Short Communication

LEVOTHYROXINE UP-REGULATES P-GLYCOPROTEIN INDEPENDENT OF THE PREGNANE X RECEPTOR

Received February 19, 2004; accepted April 26, 2004

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

P-Glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4) constitute a physiologic barrier in the intestine for many of the same substrates. Their expression can be influenced by nuclear receptor NR112 (pregnane X receptor; PXR), which acts as a receptor for various endobiotics and xenobiotics. However, P-gp and CYP3A4 are not identical in anatomic localization, suggesting unique as well as shared regulatory mechanisms of gene expression. We used established human colon carcinoma cell lines (LS180 and Caco-2) and measured mRNA and protein levels in cells after exposures to levothyroxine (L-T4), triiodo-L-thyronine (L-T3), and rifampin. Results indicate that L-T4, L-T3, and rifampin can up-regulate the expression of P-gp mRNA and protein in LS180 cells, but only L-T4 and L-T3 can produce the same effect in Caco-2 cells, which are relatively lacking in PXR. In addition, L-T4 and L-T3 did not affect the expression of CYP3A4 in either cell line. We conclude that P-gp, but not CYP3A4, can be up-regulated by thyroid hormones in vitro by a PXR-independent mechanism. Considering the widespread prescription use of L-T4 preparations in the older adult population, these results may be important for the clinical consideration of drug-drug interactions mediated by P-gp.

Abbreviations: P-gp, p-glycoprotein; CYP3A4, cytochrome P450 3A4; PXR, pregnane X receptor (nuclear receptor NR112); L-T4, levothyroxine; L-T3, 3,3',5-triiodo-L-thyronine; TR, thyroid receptor; VDR, vitamin D receptor; RXR, retinoid X receptor; Vit D3, 1α,25-dihydroxyvitamin D3; DR, direct repeat; RAR, retinoic acid receptor; ABCB1, ATP-binding cassette protein B1; kb, kilobase(s); HPLC, high-performance liquid chromatography; PCR, polymerase chain reaction.
of PXR and nuclear hormone receptor actions (dimerization with RXR and recognition of similar consensus motifs) prompted the present study of non-PXR-mediated mechanisms of CYP3A4 and ABCB1 induction, with a focus on establishing whether TR has a role in the regulation of these genes.

Materials and Methods

Cell Lines. We cultured LS180V and Caco-2 cells (both purchased from American Type Culture Collection, Manassas, VA) in American Type Culture Collection-recommended medium (In vitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 1% penicillin/streptomycin solution. LS180V cells were selected and maintained as described previously (Störmer et al., 2002). Over the course of the experiment, we passaged LS180V cells up to 14 times since the initial acquisition from the manufacturer; the passage number for Caco-2 cells varied between 28 and 32. After the cells reached confluence, they were treated with dimethyl sulfoxide vehicle.

HPLC Assay. L-T4 was extracted from the cell culture medium with methanol, and its concentrations were determined by HPLC with ultraviolet detection at 230 nm. We used a reverse-phase C18 Bondapak column (Waters, Milford, MA), with the mobile phase containing methanol, water, and phosphoric acid (80:20:0.2).

RNA Isolation, Reverse-Transcription PCR, and Quantitative Real-Time PCR. RNA isolation procedures were performed as described previously (Vishnuvardhan et al., 2003). We isolated total RNA from LS180V and Caco-2 cells using an RNaseasy Mini Kit (Qiagen, Valencia, CA). After DNase treatment (In vitrogen), the samples were reverse-transcribed with SuperScript II (In vitrogen). cDNAs dilutions ranging from 1:5 to 1:20 were subsequently used for real-time PCR according to the SYBR Green protocol on a GeneAmp PCR System (Applied Biosystems, Foster City, CA). For ABCB1 mRNA we used primers 5'-GCAGACAGCAGGAAATGAAGT-3' and 5'-TTTCTGGTTGAAGAAGTCCTCCTAAGCT-3'. The presence of TR isoforms 1, TR2, and TR3 was determined by PCR using the primers 5'-GAGAAGTTCCTCCCTGAAAGATTCAGC-3' and 5'-GAGAAGTTCCTCCCTGAAAGATTCAGC-3'. The amount of 18S RNA which was amplified using primers 5'-GAGAAGTTCCTCCCTGAAAGATTCAGC-3' and 5'-GAGAAGTTCCTCCCTGAAAGATTCAGC-3' is used as the internal control. The amount of ABCB1 mRNA in each treatment sample was determined using the PCR primer 5'-GCAGACAGCAGGAAATGAAGT-3' and 5'-GTGTTAGCTGAGGTG-3'.

