



Altered phospholipid transfer protein gene expression and serum lipid profile by topotecan

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ABSTRACT

Camptothecin (CPT) and its structural analogues including topotecan and irinotecan, are inhibitors of topoisomerase I. These drugs are clinically active against a broad spectrum of cancers. To understand the genesis of chemotherapeutic resistance to the CPT family of anticancer drugs, we examined by gene expression profiling the pharmacological response to topotecan in the human hepatoma HepG2 cells and found a striking induction of the phospholipid transfer protein (PLTP) gene expression by topotecan. We showed that activation of PLTP gene expression is specific to CPT and its analogues including specific enantiomers that inhibit topoisomerase I. PLTP-mediated lipid transfer to high-density lipoprotein (HDL) is thought to be important for shuttling and redistribution of lipids between lipoproteins, which are normally returned to the liver for metabolism via the reverse cholesterol transport pathway. Hence, we asked whether elevated PLTP levels might increase the transfer of drugs into HDL. We observed that CPT was not accumulated in HDL and other lipoproteins. In addition, topotecan treatment in mice caused a marked reduction in serum HDL that was accompanied by an increase in triglyceride and cholesterol levels. These results showed that PLTP does not mediate the transfer of topoisomerase I inhibitors to serum lipoproteins. However, elevated serum PLTP levels following treatment with topoisomerase I inhibitors in cancer patients may serve as a biomarker for monitoring the development of hypertriglyceridemia and acute pancreatitis.

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1. Introduction

The emergence of drug resistance in cancer remains a major unresolved problem. Drug resistance may arise intrinsically during tumorigenesis, or it may be acquired through selection following exposure to chemotherapeutic agents. Whether it is intrinsic or acquired resistance, the vastly altered transcriptome of cancer cells during tumor progression or following drug exposure, could result in the expression of multiple genes that contribute to drug resistance [1]. These changes in the cancer transcriptome as well as mutation in drug targets can cause functional changes in some biological pathways including DNA repair, drug transport and metabolism, that result in drug resistance in cancer [2].

Alternatively, exposure to chemotherapy may select for tumor cells, which contained adapted transcriptome that is permissive for growth and survival in the presence of cytotoxic anticancer agents. These global changes in the cancer genome and transcriptome underlie the complexity of the drug resistance phenotype encountered in cancer treatment. Therefore, understanding the dynamic changes in the cancer transcriptome may reveal the complex pathways that contribute to clinical resistance to drug therapy [3].

The discovery of P-glycoprotein and its related proteins, encoded by the superfamily of ATP-binding cassette (ABC) genes, which are transmembrane multidrug transporters that confer resistance to multiple chemotherapeutic agents in cell culture and animal models [4], raised the possibility that targeting either its expression or disrupting its transport activity in cancer, can circumvent the multidrug resistance phenotype and improve chemotherapeutic efficacy and outcome [5]. However, most clinical studies to date showed that the addition of multidrug resistance modulators such as valsopodar (PSC 388) to chemother-

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apeutic regimens did not significantly improve treatment outcome in acute myeloid leukemia [6,7], multiple myeloma [8], advanced ovarian and primary peritoneal cancer [9,10], as well as metastatic breast cancer [11], thus prompting questions about the role of the family of ABC transporters in clinical drug resistance. This leaves open the possibility that during tumorigenesis, the genetic alteration of various genes coupled with the aberrantly perturbed biological pathways may be the underlying factors in chemotherapeutic resistance.

Camptothecin, which was discovered in a National Cancer Institute cytotoxic drug screening program almost 30 years ago, and its derivatives are anticancer agents that inhibit DNA topoisomerase I and exhibit efficacy against a broad spectrum of cancers clinically [12]. Mechanisms of resistance to CPT and its analogues have been reported, which include overexpression of the breast cancer resistance protein, BCRP/MXR/ABCP, loss of DNA mismatch repair, elevation of DNA polymerases and topoisomerase II, as well as topoisomerase I mutations and down-regulation [13], suggesting that the mechanisms of resistance to the CPT family of agents are diverse.

