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TOCOTRIENOLS ACTIVATE THE STEROID AND XENOBIOTIC RECEPTOR, SXR, AND SELECTIVELY REGULATE EXPRESSION OF ITS TARGET GENES

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ABSTRACT:

Vitamin E is an essential nutrient with antioxidant activity. Vitamin E is comprised of eight members, α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. All forms of vitamin E are initially metabolized by ω-oxidation, which is catalyzed by cytochrome P450 enzymes. The steroid and xenobiotic receptor (SXR) is a nuclear receptor that regulates drug clearance in the liver and intestine via induction of genes involved in drug and xenobiotic metabolism. We show here that all four tocotrienols specifically bind to and activate SXR, whereas tocopherols neither bind nor activate. Surprisingly, tocotrienols show tissue-specific induction of SXR target genes, particularly CYP3A4. Tocotrienols up-regulate expression of CYP3A4 but not UDP-glucuronosyltransferase 1A1 (UGT1A1) or multidrug resistance protein-1 (MDR1) in primary hepatocytes. In contrast, tocotrienols induce MDR1 and UGT1A1 but not CYP3A4 expression in intestinal LS180 cells. We found that nuclear receptor corepressor (NCoR) is expressed at relatively high levels in intestinal LS180 cells compared with primary hepatocytes. The unliganded SXR interacts with NCoR, and this interaction is only partially disrupted by tocotrienols. Expression of a dominant-negative NCoR enhanced the ability of tocotrienols to induce CYP3A4 in LS180 cells, suggesting that NCoR plays an important role in tissue-specific gene regulation by SXR. Our findings provide a molecular mechanism explaining how vitamin supplements affect the absorption and effectiveness of drugs. Knowledge of drug-nutrient interactions may help reduce the incidence of decreased drug efficacy.

The vitamin E family is comprised of eight members, α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. These compounds are characterized by a 6-chromanol ring structure and an isoprenoid side chain. Tocopherols and tocotrienols differ in their side chain in that the tocopherols have saturated phytol side chains, whereas tocotrienol side chains possess double bonds at the 3′, 7′, and 11′ positions (Kamat et al., 1997; Saito et al., 2003). α-Tocopherol is reported to be the most abundant form of vitamin E in nature and to have the highest biological activity as a vitamin in humans (Brigelius-Flohe and Traber, 1999). Tocotrienols are minor plant constituents especially abundant in palm oil, cereal grains, and rice bran that can provide a significant source of vitamin E activity (Sen et al., 2000). Compared with tocopherols, the biology of tocotrienols has been poorly studied.

Vitamin E is known to act as an antioxidant, protecting cells from potentially damaging by-products of metabolism such as free radicals (Yoshida et al., 2003). Free radicals have been linked with DNA damage and consequent induction or promotion of carcinogenesis, resistance to chemotherapy, and cardiovascular disease, to list a few. The antioxidant effects of vitamin E are thought to help prevent or delay development of cancers and chronic diseases (Karbownik et al., 2001). All eight vitamin E forms are absorbed from the intestine, transported by chylomicrons in lymph, and then incorporated into hepatic cells (Traber et al., 1992; Kayden and Traber, 1993). Unlike other fat-soluble vitamins, vitamin E does not accumulate in the liver to toxic levels. All forms of vitamin E are metabolized by degradation of the side chain via initial ω-oxidation followed by β-oxidation, resulting in the main metabolites carboxyethyl hydroxychromans and their precursors, carboxymethylbutyl hydroxychromans (Landes et al., 2003a). The initial ω-oxidation step is catalyzed by cytochrome P450 (P450) enzymes, notably CYP3A4 and CYP4F2. CYP3A4 was the first P450 suggested to be involved in vitamin E metabolism (Brigelius-Flohe, 2003). The rate of vitamin E metabolism is highest for tocotrienols and lowest for α-tocopherol, which has been suggested to

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ABBREVIATIONS: P450, cytochrome P450; SXR, steroid and xenobiotic receptor; UGT1A1, UDP-glucuronosyltransferase 1A1; MDR1, multidrug resistance protein-1; QRT-PCR, quantitative real-time polymerase chain reaction; NCoR, nuclear receptor corepressor; DN-NCoR, dominant-negative NCoR; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; EtOH, ethanol; RIF, rifampicin; RU486, mifepristone; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; SRC-1, steroid receptor coactivator-1; GRIP-1, glucocorticoid receptor interacting protein-1; ACTR, activator of thyroid and retinoic acid receptor; PBP, peroxisome proliferator-activated receptor-binding protein; SMRT, silencing mediator of retinoid and thyroid hormone; SERM, selective estrogen receptor modulator; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SR12813, 3,5-di-*tert*-butyl-4-hydroxystyrene-β,β -diphosphonic acid tetraethyl ester.

contribute to the latter's more potent biological activity (Sontag and Parker, 2002).

CYP3A4 is among the most important enzymes of the P450 family since it is responsible for the metabolism of more than 50% of clinically used drugs and a corresponding number of xenobiotic chemicals (Guengerich, 1999). Recent studies have demonstrated that the orphan nuclear receptor, SXR (Blumberg et al., 1998) [also known as pregnane X receptor (Kliewer et al., 1998), pregnane-activated receptor (Bertilsson et al., 1998), and NR1I2], plays a central role in the transcriptional regulation of CYP3A4 (reviewed in Dussault and Forman, 2002; Kliewer et al., 2002). SXR is activated by a diverse array of pharmaceutical agents including Taxol (paclitaxel), rifampicin (RIF), SR12813, clotrimazole, phenobarbital, and the herbal antidepressant St. John's wort, and peptide mimetic human immunodeficiency virus protease inhibitors such as ritonavir (reviewed in Dussault and Forman, 2002; Kliewer et al., 2002). These studies indicate that SXR functions as a xenobiotic sensor (Blumberg et al., 1998; Lehmann et al., 1998) to coordinately regulate drug clearance in the liver and intestine via induction of genes involved in drug and xenobiotic metabolism, including oxidation (phase I), conjugation (phase II), and transport (phase III) (Dussault and Forman, 2002; Kliewer et al., 2002).

