Regulation of Hepatic Cholesteryl Ester Transfer Protein Expression and Reverse Cholesterol Transport by Inhibition of DNA Topoisomerase II*

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Background: CETP expression mediates cholesterol metabolism and the development of atherosclerosis.

Results: Inhibition of Topo II activates CETP expression in HepG2 cells and CETP transgenic mouse liver, which was associated with increased reverse cholesterol transport in vivo.

Conclusion: Topo II inhibitors induced CETP expression and RCT by activating LXR pathway.

Significance: We found an important function of Topo II inhibition in regulating cholesterol metabolism.

Cholesteryl ester transfer protein (CETP) transfers cholesteryl esters from high density lipoprotein to triglyceride-rich lipoproteins. CETP expression can be transcriptionally activated by liver X receptor (LXR). Etoposide and teniposide are DNA topoisomerase II inhibitors. Etoposide and teniposide, such as low density lipoprotein (LDL) and very low density lipoprotein, in exchange for triglycerides (TG), thereby exerting a key role in cholesterol metabolism (1). Although the homozygotes with CETP deficiency in humans demonstrate elevated HDL-cholesterol (HDL-C) levels, some of the patients still suffer from increased risk of coronary heart disease (2). The clinical trial evaluations indicate that CETP inhibitors can raise serum HDL-C levels but not reduce the risk of coronary heart disease (1). Mice do not express CETP naturally. However, studies with CETP transgenic mice suggest that CETP expression may increase a direct removal of liver HDL cholesteryl esters in the manners that are independent of the established lipoprotein receptors (3). Therefore, CETP may contribute to reverse cholesterol transport (RCT) in humans through a direct hepatic selective CE uptake or indirect transfer of HDL-CE to apoB-containing lipoproteins and subsequent receptor-mediated liver uptake.

CETP is predominantly expressed by the liver and secreted into plasma where CETP is mainly associated with HDL and apoB-containing lipoproteins. CETP expression can be transcriptionally activated by liver X receptors (LXR) α and β, the ligand-activated transcription factors, as there is an LXR-responsive element (LXRE) in the CETP promoter (4).

Activation of LXR leads to formation of a heterodimer of LXR with another transcription factor, retinoid X receptor (RXR). The heterodimer of LXR/RXR can bind to LXRE in the promoter of target genes to initiate their transcription (5). LXR activates expression of macrophage ATP binding cassette transporters.

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* The abbreviations used are: CETP, cholesteryl ester transfer protein; LXR, liver X receptor; HDL-C, HDL-cholesterol; LXRE, LXR-responsive element; ABCA1, ATP binding cassette transporter A1; RCT, reverse cholesterol transport; Topo, topoisomerase; MTTP, microsomal triglyceride transfer protein; TRITC, tetramethylrhodamine isothiocyanate; RIP140, receptor interacting protein 140; Rb, retinoblastoma; pRb, phosphorylated Rb; TG, triglyceride; RXR, retinoid X receptor.
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Macrophages isolated from untreated wild type or CETPtg mice in suspension were radiolabeled in serum-free RPMI 1640 medium containing 50 μg/ml acetylated LDL and 2 μCi/ml [3H]cholesterol for 24 h, washed twice, equilibrated in RPMI 1640 medium containing 0.2% BSA for 4 h, spun down, and resuspended in serum-free RPMI 1640 medium before immediate injection. Wild type or CETPtg mice receiving teniposide or solvent treatment were then intraperitoneally injected with the radiolabeled macrophages (~2 × 10^6 cells/mouse containing 1.2 × 10^6 cpm) of the corresponding type mice and transferred into metabolic chambers. Feces from individual mouse were collected at 24, 32, 40, and 48 h after the cell injection. At the end of the experiment (48 h after the cell injection), the mice were anesthetized and euthanized in a CO2 chamber followed by collection of liver (for CETPtg mice only) and blood (for both wild type and CETPtg mice) samples. Serum was isolated from blood and used to determine lipid profiles. CETP expression in the liver of CETPtg mouse was determined by real time RT-PCR, Western blot, and immunohistochemical staining.

Inhibition of LXRα/β and Topo II A Expression in HepG2 Cells by siRNA—siRNA against LXRα, LXRβ, and Topo II A and scrambled siRNA were purchased from Santa Cruz Biotechnology (Dallas, Texas). HepG2 cells were transfected with scrambled or target siRNA using Lipofectamine 2000 in minimum Eagle’s medium. After 6 h of transfection, the cells were added with same volume of the medium and continued transfection for 24 h. The transfected cells were then switched into complete medium and cultured for 24 h followed by treatment in serum-free medium.

