Reduction of nevirapine-driven HIV mutations by carbamazepine is modulated by CYP3A activity

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Objectives: The reduction in mother-to-child transmission of HIV-1 by single-dose nevirapine given at birth onset is achieved at the expense of de novo HIV-1 resistance mutations. In the VITA1 study, single-dose carbamazepine accelerated nevirapine elimination, but the accompanying trend towards fewer de novo HIV-1 mutations was statistically non-significant.

Methods: We investigated if the effect of carbamazepine was confounded by the individual variability in nevirapine metabolism and transport.

Results: Nine of 34 (26%) single-dose nevirapine-treated women had one or more nevirapine-associated resistance mutations, compared with 3 of 34 (9%) in the single-dose nevirapine/carbamazepine arm. The genetic polymorphisms in CYP2B6 and MRP7 affected neither nevirapine kinetics nor the development of HIV-1 resistance. In contrast, the reduction in HIV-1 mutations by single-dose carbamazepine reached statistical significance at \( P = 0.04 \) with an OR of 0.1 (95% CI 0.01–0.90) upon consideration of CYP3A activity, defined as the ratio of 4β-hydroxycholesterol to cholesterol, and it was more likely in women with higher CYP3A activity. These findings were in agreement with CYP3A induction in carbamazepine-treated patients. Likewise, carbamazepine induced CYP3A4, but not CYP2B6, in vitro when combined with nevirapine.

Conclusions: The induction of nevirapine elimination reduces HIV-1 resistance mutations, but this effect is modulated by individual CYP3A activity. The study suggests that CYP3A4 activity could be monitored using an endogenous marker and, if needed, boosted to improve clinical endpoints.

Keywords: mother-to-child transmission, induction, 4β-hydroxycholesterol

Introduction

A single dose of the non-nucleoside reverse transcriptase inhibitor nevirapine given at birth onset reduces the risk of mother-to-child transmission of HIV-1 from 35%–40% to 11%–21%.

Unfortunately, the combined effect of the low genetic barrier of nevirapine and of its long elimination half-life of 61–66 h results in HIV-1 resistance mutations in 15%–75% of treated mothers.

The risk of HIV-1 mutations increases 3-fold for every 100 ng/mL in nevirapine plasma level at day 2 after delivery. In consequence, nevirapine may become ineffective in subsequent pregnancies and nevirapine-resistant HIV strains may spread in the population.

To provide a remedy, a reduction in the half-life of nevirapine through deliberate acceleration of its elimination was tested in the VITA1 study. Nevirapine was administered simultaneously with a single dose of the anticonvulsant carbamazepine, an inducer of the reported nevirapine-metabolizing P450 enzymes CYP2B6, CYP3A4 and CYP3A5. Plasma nevirapine concentrations were unchanged immediately after delivery, ensuring that the protective perinatal effect of nevirapine was unaffected. They were significantly reduced by carbamazepine 1 week post partum and this delay most likely reflected the time required for the induction of CYP3A5 transcription and protein synthesis.

The accompanying trend towards fewer nevirapine resistance mutations in the carbamazepine arm failed to reach statistical...
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Figure 1. 4β-OHC/cholesterol ratio in women treated with single-dose nevirapine (sdNVP) (a) and single-dose nevirapine + single-dose carbamazepine (sdNVP/sdCMZ) (b) at three different timepoints and the correlation of 4β-OHC/cholesterol ratios at week 2 with nevirapine plasma concentrations at week 1 for both groups (c). Shown are the IQR, median and minimum and maximum values (a and b) and single values for each participant given either nevirapine alone (black filled circles) or co-treated with carbamazepine (grey filled squares) (c). Single-dose nevirapine-treated groups (a) were compared with repeated measures ANOVA and Dunnett’s post hoc test. Groups receiving both drugs (b) were compared with a non-parametric Friedman test and related to values at delivery applying Dunn’s multiple comparison test. Non-parametric correlation (c) was calculated according to Spearman. **P < 0.01, ns, not significant.