We recently showed that another critical nutrient, vitamin K_2, is able to bind to and activate SXR, inducing SXR target gene expression (Tabb et al., 2003). Since vitamin E is also a substrate of CYP3A4 and is somewhat similar in structure to vitamin K_2, we hypothesized that vitamin E might be an SXR activator. We show here that all four tocotrienols specifically bind to and activate SXR, whereas tocopherols neither bind to nor activate SXR. Surprisingly, tocotrienols elicit tissue-specific induction of SXR target genes. Tocotrienols are able to up-regulate expression of CYP3A4 in primary hepatocytes, whereas they induce UDP-glucuronosyltransferase 1A1 (UGT1A1) and multidrug resistance protein-1 (MDR1), but not CYP3A4 expression, in intestinal LS180 cells. We found that nuclear receptor corepressor (NCoR) is expressed at relatively high levels in intestinal LS180 cells compared with primary hepatocytes. Unliganded SXR interacts with NCoR, and tocotrienols only weakly reverse this interaction compared with RIF. Expression of a dominant-negative NCoR, but not the nuclear receptor coactivator steroid receptor coactivator-1 (SRC-1), enhanced the ability of tocotrienols to induce CYP3A4 in LS180 cells. These results suggest that corepressor NCoR plays an important role in tissue-specific gene regulation by SXR.

Materials and Methods

Cell Culture and Reagents. The human intestinal epithelial cell line, LS180, and the hepatic cell line, HepG2, were obtained from American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. The cells were seeded into six-well plates and grown in DMEM-10% FBS until they reached 70 to 80% confluence. At 24 h before treatment, the medium was replaced with DMEM containing 10% resin-charcoal-stripped FBS. Immediately before treatment, the medium was removed; the cells were washed once with PBS and then treated with compounds or ethanol (EtOH) vehicle for the appropriate times. Human primary hepatocytes were obtained from the Liver Tissue Procurement and Distribution System (Pittsburgh, PA) as attached cells in six-well plates. The hepatocytes were maintained in hepatocyte medium (Sigma-Aldrich, St. Louis, MO) for at least 24 h before treatment. RIF, mifepristone (RU486), and clotrimazole were purchased from Sigma-Aldrich. The vitamin E compounds were purchased from Calbiochem (San Diego, CA) provided as DL-α, DL-β, DL-γ, and DL-δ-tocopherols and DL-α, DL-β, DL-γ, and DL-δ-tocotrienols.

Transient Transfection and Luciferase Assay. To test the ability of various compounds to activate SXR, HepG2 and LS180 cells were seeded into 12-well plates overnight and transiently transfected with the SXR expression plasmid, together with the pCYP3A4XREM-362/+53 Luciferase reporter (kindly provide by Dr. M. J. Vilarem, Institut Federatif de Recherche, Paris, France) (Drocourt et al., 2001) and CMX-β-galactosidase transfection control plasmids using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) in serum-free DMEM. Twenty-four hours post-transfection, the cells were treated with the α-, β-, γ-, and δ-tocopherols and -tocotrienols, EtOH as a negative control, or the known SXR ligands RIF, RU486, and clotrimazole as positive controls for 24 h. The cells were lysed 24 h after treatment, and β-galactosidase and luciferase assays were performed as described (Grun et al., 2002). Reporter gene activity was normalized to the β-galactosidase transfection controls, and the results were expressed as normalized relative light units per O.D. of β-galactosidase per minute to facilitate comparisons between plates. Fold induction was calculated relative to solvent controls. Each data point represents the average of triplicate experiments $\pm$ S.E.M. and was replicated in independent experiments. For mammalian two-hybrid assays, LS180 cells were transfected with GAL4 reporter, VP16-SXR, and GAL-NCoR or GAL-SMRT (silencing mediator of retinoid and thyroid hormone) (kindly provided by Dr. B. M. Forman, City of Hope National Medical Institute, Duarte, CA) (Synold et al., 2001). The cells were then treated with 10 μM RIF, tocopherols, or tocotrienols.

Ligand Binding Assays. N-terminal His_6-tagged human SXR ligand-binding domain was expressed in *Escherichia coli* together with the SRC-1 receptor interaction domain, and scintillation proximity assays were performed essentially as described (Tabb et al., 2003). Briefly, active protein was refolded from inclusion bodies solubilized in denaturation buffer [6 M guanidium-HCl, 50 mM HEPES, pH 7.4, 0.2 M NaCl, 25 mM dithiothreitol, 1% (w/v) Triton X-100] by rapid 10-fold dilution into binding buffer [50 mM HEPES, pH 7.4, 1 M sucrose, 0.2 M NaCl, 0.1 mM dithiothreitol, 0.1% (w/v) CHAPS] followed by dialysis overnight at 4°C against binding buffer. Binding assays were performed by coating 96-well nickel chelate FlashPlates (PerkinElmer Life and Analytical Sciences, Boston, MA) with a 10-fold molar excess of protein for 1 h at 22°C in binding buffer (50 mM Hepes, pH 7.4, 200 mM NaCl, 1 M sucrose, 0.1% CHAPS). Unbound protein was removed from the wells by washing four times with binding buffer. ^{3}H-SR12813 (Amersham Biosciences Inc., Piscataway, NJ) was added to a final concentration of 50 nM in each well, either alone or together with competitor ligands in binding buffer as indicated. Incubation was continued for 3 h at room temperature. Total counts were measured using a Topcount scintillation counter (PerkinElmer Life and Analytical Sciences). Counts remaining after the addition of 10 μM clotrimazole were taken as nonspecific background and subtracted from all wells. All assays were performed in triplicate and reproduced in independent experiments.

RNA Isolation and Quantitative Real-time Polymerase Chain Reaction (QRT-PCR) Analysis. Total RNA was isolated from LS180 cells and primary hepatocytes using TRIzol reagent (Invitrogen) according to the manufacturer-supplied protocol. For dominant-negative NCoR (DN-NCoR) transfection, LS180 cells were seeded into six-well plates overnight and transiently transfected with the DN-NCoR (Morrison et al., 2003) (kindly provided by Dr. C. K. Osborne, Baylor College of Medicine, Houston, TX) or control plasmid for 24 h. Cells were then treated with compounds or EtOH vehicle for another 24 h and total RNA was isolated. Two micrograms of total RNA were reverse-transcribed using SuperScript II reverse transcriptase according to the manufacturer-supplied protocol (Invitrogen). Quantitative real-time PCR was performed using gene-specific primers and the SYBRGreen PCR kit (Applied Biosystems, Foster City, CA) in a DNA Engine Opticon-Continuous Fluorescence Detection System (MJ Research, Watertown, MA). All samples were quantified using the comparative C_t method for relative quantification of gene expression, normalized to GAPDH (Livak and Schmittgen, 2001). The primer sets utilized in this study are shown in Table 1.

