ltd, Gwent, UK). Routine biochemical and haematological tests, plasma CD4+ cell count and plasma HIV RNA were performed on day 14.

#### 1H-MRS

Cerebral MRS imaging (T1- and T2-weighted images) was performed on a Philips Achieva™ 1.5 Tesla magnetic resonance (MR) scanner (Phillips NV, Best, Netherlands) and studied by an experienced neuroradiologist to ensure no imaging contraindications to LP examination were present during the study screening period. 1H-MRS was also performed during this screening scan and subsequently on day 14.

1H-MRS data were acquired by single voxel examination in the right frontal white matter (FWM), mid-frontal grey matter (FGM) and right basal ganglia (RBG) using techniques previously described. Briefly, MR spectra were obtained using a double spin echo point resolved spectroscopy (PRESS) sequence with the following settings: echo time (TE) 36 ms, repetition time (TR) 3000 ms, 2048 data points, spectral width 2500 Hz and 128 data acquisitions. The cerebral metabolites N-acetyl aspartate (NAA), myo-inositol (mI) and choline (Cho) were measured and expressed as ratios to cerebral creatine (Cr). MR spectra were analysed by a single observer (L.G.) using a Java-based version of the Magnetic Resonance User Interface package (jMRUI, version 3.0), incorporating the AMARES (Advanced Method for Accurate, Robust and Efficient Spectra) algorithm.

#### Pharmacokinetic and CSF HIV RNA analysis

On day 15, blood was sampled pre-dose (C_{trough}) and paired blood and CSF were both sampled 4 or 6 h post-dose (C_{4} or C_{6}) sequentially in enrolled subjects. LP examination was performed under aseptic techniques and samples were analysed for maraviroc and lopinavir concentrations at the University of Liverpool, UK, using HPLC tandem mass spectrometry (HPLC-MS/MS) in a good clinical laboratory practice (GCLP) accredited laboratory that participates in an external quality control programme. The lower limits of quantification were 1.23 and 0.74 ng/mL and 8.26 and 5.65 ng/mL for maraviroc and lopinavir in plasma and CSF, respectively. Intra- and inter-assay variability was <13% for each analyte at low, medium and high concentrations. CSF was additionally tested for white cell count, protein and ultrasensitive HIV RNA level using an in-house assay. Briefly, virus was pelleted by centrifugation and RNA extracted by the Qiagen MinElute method (Qiagen, Crawley, UK). The eluate was reverse transcribed and amplified for 20 cycles using the Invitrogen One-Step method (Invitrogen, Paisley, UK) and PCR products quantified in a real-time PCR using the Qiagen Probe PCR method. A standard curve was generated from dilutions of the international working reagent WR1 (NBSC, Potters Bar, UK). The lower limit of detection for this study was 10 copies/mL.

#### Statistical analysis

Mean (SD) maraviroc and lopinavir concentrations were determined, including pre-dose (C_{trough}) for plasma and 4 h (C_{4}) or 6 h (C_{6}) post-dose for both CSF and plasma. CSF:plasma concentration ratio at C_{4} or C_{6} was calculated and expressed as a percentage. As maraviroc is approximately 76% bound to plasma proteins, the CSF:unbound plasma concentration was also calculated based on this mathematical assumption (24% of total plasma concentration). Associations between plasma and CSF concentrations and the relationship to clinical parameters were determined using linear regression modelling or Spearman’s rank test.

Absolute changes to CMRs between baseline and day 14 were determined for each subject and evaluated using a paired samples t-test. Where P values <0.2 were observed, linear regression analysis was used to evaluate associations between changes to metabolite ratios over the study period and both clinical and pharmacokinetic parameters. P values <0.05 were considered statistically significant. SPSS (v18.0) software (SPSS, Chicago, IL, USA) was used for all statistical analysis.
Results

Subject characteristics

Thirteen subjects were enrolled and 12 completed all study procedures. One subject was withdrawn on day 14 due to a positive urine screening test for drugs of misuse. The mean (SD) age was 42 (8) years, nine subjects (75%) were male and seven (58%) were of black ethnicity. All had a plasma HIV RNA of <50 copies/mL throughout the study and the mean (SD) CD4+ cell count at entry was 503 (199) cells/μL (see Table 1).

Pharmacokinetic and LP results

Adherence was reported at 100% for all subjects. Ten (83%) subjects had a CSF HIV RNA level of <10 copies/mL. In the remaining two subjects CSF HIV RNA was detectable at 63 and 190 copies/mL, respectively. CSF protein ranged between 0.27 and 0.94 g/L (mean 0.46). CSF white cell count concentrations were 0–4 cells/μL in all subjects except one, in whom 10 cells/μL were detected.