Preparation of Plasmid DNA and Determination of CETP Promoter Activity—The human CETP promoter (from −834 to +28, pCETP) was constructed by PCR with human genomic DNA and the following primers: forward, 5'-GCCGCTCGAG-
The sequences of primers for real time RT-PCR analysis

| Gene    | Sense                               | Anti-sense                                |
|---------|-------------------------------------|-------------------------------------------|
| CETP    | 5'-CTGCTCCTGCTGGCTCATT-3'           | 5'-GCAATGGTCCGACCTAC-3'                   |
| LXRα    | 5'-GAAGAAGCTGAGCCGACAGAA-3'         | 5'-ACTGGAGCGCTGCGAAAG-3'                  |
| LXRβ    | 5'-CAGCTGCTGCTGGCTCATT-3'           | 5'-AGATTTTGAACGGAGCA-3'                   |
| RIP140  | 5'-TCTCTCCTCCTCCTCCTGCT-3'          | 5'-GAGCTCATTGACGAGCAAC-3'                 |
| Topo IIα| 5'-CTAAGTTGTACGCTGGAGACA-3'         | 5'-CATTTGACGACCTGCT-3'                    |
| p21     | 5'-ACGTGACCTTGGGGTATGACCCC-3'       | 5'-CATCTGCTTGGGGTATGACCC-3'               |
| RipA    | 5'-TGTGAGCAATGCAATCAGAAA-3'         | 5'-TCAGGGTTGCTCTCCTGCT-3'                 |
| E2F1    | 5'-GACCTGCTGCTGGCTCATT-3'           | 5'-GAGCCAGTACGGTTCA-3'                    |
| MTTTP   | 5'-CTGAGGCTCCTATGACGTT-3'           | 5'-TGATTCCTAAATGACGTTG-3'                 |
| GAPDH   | 5'-GOTGCTGCTGCTGAGCTCTCAACA-3'      | 5'-GTTGCTGCTGAGCTCTCAACATTTGCTT-3'       |

The nuclear proteins were used to determine LXRα/β protein by Western blot or LXRα/β DNA binding activity by ChIP assay as described (21) with anti-LXRα, LXRβ, or SREBP1 (used as a negative control) polyclonal antibody. The primers for ChIP assays were: LXRE (from −384 to −399), 5'-ATCACAGCAAGGGAAAGTCC-3' (forward) and 5'-GACACATTTTTGCCCATCCCT-3' (reverse); SRE (sterol responsive element, from −191 to −207), 5'-GGCGCAAGTATTCGTCGTGG-3' (forward) and 5'-GAGATTCCACCTCTCCTGCG-3' (reverse).

**Immunofluorescent Staining**—After treatment, HepG2 cells on coverslips in a 24-well plate were washed twice with PBS and then fixed with 4% paraformaldehyde (400 μl/well) for 30 min at room temperature. The fixed cells were incubated in 0.5% Triton X-100, PBS for 10 min and then blocked with 2% BSA, PBS for 2 h at room temperature followed by incubation with anti-LXR or CETP rabbit polyclonal antibody overnight at 4 °C. After the primary antibody was removed, the cells were incubated with a TRITC-conjugated goat anti-rabbit IgG for 2 h at room temperature. After washing with PBS, the sections were stained with a DAPI solution for nuclei. Images of the sections were obtained with a fluorescence microscope (Leica).

**Data Analysis**—All experiments were repeated at least three times, and the representative results are presented. The data are presented as the mean ± S.D. and were analyzed by Student’s *t* test using Prism (GraphPad Software). The differences were considered significant if *p* < 0.05.

**Results**

**Etoposide and Teniposide Induce CETP Expression and Secretion from HepG2 Cells**—To test if Topo II inhibitors can influence CETP expression in hepatocytes, HepG2 cells were treated with etoposide or teniposide at different concentrations for 16 h followed by determination of CETP protein expression. Fig. 1A demonstrates that both etoposide and teniposide increased CETP expression in a semi-concentration-dependent manner. The maximal induction of CETP expression was observed with 5 μM etoposide and 500 nM teniposide. The time course study demonstrates that induction occurred quickly after treatment (Fig. 1B) with the maximum at 16 h after treatment. Associated with increased CETP protein expression, both etoposide and teniposide induced CETP mRNA expression in a concentration- and a time-dependent manner (Fig. 1, C and D), respectively. The induction of CETP expression by Topo II inhibitors was further confirmed by immunofluorescent staining (Fig. 1E), which shows that etoposide or tenipo-
side increased HepG2 CETP expression in a concentration-dependent manner.

