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Interaction Potential of Etravirine with Drug Transporters Assessed *In Vitro*▽

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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 antiretroviral drugs (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 pheophorbide A 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 (IC_{50}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 (Intelence) 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 IC_{50}s below 5 μmol/

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TABLE 1. IC_{50} values for proliferation inhibition in MDCKII cells overexpressing P-gp/ABCB1, BCRP/ABCG2, MRP1/ABCC1, MRP2/ABCC2, or MRP3/ABCC3

Cell line	IC_{50} etravirine (μmol/liter)[a]
MDCKII	9.4 ± 0.4
MDCKII-MDR1	7.6 ± 1.5*
MDCKII-BCRP	6.0 ± 0.9**
MDCKII-MRP1	8.5 ± 0.5
MDCKII-MRP2	7.8 ± 1.1
MDCKII-MRP3	5.1 ± 0.4**

[a] Data are expressed as means ± standard deviations (SD) from three to six experiments, with each concentration tested in octuplet. Statistical significance was evaluated using ANOVA with Dunnett's *post hoc* test. *, $P \leq 0.05$; **, $P \leq 0.01$ (compared to the untreated control).

liter, a concentration at which etravirine did not inhibit P-gp/ABCB1.

Our data for the first time demonstrate that etravirine is a very potent BCRP/ABCG2 inhibitor. In the BCRP/ABCG2 inhibition assay, etravirine increased pheophorbide A fluorescence in MDCKII-BCRP cells but not in the parental cell line MDCKII, indicating BCRP/ABCG2 inhibition (IC_{50} of 1.0 ± 0.4 μmol/liter). The IC_{50} was similar to the IC_{50} for fumitremorgin 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 nmol/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 $[I_2]/IC_{50}$ is greater than 10 (with I_2 equal to a dose/volume of 250 ml, which is the estimated intestinal concentration) (27). However, thus far the interaction between etravirine and typical BCRP/ABCG2 substrates has 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 3.0, 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). Semiquantification 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

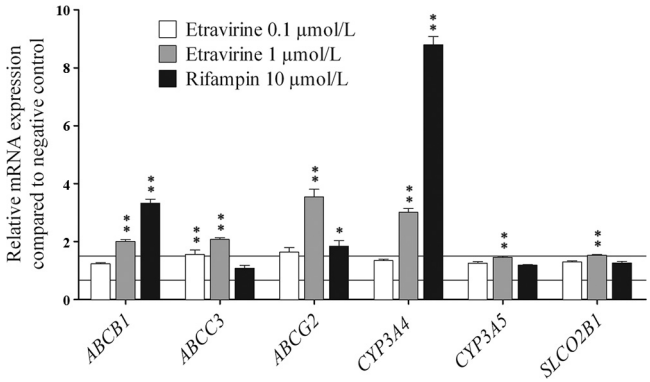

FIG. 1. Induction of mRNA expression by etravirine (0.1 and 1.0 μmol/liter) and the positive control rifampin (10 μmol/liter) in LS180 human adenocarcinoma cells, compared to expression for the medium control. Expression data were normalized to data for the reference gene *glucuronidase* β. Data are expressed as means ± standard errors of the means (SEM) from 8 to 12 experiments (4 biological replicates and 2 to 3 PCR runs for every sample). *, $P \leq 0.05$; **, $P \leq 0.01$.

induces the mRNA expression of *ABCB1*, *ABCC3*, *CYP3A5*, 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 indicated that etravirine not only is a PXR ligand but might also be a ligand for the aryl hydrocarbone 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

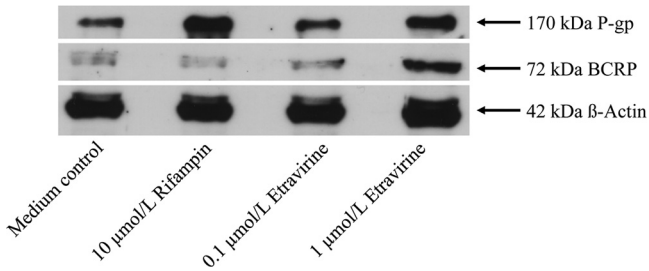

FIG. 2. Representative P-gp/ABCB1 and BCRP/ABCG2 Western blot after 1 week of incubation in LS180 cells.

TABLE 2. *In vitro* transporter interaction profiles of NNRTIs[a]

Tested function and transporter	Profile[b] for:			
	Etravirine	Delavirdine	Efavirenz	Nevirapine
Substrate				
P-gp/ABCB1	−	−	−	−
MRP1/ABCC1	−	−	−	−
MRP2/ABCC2	−	−	−	−
MRP3/ABCC3	−	−	−	−
BCRP/ABCG2	−	−	−	−
Inhibitor				
P-gp/ABCB1	−	++	+	+/−
BCRP/ABCG2	+++	+	+	+
Inductor				
P-gp/ABCB1	++	+	++	+
MRP1/ABCC1	−	NT	+	NT
MRP3/ABCC3	++	NT	++	NT
BCRP/ABCG2	+++	NT	++	NT
SLCO1B1	−	NT	NT	NT

[a] Data for delavirdine, efavirenz, and nevirapine were published in more detail previously (24).

[b] −, no substrate/inhibitor/inductor; +/−, very weak inhibitor; +, substrate or weak inhibitor/inductor; ++, moderate inhibitor/inductor; +++, strong inhibitor/inductor; NT, not tested.

identified inhibition and induction of BCRP/ABCG2 by etravirine.

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