In the course of studying the pharmacological effects of topotecan, a semi-synthetic analogue of CPT, in the human hepatoblastoma HepG2 cell, we found a striking induction of the phospholipid transfer protein (PLTP) gene expression in a dose- and temporal-dependent manner. PLTP is a major serum protein involved in reverse cholesterol transport by shuttling phospholipids and cholesterol from triglyceride-rich lipoproteins to the high-density lipoprotein (HDL) for hepatic uptake by the BI class of scavenger receptor (SR-BI) [14–16]. We showed that CPT is not a substrate for PLTP-mediated shuttling into HDL in mice. We speculate that the induction of PLTP expression by CPTs may serve as a biomarker for predicting the development of hypertriglyceridemia and acute pancreatitis in cancer patients treated with these agents.

2. Materials and methods

2.1. Cell culture and reagents

HepG2 cells were purchased from the American Type Culture Collection and cultured at 37 °C with 10% CO₂ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Invitrogen), 2 mM L-glutamine, 100 µg/ml streptomycin sulfate, and 100 U/ml penicillin. A HepG2 subline harboring a luciferase reporter driven by the promoter of the PLTP gene was generated by transfection of the reporter into HepG2 cells followed by selection in 1.6 mg/ml G418, and a G418 resistant subline stably expressing the reporter was derived and maintained in selection for drug-induced retransfection experiments.

Camptothecin, topotecan, mitoxantrone, cisplatin, cholesterol, taxol, melphalan, Adriamycin, aldosterone, methyl jasmonate, cytarabine (Ara-C), puromycin, rolipram, and staurosporine, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), phorbol 12-myristate 13-acetate 4-O-methyl ether, and 4 α -TPA were purchased from Sigma-Aldrich (St. Louis, MO). 9- and 12-amino-20(S)-CPT, 9-amino-10,11-methylenedioxy-20(R,S)-CPT, and coralyne were gifts of Dr. Leroy Liu (Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ).

2.2. Gene expression profiling of topotecan in HepG2 cells

HepG2 cells were treated with topotecan and RNAs were isolated using the TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. cDNA targets were synthesized from the isolated total RNA with ³³P-dCTP by oligo-dT-primed polymerization using Superscript II reverse transcriptase

and the labeled targets were hybridized to nylon membrane arrays, containing approximately 2500 cDNAs corresponding to known genes in the GenBank database, washed and exposed on phosphorimage screen for approximately 15 h, and then scanned on a Molecular Dynamics Typhoon Phosphorimager. Scanned images of microarrays were analyzed using Imagene (Biodiscovery) and the output intensity data were subjected to cluster analysis using the Cluster and TreeView software suite [17].

2.3. Viral infection and PLTP promoter

Adenovirus harboring either the wild-type or the dominant negative mutant of PKC α , β II, and δ has been previously described [18,19]. In brief, adenovirus harboring the various PKCs was obtained from culture supernatant of HEK293a cells previously infected with the corresponding viruses following characterization of virus titers. HepG2 cells were subsequently infected with the PKC adenovirus, and 24 h following infection, cells were treated with 500 nM topotecan for an additional 15 h, and then harvested for luciferase reporter assay. Transactivation was compared to control cells infected with adenovirus harboring the green fluorescence protein (GFP).

PLTP gene promoter was isolated as previously described [20,21] and amplified by polymerase chain reaction from the human PAC clone RP3-337018, and subcloned into the pGL3-Basic luciferase reporter construct (Promega) that was modified to contain the geneticin (G418)-resistant marker and sequence verified. HepG2 cells were then transfected with the PLTP promoter reporter and selected for G418 resistant clones at 1.6 mg/ml G418, to generate a subline that stably expressed the promoter reporter.

2.4. Northern and Western blot analysis

Time-course and dose-response experiments were conducted with either the HepG2 cells or the luciferase reporter subline by exposure to either 500 nM topotecan for various times (1–24 h), or with various doses of topotecan (1–1000 nM) for 24 h. For Northern blot analysis, total RNA prepared from these cells was subsequently fractionated on 1% formaldehyde denaturing agarose gel and then transferred to nylon membrane for hybridization with radiolabelled cDNA probe of PLTP.

