Efavirenz and metabolites in CSF; relationship with CYP2B6 c.516G>T genotype and perturbed blood-brain barrier due to tuberculous meningitis

Running title: Pharmacokinetics of EFZ metabolites in CSF

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**Objective:** Efavirenz (EFZ) has been associated with neuropsychiatric side effects. Recently the 8-hydroxy (8OH)-EFZ metabolite has been shown to be a potent neurotoxin *in vitro*, inducing neuronal damage at concentrations of 3.3ng/ml. EFZ induced similar neuronal damage at concentrations of 31.6ng/ml. We investigated the effect of genotype and blood-brain barrier integrity on EFZ metabolite concentrations in cerebrospinal fluid (CSF).

**Methods:** We measured CSF drug concentrations from two separate studies: 47 subjects with tuberculous meningitis (TBM) co-infection in Vietnam receiving EFZ 800mg with standard anti-tuberculous treatment and 25 subjects from the PARTITION study in the UK without central nervous system infection receiving EFZ 600mg. EFZ and metabolite concentrations were measured in CSF and plasma and compared with estimates of effectiveness and neurotoxicity from available published *in vitro* and *in vivo* data. The effect of *CYP2B6* c.516G>T genotype (GG=fast; GT=intermediate; TT=slow EFV metaboliser status) was examined.

**Results:** Mean CSF concentrations of EFZ and 8OH-EFZ in the TBM group were 60.3 and 39.3ng/ml respectively, and in the no-TBM group were 15.0 and 5.9ng/ml. Plasma EFZ and 8OH-EFZ concentrations were similar between groups. CSF EFZ concentrations were above the *in vitro* toxic concentration in 76% of samples (GG 61%, GT 90% and TT 100%) in the TBM group, and 13% (GG 0%, GT 18% and TT 50%) in the no-TBM group. CSF 8OH-EFZ concentrations were above the *in vitro* toxic concentration in 98% of the TBM group and 87% of the no-TBM group; levels were independent of genotype but correlated with CSF:plasma albumin ratio.

**Conclusion:** Potentially neurotoxic concentrations of 8OH-EFZ are frequently
observed in CSF, independent of CYP2B6 genotype, particularly in those with impaired blood-brain barrier integrity.
Despite concerns over central nervous system (CNS) toxicity, efavirenz (EFZ) is widely deployed within first-line combination HIV treatment regimens worldwide because of its effectiveness, established safety record and resilience to hepatic enzyme induction by rifampicin in patients who require concomitant tuberculosis (TB) therapy. EFZ undergoes rapid absorption, with maximum plasma concentrations reached in 3–6 hours and therapeutic levels achieved within a few days of commencing treatment. There is large interindividual variability in EFZ pharmacokinetics, placing patients with low plasma concentrations at risk of losing virological control and developing resistance, and those with high plasma concentrations at risk of developing adverse effects. EFZ is primarily metabolised by cytochrome P450 CYP2B6, to yield the most abundant metabolite 8-hydroxy (8OH)-EFZ. Comparatively minor alternative metabolic pathways are through CYP2A6 (leading to the 7OH-EFZ metabolite) and CYP3A. EFZ plasma concentrations relate strongly to genetic polymorphism in CYP2B6 metabolism, including the most commonly studied CYP2B6 single nucleotide polymorphism c.516G>T (rs3745274), which encodes a Gln172His amino acid substitution. The CYP2B6 c.516G>T GG genotype is associated with fast EFV metaboliser status, GT intermediate and TT slow. Preliminary data suggests that in CYP2B6 slow metabolisers, CYP2A6 represents the dominant route of elimination and may be affected by enzyme inhibition through concomitant isoniazid administration. This may have pharmacogenetic implications as CYP2A6 has considerable copy number variation in Southeast
Asian populations. The effect of CYP2A6 copy number on CSF EFZ and metabolite concentrations in those with and without slow CYP2B6 metaboliser status is not known.