CETP is mainly produced by hepatocytes followed by secretion into circulation system. To determine if the induction of CETP expression by Topo II inhibitors can result in increased CETP secretion, HepG2 cells were treated with 0.5 \( \mu \)M teniposide. The treatment medium was collected at different time points, concentrated, and normalized by cellular protein content before determination of secreted CETP protein by Western blot. Fig. 1F demonstrates that teniposide increased CETP protein levels in the treatment medium in a time-dependent manner, indicating that the CETP secretion is enhanced. Taken together, the results in Fig. 1 demonstrate that treatment of HepG2 cells with Topo II inhibitors activates CETP expression and secretion.

**FIGURE 1. Etoposide and teniposide induce CETP expression and secretion from HepG2 cells.** A–D, HepG2 cells received the following treatment, and expression of CETP protein or mRNA was determined by Western blot or real time RT-PCR. A, etoposide or teniposide at the indicated concentrations for 16 h. B, etoposide (5 \( \mu \)M) or teniposide (500 nM) for the indicated times. C, etoposide or teniposide at the indicated concentrations for 12 h. D, etoposide (10 \( \mu \)M) or teniposide (100 nM) for the indicated times. *, versus control, \( p < 0.05 \) (n = 3). E, after the indicated treatment for 16 h, CETP protein expression was determined by immunofluorescent staining. F, HepG2 cells were treated with teniposide (0.5 \( \mu \)M) for the indicated times. The treatment medium was collected, concentrated, and normalized by cellular protein content before the secreted CETP protein was determined by Western blot.

Etoposide and Teniposide Activate CETP Transcription in an LXR-dependent Manner—CETP is a transcriptional target of LXR. Activation of RXR by retinoic acid can coordinate LXR to induce target gene expression. To determine the role of RXR in Topo II inhibitor-induced CETP expression, HepG2 cells were treated with retinoic acid or plus etoposide/teniposide. Fig. 2A and B, demonstrates that retinoic acid alone activated CETP mRNA expression but had little effect on CETP protein expression. However, retinoic acid clearly further increased etoposide- and teniposide-induced CETP mRNA and protein expression.

To determine if the induction of CETP expression occurs at the transcriptional level, we constructed a CETP promoter (pCETP) that included the LXRE and determined the promoter activity in response to etoposide or teniposide treatment. Fig. 2C shows that both etoposide and teniposide increased pCETP activity in a concentration-dependent manner.

The role of LXR in Topo II inhibitor-induced CETP transcription was determined by the following experiments. 293T cells were transfected with a fixed concentration of LXR\( \alpha \) or LXR\( \beta \) expression vector plus pCETP followed by treatment with etoposide or teniposide. Fig. 2D indicates that high expressing LXR\( \alpha \) or LXR\( \beta \) increased pCETP activity, and the induction was further enhanced by etoposide and teniposide. Reciprocally, etoposide or teniposide further enhanced the high expressing LXR\( \alpha \)- or LXR\( \beta \) induced-pCETP activity (Fig. 2E). High expressing RXR also increased pCETP activity, which was further enhanced by high expressing LXR and RXR ligand (retinoic acid) and etoposide/teniposide (Fig. 2F).
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Treatment of the 3xLXRE-TK-Luc plasmid (Fig. 2G), which included a three tandem of LXRE in the CETP promoter, with etoposide and teniposide increased its activity (left panel, Fig. 2H). In the presence of high expressing LXRα, the induction of 3xLXRE-TK-Luc promoter activity was enhanced by etoposide and teniposide (right panel, Fig. 2H), which suggests that Topo...
II inhibitors can directly target LXRE motif. For further determination, we conducted a ChIP assay to assess the binding activity of LXR protein to the LXRE in CETP promoter in response to Topo II inhibitor treatment. Fig. 2, I and J, demonstrated that the binding of LXRα and LXRβ with LXRE was increased by etoposide and teniposide, respectively.