Results

Tocotrienols, But Not Tocopherols, Efficiently Activate SXR in Different Cell Lines. SXR is activated by a diverse array of pharmaceutical agents including Taxol, RIF, SR12813, clotrimazole, phenobarbital, and hyperforin. We tested the ability of vitamin E to activate SXR in transfection assays. Since SXR functions as a steroid and xenobiotic sensor primarily in liver and intestine tissue in vivo,

TABLE 1

Primer sets used for QRT-PCR

Gene	5′ Primer	3′ Primer
CYP3A4	GGCTTCATCCAATGGACTGCATAAAT	TCCCAAGTATAACACTCTACACAGACAA
UGT1A1	TGCTCATTGCCTTTTCACAG	GGGCCTAGGGTAATCCTTCA
MDR1	CCCATCATTGCAATAGCAGG	GAGCATACATATGTTCAAACTTC
GAPDH	GGCCTCCAAGGAGTAAGACC	AGGGGAGATTCAGTGTGGTG
SRC-1	CATGGTCAGGCAAAAACCTT	GCTTGCCGATTTTGGTGTAT
GRIP-1	TCTGATGGCACTCTTGTTGC	TCAGATCCGGATTCATCACA
PBP	TGCGTCAAGTCATGGAGAAG	CCACTGGCACTGAGATGAGA
ACTR	AAGGCCAGAGTGACGAAAGA	GGCATCTGTGTTGCTGAGAA
SMRT	CCTGTACCCACCCTACCTCA	GCTGCGAGGTGATGTAGTCA
NCoR	CAAGTTTCCTCGCAGACTCC	CTGGGCTGTAACGGTTTGAT

we chose human hepatic cell line HepG2 and human intestinal epithelial cell line LS180 as models. As shown in Fig. 1A, all four tocotrienols significantly activated full-length SXR in both HepG2 and LS180 cell lines at 10 μM concentration. β- and δ-Tocotrienols were somewhat more efficacious than α- and γ-tocotrienols as SXR activators. β- and δ-Tocotrienols elicited 4- to 5-fold activation in LS180 cells and 11- to 13-fold in HepG2 cells. In contrast, α- and γ-tocotrienols showed ~3-fold activation in LS180 cells and ~7-fold activation in HepG2 cells (Fig. 1A). Although stronger activation was observed in HepG2 cells, both cell lines showed the same activation profiles in response to tocotrienol treatment. Consistent with these results, activation by the control SXR ligands, clotrimazole, RIF, and RU486, ranged from 22- to 47-fold in HepG2 cells and from 5- to 18-fold in LS180 cells. None of the four tocopherols activated SXR significantly in either cell line, although a recent report showed that α-, γ-, and δ-tocopherol were able to slightly induce SXR activity (Landes et al., 2003b). Indeed, dose-response analysis in LS180 cells indicated that tocopherols failed to activate SXR at concentrations up to 100 μM, whereas tocotrienols were able to activate SXR at concentrations as low as 1 μM, reaching peak activation at 10 μM (Fig. 1B).

To further confirm that tocotrienols activated SXR, we also transfected COS-7 cells with a GAL4 reporter along with a vector expressing the SXR ligand-binding domain linked to the DNA-binding domain of GAL4 (GAL-SXR). Consistent with the results obtained using the full-length SXR, tocotrienols elicited between 6- and 8-fold activation of GAL-SXR, whereas the tocopherols had little effect (Fig. 1C). Tocotrienols also active GAL-SXR about 2-fold at 1 μM (data not shown). Therefore, we conclude that tocotrienols but not tocopherols efficiently activate SXR.

Tocotrienols Specifically Bind to SXR in Vitro. Since tocotrienols activated SXR in transient transfections (Fig. 1), we next sought to determine whether tocotrienols bind to purified SXR protein in vitro, using a sensitive scintillation proximity ligand-binding assay (Tabb et al., 2003). This assay used the high-affinity SXR ligand ^{3}H-SR12813 and recombinant histidine-6-tagged-SXR coexpressed with the SRC-1 receptor interacting domain. SR12813 interacts specifically with SXR with a dissociation constant of 40 nM (Jones et al., 2000). As seen in Fig. 2, β-tocotrienol, as well as clotrimazole (positive control), was able to displace ^{3}H-SR12813 from the SXR ligand-binding domain in a dose-dependent manner, whereas the β-tocopherol did not compete for receptor binding, consistent with our transfection results. α-, γ-, and δ-Tocotrienols were also able to specifically bind to SXR, but none of the tocopherols could effectively bind to SXR (Table 2). The K_i for tocotrienols binding to SXR was determined to be ~5 to 8 μM, a value in the range of other known SXR ligands (Tabb et al., 2004), and the rank order binding affinity was similar to the transfection results: β- and δ-tocotrienol were slightly more effective than α- and γ-tocotrienol. We infer from these results that tocotrienols (but not tocopherols) specifically bind to and activate SXR and conclude that tocotrienols are bona fide ligands for SXR.

Tocotrienols Selectively Regulate SXR Target Gene-CYP3A4 Expression. SXR is a master regulator of xenobiotic metabolism and regulates genes involved in the oxidation (phase I), conjugation (phase II), and transport (phase III) of xenobiotics (reviewed in Dussault and Forman, 2002; Kliewer et al., 2002). This prompted us to test the effect of tocotrienols on SXR target gene expression in human primary hepatocytes and intestinal LS180 cells (Synold et al., 2001; Tabb et al., 2004). Both cell lines were treated for 24 h with the solvent control, 10 μM positive control (clotrimazole, RIF, or RU486), or various vitamin E compounds. Total RNA was isolated and QRT-PCR was performed to detect the expression levels of SXR target genes involved in phase I (CYP3A4), phase II (UGT1A1) and phase III (MDR1) metabolism.

