Interaction Potential of Etravirine with Drug Transporters Assessed In Vitro

Nadine Cécile Luise Zembruski, Walter Emil Haefeli, and Johanna Weiss*

Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

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Etravirine is a novel nonnucleoside reverse transcriptase inhibitor (NNRTI) for the treatment of HIV-1 infections. ABC transporters potentially mediate clinically relevant drug-drug interactions. We assessed substrate characteristics and the inhibitory and inductive potential of etravirine on ABC transporters. Etravirine did not inhibit P-gp/ABCB1 and was not transported by the tested ABC transporters but was a potent inhibitor of BCRP/ABCG2. Etravirine induced several ABC transporters, especially BCRP/ABCG2. These data demonstrate that etravirine has the potential for drug-drug interactions by modulation of expression and function of several ABC transporters.

Etravirine is a novel nonnucleoside reverse transcriptase inhibitor (NNRTI) for the treatment of HIV-1 infections. It is active against wild-type and some NNRTI-resistant HIV strains (1, 2) and offers a new treatment option for treatment-experienced patients. Pharmacokinetic drug-drug interactions considerably influence efficacy and safety of antiretroviral therapy. Interactions might lower concentrations of antiretrovirals below therapeutic concentrations (5, 12, 14) and cause treatment failure and viral resistance. Interactions may also increase drug exposure and augment toxicity.

The main mechanisms of interaction in antiretroviral combination therapy involve the drug-metabolizing cytochrome P450 enzymes (CYPs) as well as efflux and uptake transporters. Crucial efflux transporters are several ATP-binding cassette (ABC) transporters that have been identified as important interaction sites of antiretrovirals (9–11). Relevant uptake transporters are the organic anion-transporting polypeptides (OATPs/SLCOs) (6, 16, 20). Information on interactions of etravirine is sparse. We therefore investigated whether etravirine is a substrate of P-gp/ABCB1, BCRP/ABCG2, MRP1/ABCC1, MRP2/ABCC2, or MRP3/ABCC3 and whether it inhibits P-gp/ABCB1 and BCRP/ABCG2. Furthermore, we investigated etravirine’s potency to induce ABC transporters, important OATPs/SLCOs, CYPs, and the transcription factor pregnane X receptor.

Etravirine was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, as etravirine TMC125 (catalog no. 11609) from Tibotec, Inc. Other materials were used as described previously (13). Etravirine was tested for cytotoxic effects prior to P-gp/ABCB1 and BCRP/ABCG2 inhibition assays with a cytotoxicity detection kit (Roche Applied Science, Mannheim, Germany) according to manufacturer’s instructions. Cytotoxic concentrations were excluded in the respective assays. P-gp/ABCB1 and BCRP/ABCG2 inhibition was quantified by calcein and phorbol ester efflux assays as described previously (23, 26). We used the growth inhibition assay in MDCKII cells overexpressing human P-gp/ABCB1 (7), BCRP/ABCG2 (17), and MRP1-3/ABCC1-3 (8) as a surrogate for substrate characteristics of etravirine as it has been described for other antiretroviral drugs (3, 13, 26). The induction assay, quantification of mRNA expression by real-time reverse transcriptase PCR (RT-PCR), and data evaluation by calibrator-normalized relative quantification with efficiency correction were also performed as published earlier (26).

Data were analyzed using GraphPad Prism version 5.02 and InStat version 3.06 (GraphPad Software, San Diego, CA). Statistical differences in mRNA expression and in 50% inhibitory concentrations (IC₅₀/s) of proliferation assays were tested using analysis of variance (ANOVA) with Dunnett’s post hoc test. Induction and repression were considered relevant only if mRNA expression differed from the baseline level by a factor of 1.5 or 0.67. A P value of ≤0.05 was considered significant.

Proliferation assays in MDCKII cells and MDCKII cells overexpressing P-gp/ABCB1, BCRP/ABCG2, MRP1/ABCC1, MRP2/ABCC2, and MRP3/ABCC3 suggest that etravirine is not transported by these ABC transporters. P-gp/ABCB1-, BCRP/ABCG2-, and MRP3/ABCC3-overexpressing cells were even slightly less resistant toward etravirine than the parental cell line (Table 1).

Moreover, our results demonstrate that etravirine did not inhibit P-gp/ABCB1 up to the maximum tested concentration of 5 μmol/liter (maximum solubility in the buffer used) either in P388/dx or in L-MDR1 cells. These findings disagree with the summary of product characteristics of etravirine (Intellence) reporting weak inhibition of P-gp/ABCB1 by etravirine (22).