*In-vitro* experiments have reported that 8OH-EFZ is associated with cytotoxicity via stimulation of mitochondrial dysfunction and stress activated signaling pathways. In addition 8OH-EFZ has been shown to be prone to oxidative degradation with potentially toxic quinone-imine derivatives. Recently 8OH-EFZ was shown to be neurotoxic *in vitro* at a concentration similar to those found in cerebrospinal fluid (CSF). This study demonstrated 8OH-EFZ concentrations of just 3.3 ng/ml caused neuronal damage, inducing calcium flux, apoptosis and considerable damage to dendritic spines. These changes were not observed for EFZ or 7OH-EFZ at this level. Concentrations of EFZ and 7OH-EFZ approximately ten times that of 8OH-EFZ were required to induce similar damage. The role of 8OH-EFZ in EFZ-associated CNS toxicity has not been elucidated.

In this study we developed sensitive, accurate and precise assays for measuring EFZ and its metabolites in CSF. We aimed to characterise the disposition of EFZ and its metabolites within CSF in HIV-infected patients with and without TB meningitis (TBM), and to evaluate the impact of pharmacogenetic variability on drug disposition.

**Methods**

**Participants and sampling**
The CSF pharmacokinetics of EFV was studied in two separate patient populations. Since these cohorts differ in several characteristics, no statistical comparisons between both groups was undertaken.

**TBM group:** In Vietnam, HIV-infected patients aged over 15 years with newly diagnosed TBM (ISRCTN63659091) were randomised to receive immediate (within 7 days) versus deferred (after 2 months) initiation of antiretroviral therapy as previously described. From this cohort 47 subjects had paired CSF and blood samples available while on EFZ at steady state (>10 days). Sampling was mean 97 days after commencing treatment. EFZ was dosed at 800 mg, together with zidovudine plus lamivudine in fixed-dose combination. Anti-tuberculous therapy comprised isoniazid (5mg/kg/day; maximum 300mg), rifampicin (10mg/kg/day; maximum 600mg), pyrazinamide (25mg/kg/day; maximum 2g), and ethambutol (20mg/kg/day; maximum 1.2g) for 3 months followed by isoniazid plus rifampicin for 6 months. Unless contraindicated, all patients received dexamethasone as described elsewhere. Mean age was 30 years (SD 5.4) and median CD4 at sampling was 81 cells/mm³ (IQR 46, 159). All were of Southeast Asian ethnicity. Ethics approval was obtained from the Oxford Tropical Research Ethics Committee and the Hospital for Tropical Diseases Scientific and Ethical Committee.

**No-TBM group:** In the UK, paired plasma and CSF was obtained from a single time point in 25 subjects without CNS infection from the UK PARTITION study (Penetration of AntiRetroviral Therapy INTO the Nervous system). Participants were HIV-1 infected adults (over 16 years) prospectively enrolled from 2 groups: those undergoing lumbar puncture for a clinical indication, or
those with a history of unexplained intermittently or persistently detectable plasma HIV-1 RNA within the past 12 months. In all patients the treating clinician felt that CNS infection had been excluded on the basis of CSF testing and clinical findings. All patients received 600mg of EFZ once-daily; in 25 subjects this was with tenofovir and emtricitabine, in one subject with lamivudine and abacavir and in one subject with darunavir and ritonavir. Mean age was 46 years (SD 8.6) and median CD4 at sampling was 432 cells/mm$^3$ (IQR 292, 649). 20 (80%) were of white ethnicity, 3 (12%) were of black ethnicity and 2 (8%) were of Asian ethnicity. No subject was receiving antituberculous therapy or other enzyme inducing medication at the time of sampling. The study was approved by the North Wales Research Ethics Committee (Central and East).

