Impact of CYP2B6 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients

Christoph Wyen1, Heidy Hendra1, Martin Vogel2, Christian Hoffmann3, Heribert Knechten4, Norbert H. Brockmeyer5, Johannes R. Bogner6, Jürgen Rockstroh2, Stefan Esser7, Hans Jaeger8, Thomas Harrer9, Stefan Mauss10, Jan van Lunzen11, Nicole Skoetz12, Alexander Jetter13, Christiane Groneuer1, Gerd Fätkenheuer1, Saye H. Khoo14, Deirdre Egan14, David J. Back14 and Andrew Owen14* on behalf of the German Competence Network for HIV/AIDS

1Department of Internal Medicine, University of Cologne, Köln, Germany; 2Department of Internal Medicine, University of Bonn, Bonn, Germany; 3IPM Study Center, Hamburg/University of Schleswig Holstein, Kiel, Germany; 4PZB, Aachen, Germany; 5Department of Dermatology, St Josef Hospital, Bochum, Germany; 6Department of Internal Medicine, University of Munich, München, Germany; 7Department of Dermatology, University of Essen, Essen, Germany; 8HIV Research and Clinical Care Centre Munich, Munich, Germany; 9Department for Internal Medicine 3, University Hospital Erlangen, University of Erlangen-Nuremberg, Germany; 10Gemeinschaftspraxis Mauss und Kollegen, Düsseldorf, Germany; 11Infectious Diseases Unit, University Medical Center Hamburg Eppendorf, Hamburg, Germany; 12ZKS, Köln, Germany; 13Division of Clinical Pharmacology and Toxicology, Department of Internal Medicine, University Hospital Zürich, Zürich, Switzerland; 14Department of Pharmacology and Therapeutics, University of Liverpool, 70 Pembroke Place, Liverpool L69 3GF, UK

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Objectives: The aim of this study was to investigate the frequency of CYP2B6 polymorphisms (according to ethnicity) and the influence of heterozygosity and homozygosity on plasma concentrations of efavirenz and nevirapine.

Methods: Following written informed consent, 225 Caucasians and 146 Blacks were recruited from the German Competence Network for HIV/AIDS. Plasma concentrations of efavirenz and nevirapine were assessed by HPLC, and genotyping for 516G>T, 983T>C and 1459T>C polymorphisms in CYP2B6 was conducted by real-time PCR-based allelic discrimination.

Results: The minor allele frequency for 516G>T, 983T>C and 1459T>C was 0.29, 0 and 0.08 in Caucasians and 0.34, 0.07 and 0.02 in Blacks, respectively. Two Black patients with the 983C allele receiving efavirenz were identified and both were withdrawn from therapy within 1 week of sampling due to toxicity. In multivariate analyses, efavirenz and nevirapine plasma concentrations were significantly associated with 983T>C (P < 0.0001 and P = 0.02, respectively), 516G>T (P < 0.0001 and P = 0.02, respectively) and time of drug analysis post-dose (P < 0.0001 for both). Body mass index was independently related to efavirenz (P = 0.04) but not nevirapine concentrations, and age was related to nevirapine (P = 0.05) but not efavirenz concentrations. Consistent with other studies, 1459C>T was not associated with plasma concentrations of either drug (P > 0.05 for both drugs).

Conclusions: This is the first report that the 983T>C genotype (part of the CYP2B6*18 haplotype) impacts on nevirapine plasma concentrations and the first study to assess the impact of 983C homozygosity on efavirenz concentrations. These data have implications for administration of non-nucleoside reverse transcriptase inhibitors to Black patients.

Keywords: NNRTIs, pharmacogenetics, pharmacokinetics, metabolism, drug disposition

*Corresponding author. Tel: +44-151-794-5919; Fax: +44-151-794-5656; E-mail: aowen@liv.ac.uk

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CYP2B6 SNPs and NNRTI concentrations

Introduction

Drug treatment in HIV disease is characterized by variable responses, in terms of both efficacy and toxicity. Genetic and environmental factors are important determinants of this variability, although the relative contributions are unclear and likely to vary with different drugs (see Owen et al.1 for review).