However, these data are not publicly accessible, and thus assay conditions cannot be compared. Although we cannot exclude that etravirine inhibits P-gp/ABCB1 at higher concentrations, substantial inhibition appears unlikely because strong inhibitors like verapamil or quinidine exhibit IC₅₀/s below 5 μmol/liter. 

* Corresponding author. Mailing address: Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. Phone: 49-6221/56-39402. Fax: 49-6221/56-4642. E-mail: johanna.weiss@med.uni-heidelberg.de.

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Our data for the first time demonstrate that etravirine is a very potent BCRP/ABCG2 inhibitor. In the BCRP/ABCG2 inhibition assay, etravirine increased phosphorhodine A fluorescence in MDCKII-BCRP cells but not in the parental cell line MDCKII, indicating BCRP/ABCG2 inhibition (IC50 of 1.0 ± 0.4 μmol/liter). The IC50 was similar to the IC50 for furmitremorgin C (FTC) (0.7 ± 0.3 μmol/liter [25]), one of the most potent BCRP/ABCG2 inhibitors known. Effective etravirine concentrations match maximum plasma concentrations of etravirine, which have been reported to be in the range of 1.34 ± 0.36 μg/ml (equal to 3.1 μmol/liter, yielding 3.1 mmol/liter unbound drug) on day 8 after administration of the approved dosage of 200 mg twice daily (18). According to the FDA criteria developed for P-gp/ABCB1, this justifies further in vivo evaluation, because [Ic]/IC50 is greater than 10 (with Ic equal to a dose/volume of 250 ml, which is the estimated intestinal concentration) (27). However, for the interaction between etravirine and typical BCRP/ABCG2 substrates, clinical studies have not been examined in clinical studies. The overall effect of induction and inhibition of ABCG2 as well as the abrogative influence of increased expression on inhibition is unknown.

Due to proliferation inhibition of LS180 cells at higher concentrations, etravirine was tested at 0.1 and 1.0 μmol/liter in the induction experiments. Induction of mRNA was analyzed after 4 days of treatment. Etravirine did not induce CYP2B6, ABCC1, ABCC2, ABCC4, ABCC5, SLCO1B3, SLCO1B1, or PXR. It slightly induced ABCB1, ABCC3, CYP3A5, and SLCO2B1. mRNA expression of ABCG2 and CYP3A4 was strongly induced at a concentration of 1 μmol/liter by factors of 3.5 and 5.0, respectively (Fig. 1). Induction of P-gp/ABCB1 and BCRP/ABCG2 was also verified at the protein level by Western blotting after 7 days of incubation (Fig. 2). Semi-quantification of the band intensities and normalization to the untreated medium control demonstrated an increase in P-gp/ABCB1 and BCRP/ABCG2 protein expression by factors of 1.8 and 2.8, respectively (at 1 μmol/liter etravirine). Evidence from clinical trials suggests that etravirine is less prone to drug-drug interactions than other NNRTIs (4, 15). Nevertheless, many drug-drug interactions have been described for this NNRTI, and for some interactions the mechanism is not clear. Our results demonstrate that etravirine induces the mRNA expression of ABCB1, ABCC3, CYP3A4, and SLCO2B1. So far, the clinical impact is not clear, because there are no clinical studies addressing this issue. In contrast, several in vivo studies confirm the induction of CYP3A4 that we observed in vitro. Induction of CYP3A4 manifests itself in reduced exposure of several concomitantly applied CYP3A4 substrates (4, 12). However, this induction appears to be less pronounced than that for other NNRTIs, because coadministration with etravirine does not lead to clinically relevant drug-drug interactions in all cases (12, 19).

The strong induction of BCRP/ABCG2 at the mRNA and protein levels indicates that etravirine not only is a PXR ligand but might also be a ligand for the aryl hydrocarbon receptor (AhR), being an important regulator of ABCG2 gene expression (21).

Comparing etravirine’s drug interaction potential with those of older NNRTIs, these in vitro results as well as already published in vivo results indicate that etravirine has a lower susceptibility to drug-drug interactions than the older NNRTIs (Table 2) (4, 15).

In conclusion, our study demonstrated that etravirine induces important pharmacological targets beyond CYP3A4 and strongly inhibits BCRP/ABCG2. Clinical drug-drug interaction studies should now evaluate the clinical significance of the interactions, with particular attention given to the interactions with CYP3A4 and BCRP/ABCG2.
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identified inhibition and induction of BCRP/ABCG2 by etravirine.

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