**EFZ and metabolite measurement**

EFZ concentrations were determined in plasma and CSF samples taken from subjects receiving EFZ at steady-state (>10 days), sampled at mid-dosing interval. EFZ metabolite concentrations were determined in a single paired CSF/plasma sample per subject. Measurements were repeated with and without β-glucuronidase in the TBM group to determine the amount of glucuronidated versus free compound. The ratio between albumin concentration in CSF and plasma/serum was determined as a marker of blood-brain barrier integrity.

EFZ concentrations in plasma and CSF were measured by a validated tandem liquid chromatography-mass spectrometry method as previously described. Freshly prepared standards and quality control samples (prepared in artificial CSF) and clinical samples (100 µL) were transferred into 7mL stoppered glass tube to which 100 µL of acetonitrile was added. The samples were the
evaporated to dryness at room temperature in a stream of nitrogen. The samples were then incubated at 37°C for 2h with 400 µL of a solution containing 200 units of β-glucuronidase from *H. pomatia* in 0.2 M sodium acetate buffer (pH = 5).(27) The samples were subsequently alkalized with 20 µL of potassium carbonate buffer (0.1 M, pH = 9·4) and extracted with 3 mL of a mixture of organic solvents ethylacetate:hexane (60:40 v/v). After centrifugation, the organic phase was evaporated to dryness, the residue reconstituted in 100 µL of a mobile phase (50/50 v/v ACN/H₂O in 1mM ammonium Acetate) and 20 µL of this solution was analysed directly by LC-MS/MS on a Thermo Access Triple Quadrupole mass spectrometer. Hexobarbital was used as internal standard. Gradient elution was on a reverse-phase C₁₈ column using 1 mM ammonium acetate in water and acetonitrile. Quantification was by selective reaction monitoring in negative ionisation mode. Accuracy and precision were satisfactory with mean bias 4·8% and intra-assay coefficient of variability 6.5%.

*Albumin ratio*

Albumin concentrations in CSF and blood (plasma/serum) were determined by radial immunodiffusion (Bindarid™). CSF: blood albumin ratio indicative of a breach in integrity of the blood:brain barrier was taken as ≥6.8 for subjects less than 45 years old and ≥10.2 for subjects over 45 years.(28)

*Neurotoxic concentrations*

Measured plasma and CSF concentrations were compared to the following concentrations associated with neurotoxicity. Plasma EFZ concentrations greater than 4000 ng/mL are associated with an increased risk of CNS side effects.(8) Plasma EFZ concentrations less than 1000 ng/ml have historically been
associated with virological failure. Concentrations of EFZ, 8OH-EFZ and 7OH-EFZ associated with neuronal damage in vitro were 31.6, 3.3 and 33.2 ng/ml respectively.

**Genetic analysis**

Genomic DNA was purified from whole blood using standard phenol-chloroform extraction methods. Allelic discrimination by TaqMan real-time PCR was performed for CYP2B6 c.516G>T and CYP2A6 copy number using validated commercially available assays (Life Technologies, Paisley, UK).

**Statistical analysis**

The geometric mean of log\(_{10}\) drug/metabolite concentrations were compared using Student's t test and 1 way ANOVA. Pearson r was used to determine the correlation between continuous variables. CD4 count and CSF:plasma ratio of EFZ were non-parametrically distributed and analysed using Mann Whitney U test. Fishers exact and Chi squared tests were used for categorical demographic data. All analysis was performed using SPSS version 22.

**Results**

Plasma EFZ concentrations correlated with CSF EFZ concentrations in both groups, however there was no correlation of plasma EFZ with CSF 8OH-EFZ concentrations (figure 1). The median ratio of CSF:plasma EFZ concentration was 0.027 [IQR 0.013, 0.056] in the TBM group and 0.010 [IQR 0.007, 0.012] in the no-TBM group.