As shown in Fig. 3A, in primary human hepatocytes, the four tocotrienols induced the expression of mRNA encoding the phase I SXR target gene CYP3A4 3- to 5-fold compared with solvent controls, whereas tocopherols did not. No significant induction was observed in either the phase II UGT1A1 or phase III MDR1 gene expression after treatment with tocotrienols or RIF. In contrast, tocotrienols were able to induce the expression of the phase II gene, UGT1A1 (1.5- to 2-fold except γ-tocotrienol) and the phase III gene, MDR1 (2- to 4-fold) in the intestinal LS180 cells, whereas they did not induce the expression of CYP3A4 (Fig. 3B). Clotrimazole, RIF, and RU486 were able to induce all three SXR target genes, suggesting that tocotrienols are cell-type specific SXR activators. In liver cells, tocotrienols activate SXR and induce CYP3A4 expression but have no effect on UGT1A1 and MDR1 expression. Tocotrienols induce UGT1A1 and MDR1 gene expression in intestinal cells but have no effect on CYP3A4 expression. Thus, tocotrienols provide the first example of a novel class of SXR ligands that we refer to as gene-selective SXR modulators.

NCoR Is Expressed at High Levels in LS180 Cells and Overexpression of SRC-1 Does Not Enhance the Ability of Tocotrienols to Induce CYP3A4. Nuclear receptor coregulators play critical roles in nuclear receptor activation and are also involved in the mechanisms underlying the divergent activities of selective estrogen receptor modulators (SERMs) (Glass and Rosenfeld, 2000; McDonnell et al., 2002). Several coregulators have been shown to be important for SXR activation, including the coactivators SRC-1, glucocorticoid receptor interacting protein-1 (GRIP-1), activator of thyroid and retinoic acid receptor (ACTR), and peroxisome proliferator-activated receptor-binding protein (PBP), and the corepressors NCoR and SMRT (Synold et al., 2001; Dussault and Forman, 2002; Tabb et al., 2003). Therefore, it was of interest to determine whether coregulators have any effect on the selective regulation of SXR target genes by tocotrienols in different tissues.

We first evaluated the expression levels of mRNAs encoding these

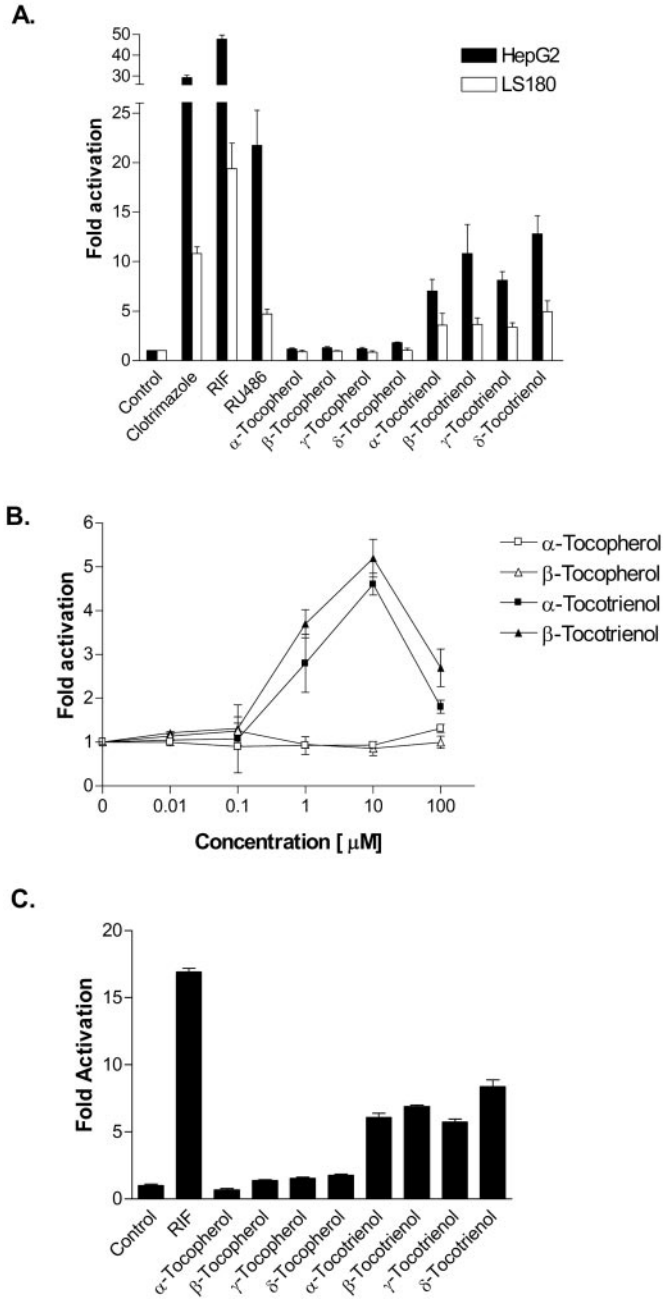


FIG. 1. SXR is activated by tocotrienols but not tocopherols. A, LS180 and HepG2 cells were transiently transfected with full-length SXR together with an SXRE-luc reporter and a CMX-β-galactosidase transfection control plasmid. After transfection, cells were treated with control medium or medium containing 10 μM clotrimazole, RIF, RU486, or the various vitamin E compounds for 24 h. B, LS180 cells were transiently transfected as described above. Cells were treated with the indicated concentrations of tocopherols or tocotrienols for 24 h. C, COS-7 cells were transiently transfected with GAL4-SXR, a GAL4 reporter fused to luciferase and CMX-β-galactosidase transfection control plasmid. Cells were then treated with 10 μM concentrations of the indicated ligands for 24 h. Data are shown as fold induction of normalized luciferase activity relative to solvent controls and represent the mean of triplicate experiments. Error bars indicate S.E.