Selective inhibition of LXR expression can determine if the induction of CETP expression by Topo II inhibitor is directly through LXR. HepG2 cells were transfected with scrambled siRNA or a mixture of LXRα and LXRβ (LXRα/β) siRNA followed by treatment with etoposide and teniposide. Fig. 3, A and B, shows that both LXRα and LXRβ mRNA expression were substantially reduced by LXRα/β siRNA. Treatment of the scrambled siRNA-transfected cells with etoposide or teniposide increased expression of LXRα mRNA but not LXRβ mRNA (Fig. 3, A and B). Similar changes of LXRα and LXRβ protein expression were caused by etoposide and teniposide (Fig. 3D). Associated with induction of LXRα expression in the scrambled siRNA-transfected cells, expression of CETP mRNA and protein was similarly induced (Fig. 3, C and D). In contrast, etoposide and teniposide were not able to influence LXR (Fig. 3, A and B) and CETP (Fig. 3C) expression in the LXR siRNA-transfected cells, indicating that the existence of LXR is critical for induction of CETP expression by Topo II inhibitors.

Etoposide and Teniposide Activate LXR by Increasing LXR Expression/Nuclear Translocation and Inhibiting RIP140

Etoposide and teniposide increased LXR but not LXRγ expression or nuclear translocation and inhibition of LXR co-repressor expression. Similar to the scrambled siRNA-transfected cells (Fig. 3, A, B, and D), treatment of normal HepG2 cells with etoposide and teniposide increased LXRα, but not LXRβ levels, in whole cellular extracts (Fig. 3E), suggesting that expression of LXRα is selectively induced. However, determination of LXRα and LXRβ protein in nuclear extract shows that both LXRα and LXRβ were increased (Fig. 3F), which suggests that LXR nuclear translocation is enhanced. The effects of etoposide and teniposide on induction of LXRα expression and enhancement of LXRα/β nuclear translocation were further confirmed by immunofluorescent staining (Fig. 3G).

To directly link the induction of CETP expression to the inhibition of Topo II expression, we transfected HepG2 cells with Topo IIA siRNA and then determined the effect of decreased Topo II protein expression on CETP levels. The results demonstrate that Topo IIA siRNA decreased Topo IIA protein expression (top panel, Fig. 4A) while increasing CETP protein expression (middle panel, Fig. 4A). At the transcriptional level, decreased Topo IIA mRNA expression by the siRNA (left half, Fig. 4B) increased CETP mRNA expression (right half, Fig. 4B). In addition, Topo IIA siRNA activated CETP promoter activity, and the activation can be further enhanced by etoposide and teniposide (Fig. 4C).

The interaction between LXR and its co-repressor, RIP140, results in decreased LXR activity (22). To determine if the induction of hepatic CETP expression by etoposide and teniposide is related to inactivation of RIP140 pathway, we initially determined RIP140 expression in response to etoposide and teniposide treatment. The results in Fig. 5, A and B, demonstrate that both etoposide and teniposide inhibited RIP140 mRNA and protein expression in a concentration-dependent manner, respectively.

RIP140 has been reported as a novel target gene of the E2F1 transcription factor (23). p21 inhibits phosphorylation of Rb protein, which results in inactivation of E2F1 (24–26). At the transcriptional level, we determined that treatment of HepG2 cells with Topo II inhibitors increased p21 mRNA expression but had little effect on both Rb and E2F1 mRNA levels (Fig. 5C). Similarly, teniposide increased p21 protein expression. The increased p21 protein decreased pRb and E2F1 levels (Fig. 5D).

To further determine if Topo II inhibitors can inhibit RIP140 transcription by inactivating E2F1, we constructed a plasmid that contained a three-tandem of the E2F1 motif in the RIP140 promoter (PGL-3xE2F1-TK-Luc (Fig. 5E)). Both teniposide and etoposide reduced PGL-3xE2F1-TK-Luc promoter activity (Fig. 5F). Taken together, the results in Fig. 5 suggest that treatment of HepG2 cells with Topo II inhibitor induced p21 expression and subsequently inactivated the RIP140 pathway, which can result in release of RIP140 suppression on LXR activity. Thus, the induction of CETP expression by Topo II inhibitors may partially be contributed by inactivation of RIP140 pathway.

Administration of Teniposide Induces Liver CETP Expression and Enhances RCT in CETP Transgenic Mice—Wild type mice do not express CETP naturally; therefore, we used CETP transgenic mice (CETPtg) to determine the effect of teniposide-induced CETP expression on cholesterol metabolism. Both wild type mice and CETPtg mice (background matched) were subcutaneously injected with teniposide solution once every 4 days twice. After 8 days of the initial injection, mice were conducted an RCT assay by intraperitoneal injection of pre-radiolabeled corresponding peritoneal macrophages, and following determination of excreted radioactivity in feces.