**CYP2B6 genotype**
Forty-six samples in the TBM group and 22 samples in the no-TBM group were successfully genotyped for \( CYP2B6 \) c.516G>T (call rates 98% and 88% respectively). Allele frequencies were 50% GG, 43% GT and 7% TT in the TBM group and 43% GG, 48% GT and 9% TT in the no-TBM group (table 1). Only 5 patients had the TT (i.e. slow metaboliser) genotype. \( CYP2B6 \) c.516G>T was in Hardy-Weinburg equilibrium in both groups (p=0.912 TBM and 0.67 no-TBM group). \( CYP2B6 \) c.516G>T genotype related to the concentration of EFZ in CSF and plasma in both groups. This relationship was not present for the concentrations of the 8OH-EFZ metabolite (table 1). Concentrations of 7OH-EFZ in plasma and CSF were also not related to genotype. There was no difference in CSF:plasma EFZ ratio according to genotype. The effect of \( CYP2B6 \) genotype on EFZ and 8OH-EFZ concentrations with respect to the estimated therapeutic range in plasma, and the \textit{in vitro} toxic concentrations in CSF, are shown in figure 2. The number and proportion of CSF samples with concentrations above estimated \textit{in vitro} toxic concentrations are given in table 2.

Plasma EFZ concentrations were similar between the TBM and no-TBM groups and mostly fell within the estimated therapeutic range, regardless of genotype. CSF EFZ concentrations exceeding the estimated \textit{in vitro} neurotoxic level were observed mainly in the TBM group, particularly in those with one or more \( CYP2B6 \) c.516G>T mutation (i.e. GT or TT genotype corresponding to intermediate or slow EFZ metabolisers). CSF 8OH-EFZ concentrations tended to be above the estimated \textit{in vitro} neurotoxic level in both groups regardless of genotype.

\textit{CYP2A6 copy number variation}
Forty-six samples in the TBM group were successfully genotyped for \(\text{CYP2A6}\) copy number (call rate 98%). The \(\text{CYP2A6}\) gene deletion occurred in 8 (17%) subjects and was in Hardy-Weinburg equilibrium (\(p=0.394\)). There was no association of \(\text{CYP2A6}\) copy number with the concentration of EFZ or metabolites in plasma or CSF either singly or in combination with \(\text{CYP2B6}\) genotype. A single subject had the \(\text{CYP2A6}\) gene deletion in combination with homozygous \(\text{CYP2B6}\) c.516G>T mutation; in this subject EFZ concentration was 6319.5 ng/ml in plasma and 54.7 ng/ml in CSF.

**Addition of \(\beta\)-glucuronidase**

In the TBM group the addition on \(\beta\)-glucuronidase did not significantly alter the concentrations of EFZ (not tested in the no-TBM group as levels were much lower). In contrast, concentrations of 8OH-EFZ we much higher following \(\beta\)-glucuronidase. The mean free:total ratio of 8OH-EFZ was 0.064 in plasma and 0.075 in CSF. Without \(\beta\)-glucuronidase, free 8OH-EFZ concentrations were low; mean 87.3 ng/mL (95% CI 63.8-122.5) in plasma and 3.7 ng/mL (95% CI 2.7-5.7) in CSF.

Mean 7OH-EFZ concentrations in the TBM group with \(\beta\)-glucuronidase were 75.3 ng/ml in plasma and 3.5 ng/ml in CSF; without \(\beta\)-glucuronidase, 7OH-EFZ levels were below the lower limit of quantification. In the no-TBM group mean 7OH-EFZ concentrations were 236.6 ng/ml in plasma and 1.3 ng/ml in CSF.

**Albumin ratio**

CSF:serum/plasma albumin ratio was abnormal in 35 (90%) subjects in the TBM group and 4 (21%) in the no-TBM group. In the TBM group CSF:plasma albumin
ratio was positively correlated with CSF 8OH-EFZ concentration (figure 3c). A non-significant trend was observed with CSF EFZ concentration (figure 3a). In the no-TBM group, no correlation was observed between CSF:serum albumin ratio and CSF EFZ or 8OH-EFZ concentrations (figure 3b and 3d).