Efavirenz and nevirapine are metabolized by CYP2B6 (although CYP3A also contributes to nevirapine metabolism). Single nucleotide polymorphisms (SNPs) and haplotype organization of CYP2B6 in Caucasians were originally described by Lang et al.,2 who showed a reduced hepatic CYP2B6 protein expression and activity in carriers of the 516G>T (rs3745274) and 1459T>C (rs3211371) polymorphisms. However, the association with protein expression and activity was not statistically significant for 516G>T, although the authors acknowledged the limitation of the sample size. With respect to HIV, the role of the 516G>T SNP in disposition and treatment response to non-nucleoside reverse transcriptase inhibitors (NNRTIs) is now well established.3–9 Moreover, a recent study indicated that efavirenz dose reduction according to 516G>T is feasible.10

Recently, the 983T>C SNP (rs28399499) was described in Black populations. This polymorphism results in an amino acid change in the CYP2B6 protein (Ile328Thr), and heterozygosity has been shown to impact upon efavirenz pharmacokinetics.11,12 Furthermore, a laboratory-based assessment of this polymorphism indicated that it may represent a null allele.11 Investigation of the 983T>C polymorphism in various populations has shown that the allele is absent in Caucasian populations, yet its frequency is as high as 7.5% in African-Americans and Ghanaians.13 However, to date, no homozygotes for this polymorphism have been described.

The aim of this study was to investigate the frequency of CYP2B6 polymorphisms (516G>T, 983T>C and 1459T>C) and the influence of heterozygosity and homozygosity for these polymorphisms on plasma concentrations of efavirenz and nevirapine in a cohort of Caucasian and Black patients from the German Competence Network for HIV/AIDS. In addition, the association with gender, ethnicity, weight, height, alcohol consumption, smoking status, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) was also determined. Finally, a multivariate analysis was conducted using best subset analysis.

Genotyping

Total genomic DNA was isolated using the QIAamp DNA mini kit according to the manufacturer’s instructions. Following extraction, purity was assessed by comparing the A260 and A280 ratio. DNA was quantified using PicoGreen® (PG) dsDNA Quantiﬁcation Reagent (Molecular Probes, CA, USA) and normalized to 20 ng/μL. Pre-amplification for exon 4, exon 9 and exons 7 and 8 (combined) was first conducted to discriminate from the CYP2B6 pseudogene (CYP2B7) by modification of previously reported methods.2 Genotyping for 516G>T, 983T>C and 1459C>T was then performed on the resultant amplicons by real-time PCR allelic discrimination using standard methodology (95°C for 15 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min) in a DNA Engine Opticon® 2 system (MJ Research Inc., USA). Full PCR conditions as well as primer and probe sequences are available on request.

Quantification of drug levels

Plasma obtained from blood samples was heat-inactivated, and efavirenz and nevirapine concentrations were determined (median time post-dose was 10 h) using HPLC with UV-Detection using previously validated assays as described elsewhere.14,15 The Liverpool Laboratory participates in an external quality assurance scheme (KKGT, The Netherlands).

Statistical analysis

All data are given as median (range), unless otherwise stated. Genotypes were tested for Hardy–Weinberg equilibrium by χ2 test of observed versus predicted (from allele frequency) genotype frequencies. Normality of the data was assessed using a Shapiro–Wilk test. Subsequently, univariate statistical analysis was conducted by simple linear regression (continuous data) or one-way analysis of variance (categorical data). Missing values were imputed by regression against efavirenz concentrations, and multivariate analysis was conducted by multiple linear regressions using best subset selection.

Results

Genotype frequencies

When χ2 test of observed versus predicted genotype frequencies was conducted, all polymorphisms were found to be in Hardy–Weinberg equilibrium. The frequencies of 516G>T, 983T>C and 1459C>T are given in Figure 1.