Methods

A detailed method description is provided in the Supplementary data available at JAC Online. Briefly, CYP2B6, CYP3A4/5 and MRP7 expression status was determined using five single-nucleotide polymorphisms (SNPs) previously reported to affect nevirapine pharmacokinetics (Table S1, available as Supplementary data at JAC Online) and three SNPs determining CYP3A5 expression status in Africans (Table S2, available as Supplementary data at JAC Online), combined with the ratio of CYP3A activity marker 4β-hydroxycholesterol (4β-OHC) to cholesterol in plasma (Table S3, available as Supplementary data at JAC Online).10 The study was approved by institutional review boards of KCMM, Moshi, Tanzania, and Radboud University Nijmegen Medical Centre, the Netherlands.

The inducing effect of carbamazepine and nevirapine on CYP3A and CYP2B6 expression was investigated in colon adenocarcinoma-derived LS174T cells transfected with CYP3A and CYP2B6 reporter gene constructs and with the transcriptionally active drug sensors and nuclear receptors PXR (pregnane X receptor, NR1I2) and CAR (constitutive androstane receptor, NR1I3).11 Nevirapine and/or carbamazepine were added at drug concentrations measured in human plasma after 400 mg single-dose carbamazepine or 200 mg single-dose nevirapine.

Haplotypes were calculated using PHASE 2.1. All other indicated statistical analyses were performed using GraphPad Prism 5.01 or SPSS (version 12.0, IBM, Chicago, IL, USA). The impact of multiple parameters on the binary variable ‘presence of nevirapine-driven induction of HIV-1 mutations’ was evaluated by logistic regression analysis. To this end, each of eight genetic polymorphisms, CYP3A4 activity, nevirapine plasma concentrations, and administration of carbamazepine were first evaluated separately in univariate fashion (Table S4, available as Supplementary data at JAC Online). Variables exhibiting a statistical trend towards HIV-1 mutation induction at P < 0.2 were jointly analysed in a multivariate logistic regression model.

Results and discussion

Carbamazepine induces CYP3A both in vivo and in vitro

CYP3A undergoes induction during pregnancy and returns to normal levels after delivery.12 In agreement, 4β-OHC/cholesterol ratios in VITA1 study participants at delivery were significantly higher (P < 0.001) than in non-pregnant women from Tanzania and from Sweden (Figure S1, available as Supplementary data at JAC Online). Likewise, 4β-OHC/cholesterol ratios were decreased 2 weeks after delivery, but only in the nevirapine arm (P < 0.01; Figure 1 and Table S3). In contrast, 4β-OHC/cholesterol ratios in women administered both nevirapine and carbamazepine remained unchanged 2 weeks post partum (Figure 1 and Table S3), indicating carbamazepine-driven CYP3A induction.
The inducing effect of carbamazepine was in a way surprising, as nevirapine itself is an inducer of its own enzymatic elimination. As the induction potentials of carbamazepine and nevirapine are controversial, they were investigated in cells transfected with CYP3A and CYP2B6 reporter gene constructs and with the transcriptionally active drug sensors and nuclear receptors PXR and CAR. We investigated both CAR splice variants (SV1-WT and SV2) shown previously to mediate carbamazepine- and nevirapine-driven CYP2B6 induction. CYP2B6 and CYP3A5 were unresponsive to either drug, whether applied separately or in combination, irrespective of the co-transfected drug sensor (Figure 2). Similar results were obtained for CYP3A4 with either drug applied alone. In contrast, nevirapine and carbamazepine applied in combination significantly induced both the CAR-SV1-WT-mediated (2.3-fold, \(P < 0.01\)) and the PXR-mediated (2.7-fold, \(P < 0.01\)) CYP3A4 promoter activity compared with solvent-treated and analogously transfected cells (Figure 2). This suggests that single-dose carbamazepine transactivates CYP3A4 promoter only, or at least particularly efficiently, in the presence of another inducer, in this case of nevirapine. Similar, additive drug effects on PXR- and/or CAR-mediated induction of CYP3A4 have been described previously for cyclophosphamide and dexamethasone. The differential response of CYP3A4 and CYP3A5 is consistent with previous observations and with the much higher number of xenosensor-responsive elements in the former gene.