Discussion

We studied the concentration of EFZ and its metabolites in plasma and CSF and observed high CSF EFZ and 8OH-EFZ concentrations in patients with TBM, which were not observed in those without TBM. These differences could not have been explained by the higher doses of EFZ used in the TBM group (800mg vs. 600mg) since plasma exposures were comparable across both studies. We observed a strong correlation between plasma and CSF EFZ concentrations and both were associated with \textit{CYP2B6} c.516G>T genotype. In contrast concentrations of the neurotoxic metabolite 8OH-EFZ were not related to plasma EFZ concentrations or \textit{CYP2B6} c.516G>T genotype, but correlated with the degree of blood-brain barrier breakdown measured by CSF:plasma albumin ratio. These data confirm the findings of a recent publication from the ENCORE CNS substudy which demonstrated an association of \textit{CYP2B6} c.516G>T genotype with plasma and CSF EFZ concentrations, but not with the metabolite 8OH-EFZ at doses of 400mg and 600mg.\textsuperscript{(29)} We demonstrate the same relationship at an EFZ dose of 800mg, albeit when prescribed with rifampicin which induces the activity of \textit{CYP2B6}.

The majority of EFZ metabolites in CSF were present as glucuronide conjugate. This is less likely to be due to CSF trapping of plasma glucuronide (percentage free compound was not significantly higher in CSF) and suggests EFZ metabolites
may be conjugated within the CNS. A number of UDP-glucuronosyltransferases have been demonstrated to be present in human brain tissue. (30, 31) EFZ metabolites may have entered the CNS by crossing the blood-brain barrier, or resulted from the CNS metabolism of EFZ. Functional CYP2B6 and CYP2A6 are present in the CNS and expression has been shown to be inducible and subject to genetic variation. (32-34) The significance of the fact that most 8OH-EFZ in CSF exists as glucuronide conjugate is unclear, in particular it is not known whether glucuronidated 8OH-EFZ induces the same neurotoxic effects as free compound or whether glucuronidation is in some way protective. We did not measure glucuronidation in the no-TBM group, however a recent study in patients without TBM found similar high levels of 8OH-EFZ glucuronidation in CSF. (35)

This is the first report of EFZ metabolites in CSF of patients with TBM. CSF concentrations of EFZ and metabolites were higher in those with loss of blood-brain barrier integrity due to TBM infection and concentrations were highest in TBM patients with the greatest loss of blood-brain barrier integrity as measured by CSF:plasma albumin ratio. As EFZ is >99.75% protein bound in blood, (36, 37) higher CSF EFZ concentrations may be due to leakage of free fraction from plasma in those with loss of integrity of the blood-brain barrier, or due to increased trapping of EFZ in those with higher albumin concentration in CSF.

CSF EFZ concentrations consistently exceeded in vitro neurotoxic concentrations in patients with a combination of TBM infection and CYP2B6 c.516G>T mutation (i.e. GT or TT genotype corresponding to intermediate or slow EFZ metabolisers). In contrast CSF total 8OH-EFZ concentrations exceeded the in vitro neurotoxic concentration in the majority of subjects with and without TBM.
regardless of genotype. This has implications for neuronal damage in TBM which could contribute to the overall neurological sequelae from this disease. Data from the recent ENCORE CNS substudy demonstrated an association of CSF 8OH-EFZ concentrations with symptoms at 1 year. The main limitation of our study is that we could not examine whether potentially neurotoxic CSF concentrations corresponded to clinical evidence of neurological dysfunction. There are several reasons why this was the case. In the TBM group adverse neurological outcomes were attributed to TBM rather than drug neurotoxicity. Higher albumin ratios may reflect more severe TBM infection and hence confound any association of CSF 8OH-EFZ with clinical outcomes. Albumin ratio would be expected to decrease over time, which may coincide with clinical improvements. In the no-TBM group detailed cognitive testing was not performed and most had clinical indication for lumbar puncture which may confound associations with clinical outcomes. Further work is needed to determine the short and long-term clinical consequences related to CSF 8OH-EFZ concentrations far exceeding in vitro neurotoxic levels as this has important clinical implications. One question is whether EFZ should be avoided in those with impaired blood-brain barrier integrity, in particular those with neurological infection such as TBM. However as discussed above, such studies will be limited by difficulties in separating EFZ neurotoxicity from the effects of neurological infection. Another question is whether CYP2B6 c.516G>T genotyping in clinical practice would lower the incidence of neurocognitive side effects. Our data suggest that avoiding EFZ in those with the GT or TT genotype would not alter CSF 8OH-EFZ concentrations and hence may not be an effective strategy.
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References