In control animals, compared with wild type mice, expression of CETP results in reduced serum total cholesterol levels, which is mainly due to decreased HDL levels (Fig. 6A), and the
decreased HDL levels are in agreement with the function of CETP in vivo. Serum TG levels were also reduced by CETP expression in control CETPtg mice. Administration of teniposide to wild type mice had little effect on serum total, LDL- and HDL-cholesterol levels but showed decreased serum TG levels. In CETPtg mice, serum total, HDL-cholesterol and TG levels were reduced by teniposide. The decreased total cholesterol levels were also mainly contributed by the decreased HDL-cholesterol levels because the changes in both of total and HDL-C levels are close (Fig. 6A). MTTP is a molecule regulating TG secretion from the liver. Compared with wild type mice, expression of MTTP in CETPtg mouse liver was reduced. Meanwhile, treatment of the animals with teniposide reduced MTTP expression in both wild type and CETPtg mouse liver. The decreased MTTP expression may lead to reduced TG secretion from the liver and contribute to the reduced serum TG levels. The decreased HDL-cholesterol levels in CETPtg mice by teniposide may be attributed to the induction of hepatic CETP

FIGURE 3. Etoposide and teniposide activate LXR expression or/and nuclear translocation. A–D, HepG2 cells were transfected with scrambled siRNA (100 nM) or a mixture of LXRα siRNA and LXRβ siRNA (50 nM of each) followed by treatment with etoposide or teniposide for 16 h. Expression of LXRα, LXRβ, and CETP mRNA and protein was determined by real time RT-PCR and Western blot. *, versus control in the same group; **, versus the corresponding sample in the scrambled siRNA-transfected group at \( p < 0.05 \) (\( n = 3 \)). E and F, after 16 h of treatment, expression of LXRα and LXRβ protein in total extract or nuclear extract was determined by Western blot. G, expression of LXRα and LXRβ protein in intact HepG2 cells was determined by immunofluorescent staining. Eto, etoposide; Teni, teniposide.
expression. Indeed, teniposide increased both CETP mRNA and protein expression in CETPtg mouse liver, which were determined by real time RT-PCR, Western blot, and immunohistochemical staining (Fig. 6, C and D).

Furthermore, we determined if teniposide can increase RCT in vivo. Fig. 6E shows that teniposide increased RCT in both wild type and CETPtg mice. The increased RCT in wild type mice should be due to the induction of ABCA1 expression by teniposide (14). However, the overall induction of RCT by teniposide was greater in CETPtg mice than in wild type mice, which should be contributed by induction of CETP expression.

**Discussion**

In this study we determined that inactivation of Topo II induced hepatic CETP expression and secretion. Mechanically, Topo II inhibitors increased LXR expression/nuclear translocation and the binding activity of LXR with the LXRE in CETP promoter but inhibited the RIP140 pathway. These findings suggest that induction of CETP expression by Topo II inhibitor is mediated by activation of LXR pathway. In vivo, administration of teniposide to CETPtg mice induced CETP expression in the liver and RCT more than wild type mice. Thus, our study suggests that Topo II inhibitors can play an important role in regulation of cholesterol metabolism by activating hepatic CETP expression.

The overall role of CETP expression in atherosclerosis is still controversial. The inverse relationship between serum CETP and HDL-C levels leads to the development of CETP pharmacological inhibitors as a potential therapy for atherosclerosis (27). Four Topo II inhibitors have been in phase III clinical trial evaluations. However, two of them (torcetrapib and dalcetrapib) failed, and the third one (anacetrapib) did not show benefits to the patients with coronary heart disease after the preliminary studies. Although these inhibitors raise HDL-C levels while decreasing LDL-C levels, compared with placebo, they either increase or have no effect on the severe cardiovascular events (1). In humans, natural CETP deficiency increases HDL-C levels, which is associated with increased coronary heart disease prevalence in this population (2). In animal models, CETP transgenic mice demonstrate reduced both HDL-C levels and atherosclerotic lesions (15). Expression of CETP also attenuates ovariectomization or testosterone deficiency-induced lesions in mice (16, 17).