C57BL/6 mice were given, by intraperitoneal injection, various doses of topotecan (2, 5, and 10 mg/kg) and serum was collected from the mice 24 h following treatment and PLTP levels were assessed by fractionating 5 µl of serum on 12% SDS-PAGE and then transferred to a 0.45 micron pure nitrocellulose membrane (Bio-Rad). Blots were probed with anti-PLTP antibodies (BioVision, #3595-100) in phosphate-buffered saline containing 5% nonfat dry milk powder and incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Amersham Biosciences, #NA934). Protein bands were resolved using HyGLO HRP detection kit (Denville, #34080 or 34094).

2.5. PLTP activity assay

PLTP activity was measured using a fluorescence-based assay kit (Cardiovascular Targets Inc., New York, NY) that contained a donor molecule comprised of a fluorescent self-quenched phospholipid that is transferred to an acceptor molecule in the presence of PLTP, resulting in an increase in fluorescence. Briefly, either 3 µl of mouse serum or 50 µg of protein concentrated from HepG2 culture conditioned media was incubated with donor and acceptor molecules and changes in fluorescence intensity were measured on a BMG Labtech POLARstar fluorescence microplate reader (Ex. = 465 nm; Em. = 535 nm).

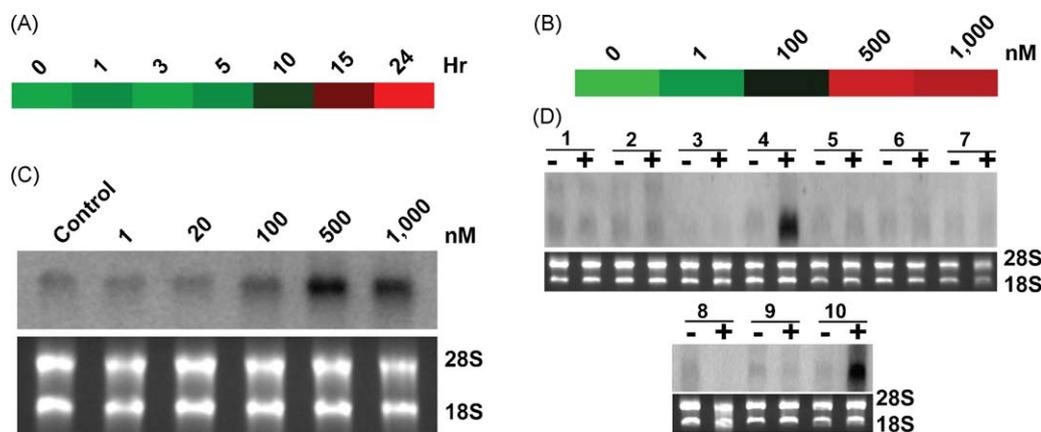


Fig. 1. Induction of PLTP gene expression by topotecan. RNA was isolated from HepG2 cells for gene expression profiling analysis following treatment with topotecan either in a time-course study (500 nM topotecan) at the various times indicated (A), or dose–response analysis (0, 1, 100, 500, and 1000 nM) for 24 h (B). The dendrograms showed the induction of PLTP expression normalized to untreated control. (C) Validation of topotecan-induced PLTP expression in a dose response study by Northern blot analysis. (D) Cell-type specific induction of PLTP expression by topotecan (+) compared to untreated control (–) in various cancer cells: 1, 2008 (ovarian carcinoma); 2, A2780 (ovarian carcinoma); 3, A549 (lung adenocarcinoma); 4 and 10, HepG2 (hepatoma); 5, KB-3-1 (oral cancer); 6, KB-V-1 (multidrug-resistant cells derived KB-3-1); 7, MCF-7 (mammary carcinoma); 8, U937 (myeloid leukemia); and 9, PC3M (metastatic prostate carcinoma).

2.6. Fractionation of plasma lipoproteins by density gradient ultracentrifugation

Male C57BL/6 mice weighing 18–20 g were given either 50 μ Ci [1,2- 3 H(N)]-cholesterol or [14- 3 H(N)]-camptothecin (3 H]-CPT) (Moravek Biochemicals, Brea, CA) intraperitoneally in 0.2 ml of saline buffer. Blood samples were collected from the animals by cardiac puncture at 0.5, 2, 6, 12, 24, and 36 h, and plasma was separated by centrifugation at 3000 rpm for 10 min at 4 °C. Lipoproteins isolation was performed as described previously [22]. In brief, plasma sample was adjusted by refractometry to a density of 1.24 g/ml with KBr, and the solution was overlaid sequentially on a stepwise NaCl-KBr-gradient (density 1.12–1.06 g/ml), followed by ultracentrifugation at 36,000 \times g in a swing-out SW-40 rotor for 9 h. The resolved very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL) were collected by bottom puncture in twenty-four 0.5 ml each fractions. Distribution of [1,2- 3 H(N)]-cholesterol and [14- 3 H(N)]-camptothecin in the lipoproteins was determined by liquid scintillation counting of each fraction. Fractions 8, 18, and 24 correspond to peak HDL, LDL and VLDL distribution respectively.