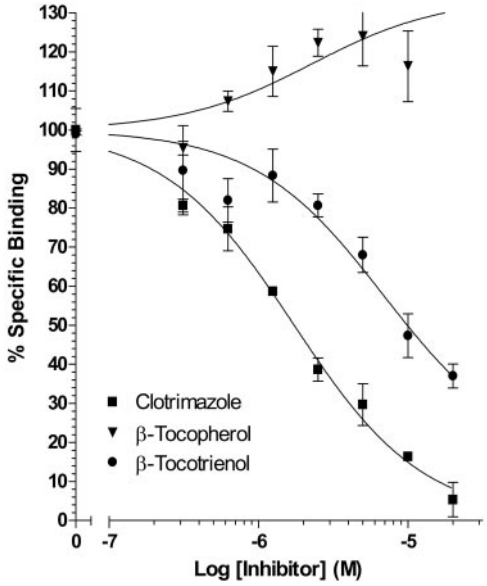


FIG. 2. β-Tocotrienol but not β-tocopherol specifically binds to the purified SXR ligand-binding domain. His_6-SXR ligand-binding domain was coexpressed with the SRC-1 receptor interaction domain and purified. The receptor complex was bound to nickel chelate FlashPlates and incubated with 50 nM ^{3}H-SR12813 in the presence of the indicated concentration of β-tocotrienol, β-tocopherol, or clotrimazole. Values represent the average of triplicates ± S.E. and were replicated in independent experiments.

TABLE 2

SXR binding constants for tocopherols and tocotrienols

K_i and ±95% confidence interval (95% CI) values were determined by nonlinear regression using the Cheng-Prussoff equation from eight-point curves performed in triplicate; for competitive ligands, the $K_i = K_d$. Assays were performed at constant ^{3}H-SR12813 concentration [50 nM; K_d = 40 nM (Jones et al., 2000)]. Total specific binding was determined as the counts displaced by 10 μM clotrimazole.

Compound	K_i	95% CI
	μM	
α-Tocopherol	NB	NB
β-Tocopherol	NB	NB
γ-Tocopherol	NB	NB
δ-Tocopherol	NB	NB
α-Tocotrienol	8.0	5.2–12.0
β-Tocotrienol	6.4	4.6–9.0
γ-Tocotrienol	7.1	3.9–13.0
δ-Tocotrienol	4.9	2.5–9.6
Clotrimazole	0.79	0.62–1.0

NB, no specific binding.

four coactivators (SRC-1, GRIP-1, ACTR, and PBP) and two corepressors (NCoR and SMRT) in primary hepatocytes and intestinal LS180 cells. As shown in Fig. 4A, all of the four coactivators were expressed at relatively high levels in primary hepatocytes from two different donors compared with LS180 cells. Of the corepressors, SMRT was expressed at very low levels in both primary hepatocytes and LS180 cells; however, expression levels of NCoR were relatively high in both cell types. Therefore, LS180 cells have a much lower coactivator/corepressor ratio compared with primary hepatocytes. This may explain why stronger activation was observed in hepatic cells in transfection assays and also suggests a possible mechanism for tocotrienols selectively regulating SXR target genes in different tissues. We then tested whether overexpression of coactivator enhances the ability of tocotrienols to induce CYP3A4 gene expression by modulating the coactivator/corepressor ratio. LS180 cells were transfected with SRC-1 expression plasmid or control plasmid, and then treated with 10 μM RIF or tocotrienols for 24 h. Overexpression of SRC-1 did not enhance the ability of tocotrienols to induce CYP3A4, although RIF-induced CYP3A4 mRNA expression was slightly enhanced (Fig. 4B).

SXR Interacts with Both NCoR and SMRT in LS180 Cells and Expression of Dominant-Negative NCoR Enhances the Ability of Tocotrienols to Induce CYP3A4. Previous reports have shown that SXR can interact with both NCoR and SMRT in CV-1 cells and can

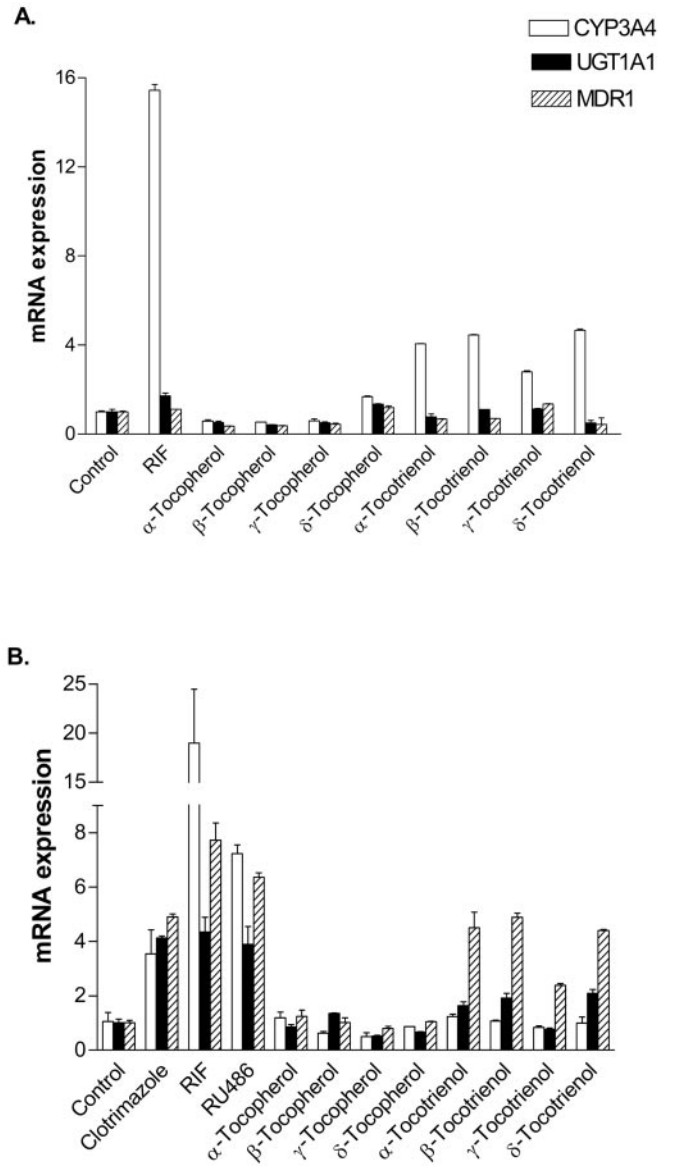


FIG. 3. Tocotrienols selectively regulate SXR target gene expression. Human primary hepatocytes (A) or LS180 intestinal epithelial cells (B) were treated with control medium or medium containing 10 μM RIF or the various vitamin E compounds. Total RNA was isolated and gene expression levels were analyzed by QRT-PCR with primers for human CYP3A4, UGT1A1, or MDR1. All values were normalized to GAPDH control.