Efavirenz plasma concentrations

In the entire cohort, efavirenz plasma concentrations were 2077 (487–21 486) ng/mL. In the univariate analysis, frequencies of 516G>T [1779 (530–26 018), 2299 (487–11 198) and 6248 (1345–23 592) ng/mL for TT, TC and CC, respectively; P = 0.001], 983T>C [2608 (530–11 198), 2076 (1269–6835) and 23 418 (20 818–26 018) ng/mL for TT, TC and CC, respectively; P = 0.0001], body mass index (BMI) (R² = 0.03; P = 0.05), alcohol consumption (R² = 0.03; P = 0.04) and time post-dose of drug (R² = 0.22; P < 0.0001) were all significantly associated with efavirenz plasma concentrations (Table 1). Following multivariate analysis, only 516G>T (P < 0.0001), 983T>C

Patients and methods

Patients

Whole blood and plasma were provided by the German Competence Network for HIV/AIDS. Three hundred and seventy-one patients (225 Caucasians and 146 Blacks), with age range from 21 to 82 (median = 43) years, who were on stable efavirenz- (87% of cohort) and nevirapine- (n = 185) containing HAART for at least 3 months, were included in the study. Median (range) CD4 counts were 530 (50–26 018), 2299 (487–11 198) and 6248 (1345–23 592) ng/mL for TT, TC and CC, respectively; P = 0.0001, 983T>C [2608 (530–11 198), 2076 (1269–6835) and 23 418 (20 818–26 018) ng/mL for TT, TC and CC, respectively; P = 0.0001], body mass index (BMI) (R² = 0.03; P = 0.05), alcohol consumption (R² = 0.03; P = 0.04) and time post-dose of drug (R² = 0.22; P < 0.0001) were all significantly associated with efavirenz plasma concentrations (Table 1). Following multivariate analysis, only 516G>T (P < 0.0001), 983T>C
516G>T efavirenz ($n=174$)

516G>T nevirapine ($n=157$)

1459C>T efavirenz ($n=174$)

1459C>T nevirapine ($n=157$)

983T>C efavirenz ($n=174$)

983T>C nevirapine ($n=157$)

Figure 1. The impact of 516G>T (a and b), 1459C>T (c and d), and 983T>C (e and f) polymorphisms in CYP2B6 on efavirenz (a, c and e) and nevirapine (b, d and f) plasma concentrations according to ethnicity (see text for experimental procedures). The genotype frequencies within each group are also given.

(P < 0.0001), BMI (P = 0.04), and time post-dose (P < 0.0001) remained statistically significant (Table 1).

Neither 1459C>T [2241 (530–26 018) and 1816 (792–4357) ng/mL for CC and CT, respectively] or 983T>C [2168 (487–26 018) and 1974 (688–23 592) ng/mL for male and female, respectively], ethnicity [2143 (669–11 198) and 1963 (487–26 018) ng/mL for Caucasian and Black, respectively], gender [2168 (487–26 018) and 1974 (688–23 592) ng/mL for male and female, respectively], and smoking status [2143 (669–11 198) and 1963 (487–26 018) ng/mL for Caucasian and Black, respectively] had a significant effect on plasma concentrations.
Efavirenz

respectively; 516G>T N/A <0.0001 <0.0001

983T>C N/A <0.0001 <0.0001

1459C>T N/A 0.06 0.78

BMI 0.02 0.05 0.04

GOT 0.01 0.15 0.52

GPT 0.002 0.55 N/A

alcohol status 0.03 0.04 0.25

smoking status 0.02 0.09 0.35

age 0.001 0.66 N/A

time post-dose 0.22 <0.0001 <0.0001

Nevirapine

gender N/A 0.62 N/A

ethnicity N/A 0.50 N/A

516G>T N/A 0.007 0.002

983T>C N/A 0.02 0.02

1459C>T N/A 0.18 N/A

BMI 0.01 0.20 N/A

GOT 0.004 0.42 N/A

GPT 0.006 0.33 N/A

alcohol status 0.002 0.55 N/A

smoking status 0.005 0.34 N/A

age 0.03 0.03 0.05

time post-dose 0.09 <0.0001 0.0004

Associations with a \(P\) value less than 0.15 in the univariate analysis and therefore included in the multivariate analysis are shown in bold, as are those associations with a \(P\) value less than 0.05 in the multivariate analysis.