1. World Health Organisation. Antiretroviral therapy for HIV infection in adults and adolescents; recommendations for a public health approach, 2010 revision.


Metabolites When Dosed at 400 mg and 600 mg Once Daily: A Randomized Controlled Trial. Clin Infect Dis 60:1026-32


**Table and figure legends:**

**Table 1.** *CYP2B6* c.516G>T allele frequency and EFZ/8OH-EFZ concentrations in CSF and plasma.

<table>
<thead>
<tr>
<th>Group</th>
<th>All genotypes</th>
<th>Allele frequency, n (%)</th>
<th>Plasma concentration, geometric mean (95% confidence interval)</th>
<th>CSF concentration, geometric mean (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>GT</td>
<td>TT</td>
</tr>
<tr>
<td>TBM</td>
<td>46 (100)</td>
<td>23 (50.0)</td>
<td>20 (43.5)</td>
<td>3 (6.5)</td>
</tr>
<tr>
<td>No-TBM</td>
<td>23 (100)</td>
<td>10 (43.5)</td>
<td>11 (47.8)</td>
<td>2 (8.7)</td>
</tr>
</tbody>
</table>

All concentrations are with β-glucuronidase. EFZ; efavirenz.

**Table 2.** Proportion of CSF samples with EFZ and 8OH-EFZ concentrations above in vitro toxic concentrations (i.e 31.6 ng/ml for EFZ and 3.3 ng/ml for 8OH-EFZ).

<table>
<thead>
<tr>
<th>Group</th>
<th>All, n (%)</th>
<th>GG, n (%)</th>
<th>GT, n (%)</th>
<th>TT, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBM</td>
<td>35 (76%)</td>
<td>14 (61%)</td>
<td>18 (90%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>No-TBM</td>
<td>3 (13%)</td>
<td>0 (0%)</td>
<td>2 (18%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>TBM</td>
<td>45 (98%)</td>
<td>22 (96%)</td>
<td>20 (100%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>No-TBM</td>
<td>20 (87%)</td>
<td>10 (100%)</td>
<td>9 (82%)</td>
<td>1 (50%)</td>
</tr>
</tbody>
</table>
Figure 1. Relationship between concentrations of EFZ in plasma (a readily accessible and more easily measured parameter) and concentrations of EFZ and 8OH-EFZ in CSF

CSF and plasma EFZ concentrations were correlated in the TBM group (fig 1a) and the no-TBM group (fig 1b). No relationship was seen for 8OH-EFZ in either the TBM group (fig 1c) or no-TBM group (fig 1d).
Figure 2. Affect of CYP2B6 genotype on estimated effective and toxic concentrations of EFZ in plasma (fig 1a and b), EFZ in CSF (fig 1c and d) and total 8OH-EFZ in CSF (fig e and f).

Error bars are geometric mean and 95% confidence interval for GG/GT genotype, and geometric mean, range for TT genotype. MTC – minimum toxic concentration, MIC – minimum inhibitory concentration, ITC – *in vitro* toxic concentration.
Figure 3: Relationship between degree of blood-brain barrier breakdown, as measured by CSF:blood albumin ratio, and CSF concentrations of EFZ and 8OH-EFZ.