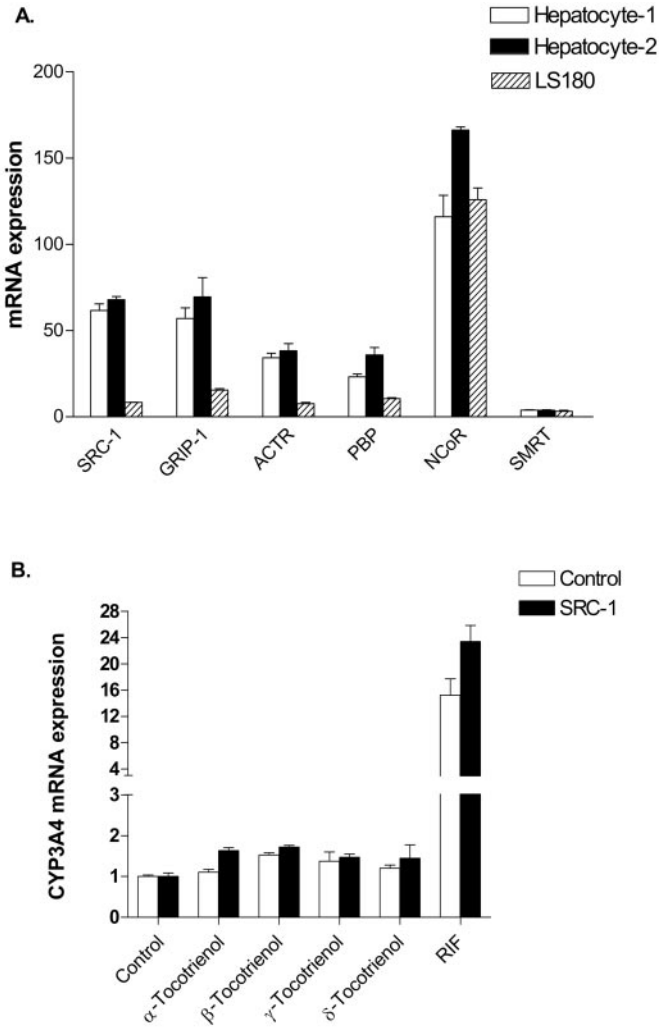
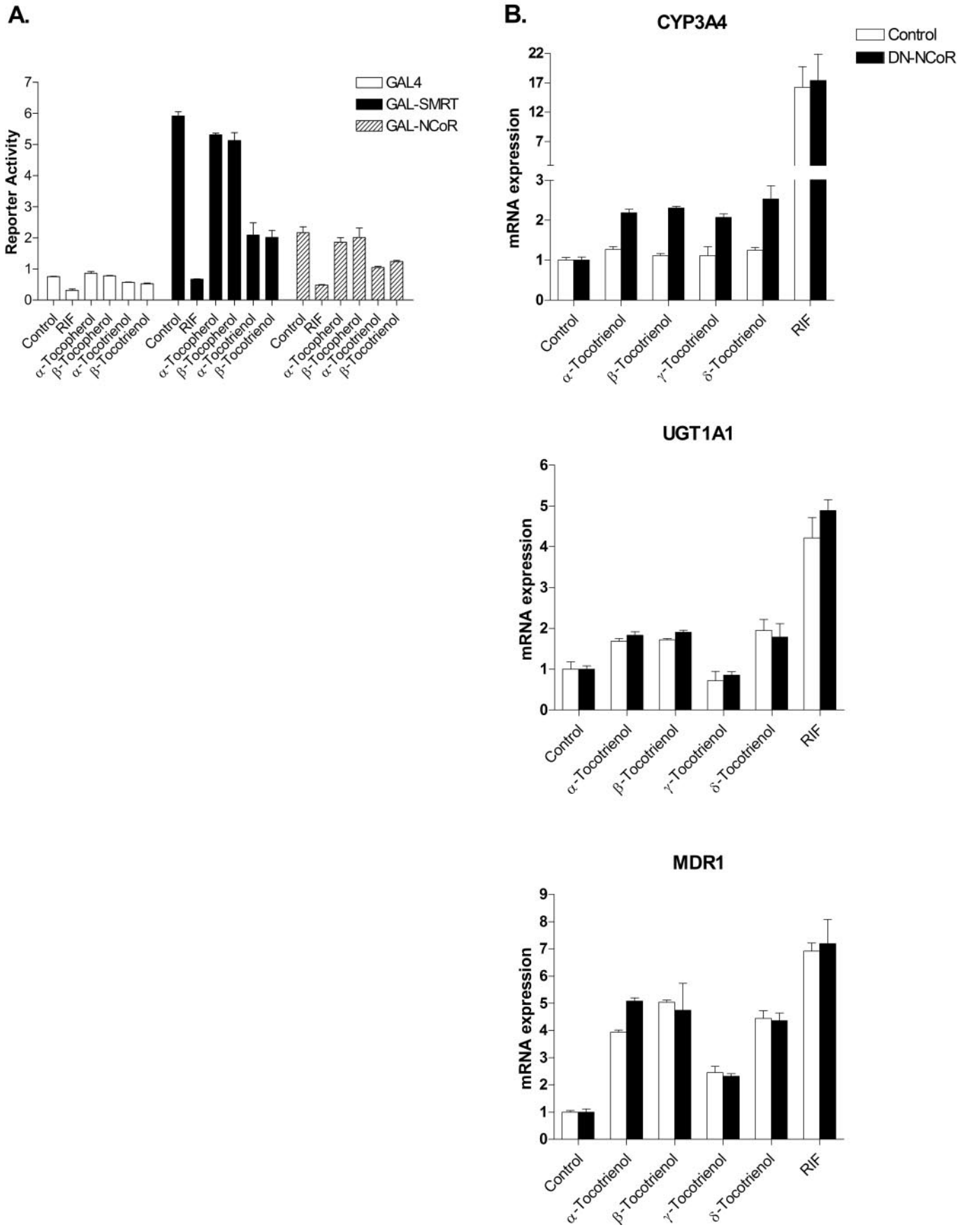