Immunoblot Analysis of P-gp. Western blot procedures were performed as described previously (Cotreau et al., 2000; Perloff et al., 2001).

Enhancer Sequence Analysis. We used the Transcription Element Search System (TESS; URL: http://www.cbil.upenn.edu/tess) to analyze the sequences (−10,000 base pairs to +100 base pairs) of ABCB1 and CYP3A4 (GenBank accession numbers X58723 and AF185589, respectively) genes.

Statistical Analysis. We used the one-tailed paired t test to assess the increase in mRNA levels after treatment of cells with L-T4 versus treatment with dimethyl sulfoxide vehicle.

Results and Discussion

LS180V cells were induced for 72 h with two different concentrations of L-T4: 100 nM, which approximates the physiologic plasma levels of total L-T4 in normal individuals, and 100 μM, which approximates the intraluminal concentration in patients receiving oral levothyroxine. Rifampin (10 μM) was used as the known PXR-mediated inducer (Pfrunder et al., 2003) of both ABCB1 and CYP3A4. The LS180V cell line has been shown in our laboratory to have abundant PXR, while lacking constitutive androstane receptor (unpublished data). Stability of L-T4 in the culture medium over this period was verified by HPLC analysis.

In LS180V cells, ABCB1 was induced in a concentration-dependent manner by L-T4 (p < 0.05), with EC50 determined at 3.6 μM, and by rifampin (Fig. 1A). Levels of CYP3A4 mRNA were undetectable in all conditions except in rifampin-induced cells. Default values for these conditions were assigned by the real-time PCR software, and rifampin-treated samples were compared. Consequently, the magnitude of the actual mRNA increase with rifampin is at least as great as reported, but may be higher. L-T4 did not produce a detectable increase in CYP3A4 mRNA.

The mechanism of ABCB1 induction by L-T4 was evaluated using the Caco-2 cell line, which lacks significant amounts of PXR (Thummel et al., 2001; T. Mitin, L. L. vom Moltke, M. H. Court, and D. J. Greenblatt, unpublished data) and therefore should not demonstrate induction of PXR-regulated genes. Rifampin failed to increase levels of ABCB1 mRNA or protein, but L-T4 significantly increased ABCB1 expression in a concentration-dependent manner (p < 0.05) (Fig. 1, B and C).

These results suggest that L-T4 differs from rifampin in its ability to induce P-gp and regulates the expression of P-gp by a PXR-independent mechanism, perhaps through the TR. Both cell lines express the three major types of TR (TRα1, TRα2, and TRβ1) (Fig. 2, and L-T4 may therefore regulate P-gp expression by a mechanism involving binding to its endogenous receptor. TRα accounts for most of the thyroid hormone secreted by the thyroid gland. TRα is converted to TRβ by target tissues, and TRβ directly interacts with TRα. TRβ also induced ABCB1 expression in LS180V cells in a concentration-dependent manner, with no effect on CYP3A4 expression (data not shown). Our data were generated in human carcinoma cell lines, and any extrapolation to normal cells must be made with great caution.