**Effects of carbamazepine, CYP3A activity and SNPs on nevirapine pharmacokinetics and HIV-1 mutations**

When tested individually, these variables had no effect on nevirapine concentrations at week 1 (non-parametric Spearman correlation, \(r = -0.26, P < 0.05\); Figure 1c). This was particularly surprising for CYP2B6 SNP 516G→T, considering the evidence for this SNP affecting nevirapine exposure (Table S1). However, in several recent studies only a weak or a population-dependent effect on nevirapine given as a single dose. The activity difference between CYP2B6 expressed from 516T and 516G alleles may become manifest only following long-term induction of CYP450 by nevirapine. CYP2B6 516G→T may be able to affect the pharmacokinetics of single-dose nevirapine in Asian populations.

Linear regression analysis of the effects of 4β-OHC/cholesterol ratios and of gene variants and CYP3A5 expression status on nevirapine concentrations at week 1 was not possible due to the non-Gaussian distribution of the latter (D’Agostino and Pearson omnibus test).

Nine of 34 (26%) single-dose nevirapine-treated women had one or more nevirapine-associated resistance mutations, compared with 3 of 34 (9%) in the single-dose nevirapine/carbamazepine arm. The impact of CYP3A activity, SNPs and carbamazepine on HIV-1 mutations was investigated using a two-step logistic regression analysis. Firstly, using univariate logistic regression we looked for independent variables affecting HIV-1 mutation status from the model had no effect on the 4β-OHC/cholesterol ratios at week 1 (\(P = 0.18\)). Thereafter, these variables were subjected to a multivariate logistic regression analysis we looked for independent variables affecting HIV-1 mutations with exploratory \(P\) values <0.20 (Table S4). This criterion was met by carbamazepine (\(P = 0.07\)), MRP7 (\(P = 0.12\)) and 4β-OHC/cholesterol ratios at week 1 (\(P = 0.18\)). Therefore, these variables changed the model had no effect on the \(P\) value. Taken together, these results demonstrated that the reduction of HIV-1 mutations by single-dose carbamazepine was more likely in women with higher 4β-OHC/cholesterol ratios.
In summary, a single dose of carbamazepine appeared capable of a substantial reduction (by two-thirds) in nevirapine-driven HIV-1 resistance mutations. Three lines of evidence suggest that this reduction was mediated primarily by CYP3A4. Firstly, unlike the CYP3A4/5 activity marker 4b-OHC/cholesterol ratio, CYP3A5 expression status considered alone had no effect on nevirapine pharmacokinetics. Secondly, CYP3A4 is more active than CYP3A5 towards nevirapine, as it is towards most other CYP3A substrates. Thirdly, CYP3A4, but not CYP3A5, was induced by the combination of the two drugs, most likely due to the much higher number of xenosensor-responsive elements.

Carbamazepine-driven CYP3A4 induction would be expected to enhance the elimination of nevirapine. This is supported by the negative correlation between the phenotypically assessed CYP3A activity and nevirapine concentrations at week 1 post partum. The associated reduction in HIV-1 resistance mutations was more likely in women with higher CYP3A enzyme activity. This may seem counterintuitive, as study participants with low initial CYP3A expression could be expected to have a higher induction potential. However, the net induction of CYP3A4 appears to be independent of the initial CYP3A4 expression level. In consequence, CYP3A4 activity following carbamazepine may have reached higher levels in women with higher initial CYP3A4 levels than in those with lower ones. It follows that nevirapine-metabolizing activity must overcome a certain threshold in order to prevent the development of HIV resistance mutations rather than simply increase by a certain fold-factor.

The direct clinical utility of our findings is limited, as single-dose nevirapine is no longer recommended for the prevention of mother-to-child transmission of HIV-1. On the other hand, the study suggests that the individually variable CYP3A4 activity can be monitored using an endogenous marker and, if needed, boosted to improve non-pharmacokinetic clinical endpoints, in the case at hand to reduce de novo HIV-1 mutations. This suggestion is derived from the retrospective analysis presented in this paper and requires prospective verification with one of the numerous CYP3A substrates. Lastly, this work further emphasizes the importance of considering interindividual differences in drug-metabolizing capacity.

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Transparency declarations
None to declare.

Author contributions

Supplementary data
Supplementary data, including Tables S1 to S4 and Figure S1, are available at JAC Online (http://jac.oxfordjournals.org).

References
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