FIG. 4. Corepressor NCoR is expressed at relatively high levels in LS180 cells, and expression of coactivator SRC-1 does not alter the ability of tocotrienols to induce CYP3A4. A, total RNA was isolated from human primary hepatocytes (two different donors) and LS180 cells. QRT-PCR was utilized to analyze the gene expression levels for SXR coactivators (SRC-1, GRIP-1, ACTR, and PBP) and corepressors (NCoR and SMRT). All values were normalized to GAPDH control. B, LS180 cells were transfected with either control plasmid or SRC-1 expression plasmid. Cells were treated with control medium or medium containing 10 μM RIF or α-, β-, γ-, or δ-tocotrienol for 24 h. Total RNA was isolated and CYP3A4 gene expression levels were analyzed by QRT-PCR.

control differential responsiveness of paclitaxel and docetaxel. In addition, SXR has a preferred interaction with SMRT over NCoR in HepG2 cells (Synold et al., 2001). Since NCoR is expressed at much higher levels than SMRT in LS180 cells, we used the mammalian two-hybrid assay to evaluate the SXR/corepressor interaction in this cell line. We transiently transfected LS180 cells with a GAL4 reporter, a vector expressing VP16-SXR, and an expression vector for the GAL4 DNA-binding domain or the GAL4 DNA-binding domain linked to the receptor interaction domains of the indicated corepressors. Consistent with previous reports, unliganded SXR is able to interact with both NCoR and SMRT, although the SXR-NCoR interaction is weaker (Fig. 5A). As expected, 10 μM RIF was able to completely reverse the SXR-corepressor interactions. Compared with RIF, α- and β-tocotrienols only weakly reversed these interactions, whereas tocopherols had no effect at all.

NCoR has been shown to be important for the varying effects of SERMs in different tissues (McDonnell et al., 2002). NCoR is expressed at much higher levels than SMRT in LS180 cells; therefore, we used a DN-NCoR to further examine the effects of NCoR on CYP3A4 expression in LS180 cells. DN-NCoR has been successfully used to inhibit endogenous NCoR activity and to relieve repression conferred by other nuclear receptors such as the retinoid acid receptor (Soderstrom et al., 1997; Morrison et al., 2003). LS180 cells were transfected with DN-NCoR or control plasmid and then treated with 10 μM RIF or 10 μM tocotrienols for 24 h. Intriguingly, CYP3A4 mRNA expression could be induced 2- to 3-fold by tocotrienols in DN-NCoR-transfected LS180 cells but not in the control plasmid-transfected cells (Fig. 5B). In contrast, RIF-induced CYP3A4 gene expression remained the same in controls or with DN-NCoR transfection. This result may be explained by RIF's strong ability to displace NCoR. Transfection of DN-NCoR did not further induce UGT1A1 or MDR1 gene expression, which may be due to different promoter context. Therefore, inhibition of endogenous NCoR in


A.
Reporter Activity
GAL4
GAL-SMRT
GAL-NCoR
Control
RIF
α-Tocopherol
β-Tocopherol
α-Tocotrienol
β-Tocotrienol
B.
CYP3A4
Control
DN-NCoR
mRNA expression
γ-Tocotrienol
δ-Tocotrienol
UGT1A1
MDR1

LS180 cells enhances the ability of tocotrienols to induce CYP3A4 expression.

Discussion

All forms of vitamin E are initially metabolized by P450-catalyzed ω-oxidation. CYP3A4 is important for oxidizing γ-tocopherols, and α- and γ-tocotrienol, whereas CYP4F2 has the highest activity for γ-tocopherol (Sontag and Parker, 2002). α-Tocopherol is metabolized at a very low rate, which has been suggested to underlie its more potent biological activity (Sontag and Parker, 2002). Here we show that α-, β-, γ-, and δ-tocotrienols, but not the corresponding tocopherols, specifically bind to and activate the nuclear receptor SXR. Tocotrienols also selectively regulate the SXR target gene CYP3A4 in hepatic and intestinal cell lines. These findings confirm and extend previous reports suggesting that some forms of vitamin E can activate SXR (Landes et al., 2003b). Therefore, tocotrienols may not only induce their own metabolism but may also alter the metabolism of other CYP3A4 substrates such as steroids, drugs, xenobiotic chemicals, and bioactive dietary compounds.

SXR is expressed at high levels in the liver and intestine where it acts as a xenobiotic sensor that regulates the expression of cytochrome P450 enzymes such as CYP3A4 and CYP2C8, conjugation enzymes such as UGT1A1, and ATP-binding cassette family transporters such as MDR1 and multidrug resistance protein 2 (Kliewer et al., 2002). SXR is thus a master regulator of xenobiotic clearance, coordinately controlling steroid and xenobiotic metabolism (Xie and Evans, 2001; Willson and Kliewer, 2002). In a previous report, Landes et al. (2003b) tested some forms of vitamin E and showed that α- and γ-tocotrienols could strongly activate SXR. α-, γ-, and δ-Tocopherols were also able to induce SXR activity (Landes et al., 2003b). In addition, it was shown that α- and γ-tocotrienols were even more effective SXR activators than RIF. We tested all eight forms of vitamin E and found that only tocotrienols could bind to and activate SXR, whereas none of the tocopherols could activate SXR or induce expression of its target genes (Figs. 1 and 2). Although tocotrienols are much weaker activators of SXR than of RIF, they act as selective modulators of the CYP3A4 gene (Fig. 3). Tocotrienols are able to induce CYP3A4 in hepatocytes but not in intestinal cells. Therefore, tocotrienols are a new class of SXR ligands that are able to selectively regulate expression of its target gene-CYP3A4 in different tissues.