Our findings may relate to the effect of 1α,25-dihydroxyvitamin D3 (Vit D3) on CYP3A4 and ABCB1 induction. The regulation of intestinal CYP3A4 expression by VDR and Vit D3 was first suggested by observations that treatment of Caco-2 cells with vitamin D analogs led to significant enhancement of CYP3A4 mRNA and protein levels and increased midazolam hydroxylation activity (Schmiedlin-Ren et al., 1997). These findings were confirmed later in LS180 cells, and ABCB1 up-regulation by Vit D3 occurred in the same study (Thummel et al., 2001), as well as in subsequent studies (Pfrunder et al., 2003).

Although ABCB1 gene expression can be up-regulated by L-T4, L-T3, and Vit D3, CYP3A4 can only be up-regulated by Vit D3. The ABCB1 upstream enhancer contains a perfect DR4 and an overlapping DR3 with only one nucleotide substitution at the fifth position (C→A) (Fig. 3). The presence of both motifs could allow binding of
TR/RXR as well as VDR/RXR to the enhancer site. The CYP3A4 enhancer region also contains a perfect DR3 and a DR4 with two nucleotide substitutions at position five (C→T), as well as at a critical position three (G/T→A). The nucleotide at the third position may be critical for transactivation of DNA by hormonal receptors (Yen, 2001). The absence of effect by L-T₄ or L-T₃ on CYP3A4 induction therefore might be the result of this nonfunctional DR4, which lacks a specific hexamer recognizable by TR/RXR. L-T₄ and L-T₃ might also lead to mRNA stabilization, resulting in the observed increase in mRNA and protein levels.

To our knowledge, this is the first report of L-T₄- and L-T₃-mediated ABCB1 gene expression up-regulation in vitro. A recent clinical study demonstrated an increase in duodenal P-gp in human volunteers after administration of L-T₄ (Siegmund et al., 2002), but with no significant pharmacokinetic changes for the P-gp substrate talinolol in individu-
als with P-gp increases. Since this study included only healthy individuals under the age of 30, the potential functional relevance of P-gp induction by thyroxine remains unclear. In clinical reports, higher doses of digitals (a prototypic P-gp substrate) were necessary to control the heart rate of patients with hyperthyroidism and atrial fibrillation (Frye and Braunwald, 1961), and this phenomenon was attributed in part to the lower plasma concentration of digoxin in hyperthyroid patients (O’Connor and Feely, 1987).

In addition, l-T₄ is widely prescribed, with prescriptions for Synthroid (one commercial formulation of l-T₄) alone ranking as number four among all drugs for 2002 (http://www.rxlist.com/top200.htm).

Reports indicate that 6.9% of a sample U.S. population over 58 years of age are treated with l-T₄ (Sawin et al., 1989).

P-gp recognizes many of the same substrates as CYP3A4 and responds to some of the same pharmacologic inducers (Schuetz et al., 1996; Dürre et al., 2000), but overlap is incomplete, and P-gp and CYP3A4 are not identical in anatomic localization. The presence of P-gp without appreciable CYP3A4 at sites such as the blood-brain barrier and in subsets of circulating lymphocytes supports our findings that P-gp is likely to have unique regulatory elements and responses that need to be elucidated.

Peroxisome proliferator-activated receptor and RXR activate estrogen-dependent genes by binding to the estrogen receptor response elements in the upstream enhancer region (Nunez et al., 1997). A similar cross talk mechanism was proposed for PXR/constituutive androstane receptor and VDR (Drocourt et al., 2002). Such regulatory overlap may have implications for treatment of conditions in which the function of a hormonal nuclear receptor is altered, such as the thyroid hormone resistance syndrome, linked to mutations in TR (Sakurai et al., 1989), or vitamin D-resistant rickets, in which a mutated VDR does not respond to Vit D₃ (Brooks et al., 1978). If orphan nuclear receptors in the presence of specific therapeutic agents increased the expression of genes that are normally regulated by the defective hormonal nuclear receptor, this could provide a paradigm for amelioration of specific clinical or physiologic manifestations of these conditions.

References