\((R^2 = 0.02)\), nor age \((R^2 = 0.001)\) was significantly associated with efavirenz plasma concentrations (Table 1).

Nevirapine plasma concentrations

In the entire cohort, nevirapine plasma concentrations were 5589 (149–21 026) ng/mL in the univariate analysis, 516G>T 5184 (1894–11 158), 6132 (149–11 689) and 6699 (4164–21 026) ng/mL for GG, GT and TT, respectively; \(P = 0.007\), 983T>C [5483 (864–21 026) and 8685 (4890–10 294) for TT and TC, respectively; \(P = 0.02\), age \(R^2 = 0.03\), \(P = 0.03\), and time post-dose of drug analysis \(R^2 = 0.09\); \(P = 0.0001\) were all significantly associated with nevirapine plasma concentrations (Table 1). Following multivariate analysis, 516G>T \(P = 0.002\), 983T>C \(P = 0.02\), age \(P = 0.05\), and time post-dose \(P = 0.0004\) all remained statistically significant (Table 1).

Neither 1459C>T [5670 (864–21 026) and 5243 (2230–7436) ng/mL for CC and CT, respectively], gender [5893 (149–11 689) and 5483 (864–21 026) ng/mL for male and female, respectively], ethnicity [5893 (1894–11 689) and 5138 (149–21 026) ng/mL for Caucasian and Black, respectively], BMI \((R^2 = 0.01)\), GOT \((R^2 = 0.04)\), GPT \((R^2 = 0.006)\), alcohol consumption \((R^2 = 0.002)\), nor smoking status \((R^2 = 0.005)\) was significantly associated with nevirapine plasma concentrations (Table 1).

Discussion

The \(CYP2B6\) gene is highly polymorphic with numerous SNPs and associated haplotypes. The association of the 516G>T SNP with efavirenz and nevirapine pharmacokinetics is well established, but a recent study indicated that the 516TT genotype was not associated with the time to failure of efavirenz-containing regimens or increases in the CD4 cell count.\(^1\) This polymorphism did predict CNS adverse effects at week 1 of therapy, but tolerance developed despite the higher plasma efavirenz exposure.\(^15\) Nonetheless, a recent manuscript described the use of 516G>T (along with 499C>G and 785A>G) in successful dose reduction of efavirenz in a Japanese cohort.\(^10\) The cost-effectiveness of this approach now requires further investigation.

Genetic variability has been assessed in different ethnicities and a number of novel functional variants discovered,\(^1,11,12\) among these, the 983T>C polymorphism is a suspected null allele. With the increasing use of NNRTIs in developing countries, it is imperative that the functional significance of these alleles on NNRTI therapy in different sub-populations be assessed. The 983T>C polymorphism has only previously been identified in Hispanic and African populations,\(^1,12\) with allele frequencies of 1.1% in Hispanic Americans and up to 7.5% in some African countries.\(^13\) We observed this allele with a frequency of 2.6% in Blacks residing in Germany.

In agreement with previous studies, we observed a significant gene dose effect between 516G>T and efavirenz and nevirapine plasma concentrations. In addition, 983T>C genotype was also associated with plasma concentrations of both drugs. Importantly, homozygote patients in the efavirenz arm discontinued therapy 1 week after blood was collected for this study (but before drug analysis) due to CNS toxicity. Previous studies have illustrated that heterozygosity of this polymorphism (present in the \(CYP2B6*18\) allele) is associated with efavirenz concentrations.\(^12\) However, no homozygotes for this polymorphism have been previously reported. Therefore, this is the first study to illustrate the phenotypic consequence of heterozygosity for efavirenz and to show an association with nevirapine plasma concentrations. Further studies are now required to determine the impact of 983T>C on long-term efficacy and toxicity of this important class of drug.

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