The divergent activities of SERMs have been studied for over two decades and the molecular mechanisms are still not completely understood. At least three factors have been proposed to contribute in combination to the divergent activities of SERMs: the conformation of the ligand-receptor complex, the promoter context, and the relative levels of expression of coactivators and corepressors (McDonnell et al., 2002; Dussault et al., 2003). Nuclear receptor coregulators play critical roles in the mechanism of tissue-specific effects of these compounds. We investigated the expression of known SXR coregulators in two different cell lines, human primary hepatocytes and LS180 intestinal cells. The corepressor NCoR is expressed at similarly high levels in both cell types, but LS180 cells have a much lower coactivator/corepressor ratio compared with primary hepatocytes (Fig. 4). This may explain the stronger activation we observed in hepatic cells in our transfection assays (Fig. 1A). However, altering the coactivator/corepressor ratio by SRC-1 overexpression failed to enhance the ability of tocotrienols to induce CYP3A4 (Fig. 4B). Therefore, the different coactivator/corepressor ratios in hepatic versus intestinal cell lines cannot solely account for the tissue-selective activation of CYP3A4 by SXR that we observe.

Previous reports suggested that corepressor NCoR controls the differential responsiveness of paclitaxel and docetaxel for SXR (Synold et al., 2001**)**. Both paclitaxel and docetaxel can recruit coactivators to SXR, but only paclitaxel is able to displace corepressors. This results in paclitaxel but not docetaxel activation of SXR and subsequent expression of its target genes. We evaluated the SXR/corepressor interaction in LS180 cells using mammalian two-hybrid assays and found that unliganded SXR is able to interact with both NCoR and SMRT. Treatment with 10 μM RIF was able to completely reverse both SXR/corepressor interactions (Fig. 5A). Compared with RIF, however, tocotrienols only weakly reversed these interactions, whereas tocopherols had no effect at all. This result may explain why overexpression of coactivators could not restore tocotrienol's ability to induce CYP3A4 gene in LS180 cells.

Since NCoR has been shown to be important for the varying effects of SERMs in different tissues and is expressed at relatively high levels in LS180 cells, we inhibited the endogenous NCoR by transfection of a DN-NCoR into this cell line. Transfection of DN-NCoR resulted in enhanced induction of CYP3A4 expression by tocotrienols. However, transfection of DN-NCoR did not further induce UGT1A1 and MDR1 gene expression in response to tocotrienols. This may be due to the different promoter contexts of UGT1A1 and MDR1 compared with CYP3A4. These results suggest that SXR coregulators, especially NCoR, play important roles in determining tissue-specific effects of selective modulators of SXR such as tocotrienols. The ability of tocotrienols to regulate SXR target genes in a tissue-specific manner suggests that future development of compounds that selectively activate SXR target genes in certain tissues will be possible.

Drug-drug interactions are a common problem in medical practice, but drug-nutrient interactions are less widely considered when prescribing medications. However, dietary supplements have the potential for potent adverse effects. For example, St. John's wort, a widely used herbal antidepressant, is able to interact with a variety of drugs. Hyperforin, the active constituent of St. John's wort, can induce drug metabolism through activation of SXR, which then regulates expression of the key xenobiotic-metabolizing enzymes such as CYP3A4 (Moore et al., 2000; Wentworth et al., 2000). This provides a molecular mechanism for the interaction of St. John's wort with various drugs. Although drug-nutrient interactions are not as common as drug-drug interactions, there is evidence to suggest that vitamin supplements can affect the absorption and effectiveness of drugs (Trovato et al., 1991). Vitamin E is taken daily by more than 35 million people in the U.S. (Traber, 2004). People also consume vitamin E in foods such as vegetable oils, nuts, green leafy vegetables, and fortified cereals. Tocopherols are found in the germ of cereal seeds and in polyunsaturated vegetable oils, whereas tocotrienols are found in the aleurone and subaleurone layers of cereal seeds and in palm oils (Yoshida et al., 2003). The components of over-the-counter vitamin E supplements vary based on the brand. Intriguingly, the so-called natural vitamin E supplements that contain mixed tocopherols and

FIG. 5. SXR interacts with both NCoR and SMRT in LS180 cells and expression of dominant-negative NCoR enhances the ability of tocotrienols to induce CYP3A4. A, LS180 cells were transfected with a GAL4 reporter and VP16-SXR as well as expression vector for GAL4 DNA-binding domain or GAL4 DNA-binding domain linked to the receptor interaction domains of the indicated SXR corepressors (GAL-SMRT or GAL-NCoR). Cells were then treated with control medium or medium containing 10 μM RIF, or α- or β-tocopherol or -tocotrienol. B, LS180 cells were transfected with either control plasmid or DN-NCoR expression plasmid. Cells were treated with control medium or medium containing 10 μM RIF, or α-, β-, γ-, or δ-tocotrienol for 24 h. Total RNA was isolated and SXR target gene expression levels were analyzed by QRT-PCR.

tocotrienols have the potential for altering drug and xenobiotic metabolism through SXR, whereas synthetic α-tocopherols would not be expected to cause such effects.

Our data suggest that tocotrienols can bind to and activate SXR and induce SXR target gene expression at low micromolar concentrations. Pharmacokinetic studies show that the plasma concentration of tocotrienols can exceed 2000 ng/ml (>5 μM) after oral administration of a single dose of 300 mg of mixed tocotrienols (Yap et al., 2001). This concentration may be even higher in the liver. This would be expected to lead to SXR activation in vivo and, therefore, to increase CYP3A4 expression in the liver and UGT1A1 and MDR1 expression in the intestine. It has been demonstrated that the vitamin E isoforms are all equally well absorbed from the intestine, transported in the lymph, and incorporated into hepatic cells (Saito et al., 2003). This leads to the inference that ingestion of foods or dietary supplements containing high levels of tocotrienols could lead to accelerated metabolism and consequent reduced efficacy of prescription drugs. Therefore, it may not be in the best interest of patients on medications necessary for disease or symptomatic treatment to supplement their diet with high doses of tocotrienol-containing vitamin E. Our findings agree with previous research that several natural foods, herbs, and beverages are able to induce drug metabolism (Sorensen, 2002). The metabolism of tocotrienols still remains unclear in vivo, and other groups showed that tocotrienols could not accumulate in the liver in the rat fed with tocotrienol-rich diets (Ikeda et al., 2001). Therefore, future experiments to examine differences in the metabolism of tocotrienols versus tocopherols in vivo and their potential for drug-nutrient interactions via SXR in vivo may help to decrease the incidence of reduced drug efficacy.

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