The inhibitory effects of CETP on atherosclerosis might be related to the enhanced RCT. Lack of SR-BI expression results in decreased RCT. However, the impaired RCT can be restored to normal by CETP expression (28). The patients with CETP deficiency or receiving CETP inhibitor treatment demonstrate slower apoA-I catabolism (29, 30). Furthermore, the plasma isolated from subjects with high endogenous CETP activity displays increased the capacity to mediate macrophages RCT, which implies that CETP can prevent macrophage/foam cell formation (31). The transfer of cholesteryl esters from HDL to apoB-containing lipoproteins by CETP also leads to reduction of HDL size and generation of lipid-poor HDL or pre-β-HDL (32, 33), a form that is a preferred acceptor of ABCA1-mediated cholesterol efflux from macrophages (34). In addition, CETP can enhance liver delivery and selective uptake of HDL-CE that may further benefit cholesterol metabolism (3, 35). Serum HDL-cholesterol levels and TG levels are reciprocal usually. However, in CETPtg mice it has been reported that changes of serum HDL-cholesterol levels are not associated with serum TG levels reciprocally. For instance, treatment of CETPtg mice
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with CETP antisense oligonucleotide or CETP inhibitor, anacetrapib, increased serum HDL-cholesterol levels, whereas results were slightly increased or had no effect on serum TG levels (36). In APOE*3-Leiden.CETP transgenic mice, activation of farnesoid X receptor by taurocholic acid increased CETP expression, which was associated with decreased HDL-C and TG levels in serum (37). Similarly, we determined that Topo II inhibitor decreased both serum HDL-C and TG levels in CETPtg mice in our study.

Synthetic LXR ligands, such as T0901317 and GW3965, can induce macrophage ABCA1 expression and cholesterol efflux, thereby inhibiting atherosclerosis. However, LXR ligands also induce hepatic lipogenesis, fatty liver, and hypertriglyceridemia. The severity of lipogenesis is correlated to the doses of LXR ligand used (7). Compared with the ligands with full LXR functions, the selective LXRβ modulators may exert antiatherogenic properties with minimal side effects. Unfortunately, the progress to discover selective LXRβ modulators is very slow due to the high similarities in both ligand and DNA binding domains between LXRα and LXRβ isoforms (38). We previously reported that etoposide and teniposide can induce macrophage ABCA1 expression. By completing a "molecular
Docking study, we determined a much weaker interaction between etoposide or teniposide and amino acid residues within the ligand binding pocket of LXR than T0901317 (14). In this study both etoposide and teniposide induce CETP expression, which is also associated with activation of LXR pathway. Recently, some of cholesterol oxidized derivatives, such as cholesteryl-5α,6α-epoxide, has been reported to specifically inhibit Topo II activity and suppress human cancer cell growth (39), which implied the similar properties between Topo II inhibitors and cholesterol-derived oxysterols on LXR activation.

Etoposide and teniposide also inhibit RIP140 expression (Fig. 5, A and B), a co-repressor of LXR. RIP140 has demonstrated as a transcriptional target of E2F1, a member of E2F transcription factor family regulating a broad spectrum of genes involved in major cellular processes, such as DNA replication, apoptosis, differentiation, and cell cycles (23). Transcriptional activity of E2F1 is regulated by pRb, whereas pRb is inversely regulated by p21. In our study we observed that etoposide and teniposide activate p21 expression and subsequently reduced pRb and E2F1, which may lead to inhibition of RIP140 transcription (Fig. 5, C–F). In fact, a few studies have reported that p21 expression can be activated by Topo II inhibitor or LXR ligand in different cell types (40, 41). Thus, induction of hepatic CETP expression by Topo II inhibitors is partially contributed by inactivation of RIP140 pathway. In addition, we observed that inhibition of Topo IIA expression by siRNA activated CETP expression (Fig. 4, A–C). Altogether, these results indicate that etoposide and teniposide activate the LXR pathway by multiple mechanisms which might distinguish the ligands with full LXR activities. Indeed, in our previous study we observed that teniposide demonstrates a greater effect than T0901317 on induction of macrophage ABCA1 expression at the same concentration (14). Although the interaction between LXR and Topo II protein and the involved precise mechanisms by which to influence LXR activity require more investigation, we anticipate that this interaction may suppress LXR activity. Thus, in the presence of Topo II inhibitors, such as etoposide or teniposide, the interaction will be reduced and consequently activates expression of LXR-targeted genes including CETP.

Compared with mice, the importance of CETP in RCT implies that Topo II inhibitors may enhance RCT with a greater effect in species expressing CETP, such as humans. Indeed, more RCT was induced in CETP transgenic mice than wild type mice by teniposide (Fig. 6E), which might be another mechanism responsible for anti-atherogenic properties of Topo II inhibitors.

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