Plasma and CSF concentrations are shown for each subject in Table 2. The mean (SD) maraviroc plasma $C_{\text{trough}}$ was 337 (74.5) ng/mL. Maraviroc plasma $C_e$ and $C_6$ were 842 (174) and 485 (100) ng/mL, respectively. Mean (SD) maraviroc CSF $C_e$ and $C_6$ were 7.54 (1.26) and 5.10 (1.21) ng/mL, respectively. The mean overall maraviroc CSF:plasma ratio was 1.01% (range 0.57%–1.61%) and, when studied by time of sampling, the mean maraviroc CSF:plasma ratio was 0.93% (range 0.57%–1.27%) for $C_e$ and 1.09% (range 0.71%–1.61%) for $C_6$. The mean overall maraviroc CSF:unbound plasma ratio was 4.20% (range 2.37%–6.70%).

The mean (SD) lopinavir plasma $C_{\text{trough}}$ was 6088 (1215) ng/mL, and $C_e$ and $C_6$ were 9048 (870) and 9253 (1441) ng/mL, respectively. The mean (SD) lopinavir CSF $C_e$ and $C_6$ were 75.1 (45.0) and 76.8 (30.8) ng/mL, respectively. The mean overall lopinavir CSF:plasma ratio was 0.85% (range 0.32%–1.83%).

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Table 1. Patient demographics and clinical parameters at study entry

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<th>Demographic/clinical parameter</th>
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<tr>
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<td>Male gender, n (%)</td>
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<td>Years since HIV diagnosis</td>
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<td>Baseline CD4 cell count (cells/μL)</td>
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<td>Baseline plasma HIV RNA level</td>
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<td>Current smoker, n (%)</td>
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Table 2. Pharmacokinetic and virological results of plasma and CSF samples by individual subject and overall

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<th>Subject</th>
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<th>Plasma HIV RNA (copies/mL)</th>
<th>CSF HIV RNA (copies/mL)</th>
<th>MVC plasma $C_{\text{trough}}$ (ng/mL)</th>
<th>MVC plasma $C_e$ or $C_6$ (ng/mL)</th>
<th>MVC CSF:plasma ratio (%)</th>
<th>MVC CSF:unbound plasma ratio (%)</th>
<th>LPV CSF:plasma ratio (%)</th>
<th>LPV CSF:plasma ratio (%)</th>
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MVC, maraviroc; LPV, lopinavir; CV, coefficient of variation.
Cerebral imaging and $^1$H-MRS metabolite ratios

No significant abnormalities of T1- and T2-weighted MR images were reported. Results of baseline and day 14 $^1$H-MRS metabolite ratios are shown in Table 3. No significant changes to CMRs in either the FGM or FWM were observed after maraviroc intensification during the study period ($P > 0.41$ all values). Although not statistically significant, changes were observed in the RBG metabolites, with absolute (%) increases in NAA/Cr, Cho/Cr and mI/Cr ratios of $+0.27$ (14.8%, $P = 0.18$), $+0.14$ (17.9%, $P = 0.07$) and $+0.24$ (34.8%, $P = 0.17$), respectively.

Associations between pharmacokinetic results, metabolite ratios and clinical parameters

No statistically significant associations between plasma or CSF maraviroc exposure and patient demographics or clinical parameters (including CSF protein) were observed ($P > 0.1$ all values, data not shown). A statistically significant association between maraviroc CSF concentration and plasma $C_{\text{trough}}$ ($P = 0.009$, $r = 0.71$) and plasma $C_{4-6}$ ($P = 0.007$, $r = 0.73$) was observed (see Figure 1).

The increase in the RBG NAA/Cr ratio was significantly associated with a higher maraviroc plasma $C_{\text{trough}}$ ($P = 0.047$, $r = 0.61$), but not CSF concentration ($P = 0.16$, $r = 0.46$). No relationship between changes to other metabolites and either plasma or CSF exposure were found ($P > 0.06$ all values).

Discussion

In this study, which enrolled HIV-infected subjects on a standardized and stable antiretroviral regimen, we observed a mean maraviroc CSF:plasma ratio of 1.01% and observed changes in neuronal (NAA/Cr) CMRs associated with maraviroc plasma exposure.
This is the first study to describe a cerebral effect, observed by measuring MR-visible CMRs, and a relationship of this effect to maraviroc exposure. We have made several interesting observations. First, unlike other cohorts, we observed maraviroc CSF concentrations greater than 5-fold the median protein-free IC_{50} (0.57 ng/mL) in all subjects. Interestingly, however, CSF:plasma ratios (range 0.57%–1.61%) were lower and less variable than previously described, where CSF:plasma ratios have ranged between 1%–10% and 0.4%–17%. High CSF maraviroc concentrations have previously been described in individuals with neurological impairment and associated CSF pleocytosis. We postulate this may be related to BBB disruption from cerebral inflammation, which may enhance drug delivery to the CSF. It is therefore likely the lower maraviroc CSF:plasma ratios that we observed are due to our strict inclusion criteria, with an absence of neurological symptoms and disease in our study population, supporting the BBB integrity hypothesis. Furthermore, our strict eligibility criteria, including standardized cART at study entry and undetectable plasma HIV RNA, may also have contributed to the lower observed CSF:plasma ratios than reported in other cohorts, for the above reasons.

A strong correlation was observed between maraviroc CSF and plasma concentrations, which has not previously been described. Such associations may only be recognized within a study designed like ours, where a lack of variability in clinical parameters, such as body mass index (BMI), cART regimen or concomitant medications, and standardized sampling times following fed-state dosing, allows such observations to become apparent.

Of interest, although not statistically significant, we observed changes in the CMRs in the RBG after 14 days of maraviroc intensification, whereas changes were not observed in the FWM or FGM of the cerebral cortex. For several reasons, we postulate these changes are related to the introduction of maraviroc. First, a statistically significant association between increases in RBG NAA/Cr ratio and maraviroc plasma C_{trough} was observed, suggesting a direct relationship. Second, we observed metabolite changes in the RBG, but in no other cerebral location. The basal ganglia has a higher blood flow per unit volume compared with other cerebral locations, suggesting greater and earlier exposure of this part of the brain, which may explain why changes were observed here in our short study but had not yet evolved in other cerebral locations, and, if associated with plasma, blood flow may also explain why CMR changes were associated with plasma maraviroc exposure and not CSF exposure. Lastly, very small absolute changes in metabolite ratios were observed in the frontal anatomical locations, which provide assuring data that the changes observed are not due to high intra-patient variability when undergoing in vivo cerebral \(^1\)H-MRS on two occasions. Indeed, the intra-patient variability between scanning in the frontal anatomical voxels in our study is within the lower variability range from previous published series assessing such variability of sequential \(^1\)H-MRS. We were not able to determine HIV co-receptor usage in this study in order to assess any associations between these CMR changes and viral co-receptor tropism.

Two subjects had detectable CSF HIV RNA in our study, despite plasma HIV RNA levels of <50 copies/mL at study entry. In one subject (subject 6), low-level CSF HIV viraemia of 63 copies/mL was detected, and in one subject (subject 9), significant CSF viraemia of 190 copies/mL was detected. The CSF concentration and CSF:plasma ratio in subject 9 were below the study mean and were not elevated as may be expected in neurologically symptomatic subjects. Furthermore, the absolute change in the RBG NAA/Cr ratio during the study period in subject 9 was low (0.02) and below the cohort mean (0.27), making it unlikely that this subject could have influenced or in any way driven the findings of our study.

NAA is a marker of neuronal integrity, and reductions in NAA/Cr are reported in advanced HIV disease stages, including AIDS dementia complex and severe neurocognitive impairment. Increases in NAA/Cr following initiation of cART have previously been described in antiretroviral-naive individuals, but over much longer treatment programmes. Increases in mI/Cho and Cho/Cr ratios also occurred in the RBG. Such metabolites are osmolyte markers of glial cell metabolism and alter in the presence of neuroinflammation. Increases have previously been observed in AIDS dementia complex, but changes occurring within an asymptomatic cohort and over such a short period of study are unlikely to represent disease progression. Osmosensitive glial markers, such as mI, play a crucial role in cell volume regulation. Organic osmolytes such as mI are also rapidly released into the extracellular space in response to cell swelling via osmoregulated membrane channels. It is possible that the increase in the mI/Cho ratio that we observed may represent an initial immune response to the short course of maraviroc.

Sampling CSF for lopinavir concentrations has in the past revealed inconsistent findings, with undetectable CSF lopinavir concentrations being described in some adherent subjects, even in the cART era. In our study, lopinavir was detected in CSF samples from all subjects and observed CSF:plasma ratios were higher than previously described. This consistent detection of CSF lopinavir may again reflect the strict inclusion criteria of our study, or may be secondary to a pharmacokinetic interaction when lopinavir and maraviroc are co-administered. Although maraviroc has no effect on the plasma pharmacokinetic profile of lopinavir, pharmacokinetic effects might occur within the CSF compartment, and as no previous study has assessed lopinavir CSF exposure in subjects also receiving maraviroc, such an interaction is plausible.

In summary, we observed small changes in CMRs after only 14 days of maraviroc intensification in neurologically asymptomatic HIV-infected subjects on stable CART. Although these changes in CMRs were not statistically significant, they were associated with plasma maraviroc exposure. These findings may have implications for clinical practice. For instance, it would be interesting to see if such changes in cerebral metabolites could be associated with changes in neurocognitive function or other functional assessments. Future work to assess these effects over longer treatment periods, in both neurologically symptomatic and asymptomatic HIV-infected subjects, is justified and studies to assess the clinical implications of these findings are needed.

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**Transparency declarations**

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**Author contributions**

A. W. conceptualized the study idea and obtained study funding. L. G., D. B., S. D. T.-R. and A. W. drafted the manuscript. L. G., J. M. A., A. M. and S. D. T.-R. analysed and assisted in the interpretation of MRS findings. V. W. and D. B. analysed and assisted in the interpretation of the pharmacokinetic results. All authors critically appraised the final version of the manuscript.

**References**