3. Results

To understand the pharmacological response to CPT and its derivatives, and the development of resistance to these agents, we examined by gene expression profiling the transcriptional changes in HepG2 cells following exposure to topotecan. Time-course and dose–response analyses showed modest changes in the expression of a handful of genes, except for the profound induction of the PLTP gene expression by topotecan in a dose- and temporal-dependent manner (Fig. 1). We observed a 20-fold induction of the PLTP gene by topotecan (0.5 μ M) (Fig. 1A) that peaked at approximately 24 h (Fig. 1B), suggesting that PLTP expression is temporally regulated as a late response gene following drug treatment. The induction of PLTP gene expression by topotecan was confirmed by Northern blot analysis in a dose–response study (Fig. 1C). We further assessed the induction of PLTP expression by topotecan in various cell types and found its expression activated by topotecan in HepG2 cells only (Fig. 1D).

We next evaluated the induction of PLTP expression by other topoisomerase I inhibitors. Northern blot analysis showed that in addition to topotecan, PLTP expression was also induced by CPT, 9-

amino-20(S)-CPT (9-NH₂-CPT(S)), 9-amino-10,11-methylene-dioxy-20(S)-CPT (9-NH₂-MD-CPT(S)), and the proto-berberine coralyne, which belongs to a new class of organic cations that are dual poisons of topoisomerase I and topoisomerase II (Fig. 2A) [23,24]. The 9-NH₂-10,11-MD-CPT(R) enantiomer and the prodrug, irinotecan (CPT-11), that are inactive against topoisomerase I, did not induce PLTP expression. The cytotoxic anti-microtubule, vinblastine, as well as cholesterol, a substrate of PLTP, also failed to induce PLTP expression (Fig. 2A).

To facilitate the characterization of the transcriptional activation of PLTP gene expression, a HepG2 subline stably expressing the PLTP promoter reporter was generated that exhibited response to topotecan induction in a time- and dose-dependent manner (Fig. 2B and C). Topoisomerase I inhibitors that induce PLTP expression (Fig. 2A) predictably transactivated the PLTP gene promoter reporter, while the inactive 9-NH₂-MD-CPT(R) enantiomer, and mitoxantrone (topoisomerase II inhibitor) did not (Fig. 2D). Transactivation from PLTP promoter seemed to be specific to the topoisomerase I inhibitors, as other anticancer agents including taxol, melphalan, and adriamycin, the mineralocorticoid aldosterone, the plant hormone methyl jasmonate, and the phosphodiesterase inhibitor rolipram, had no significant effects on the promoter activity (Fig. 2E). Modest transactivation of the promoter was observed with cytarabine (Ara-C) and puromycin. Interestingly, the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), strongly transactivated the PLTP promoter activity (Fig. 2E).

Since TPA is a potent activator of protein kinase C (PKC) [25,26], we further examined the effects of other phorbol esters including 4 α -TPA and phorbol 12-myristate 13-acetate 4-O-methyl ether (mPMA), which are incapable of activating PKC, and showed that these compounds had no effect on PLTP promoter activity compared to TPA (Fig. 3A). Staurosporine, a potent protein kinase inhibitor including PKC [27], inhibited topotecan- and TPA-mediated transactivations of the PLTP promoter (Fig. 3A), thus suggesting that topotecan- and TPA-induced PLTP gene expression may be regulated via the PKC signaling pathway.

There are at least 10 PKC isotypes in the mammalian genome that can be classified into subtypes due to their distinct structural as well as biochemical properties [28]. To further determine whether specific PKC isozymes contribute to the regulation of PLTP gene expression by topotecan, we examined the effects of some dominant negative mutant of PKCs [18,19] on the transactivation

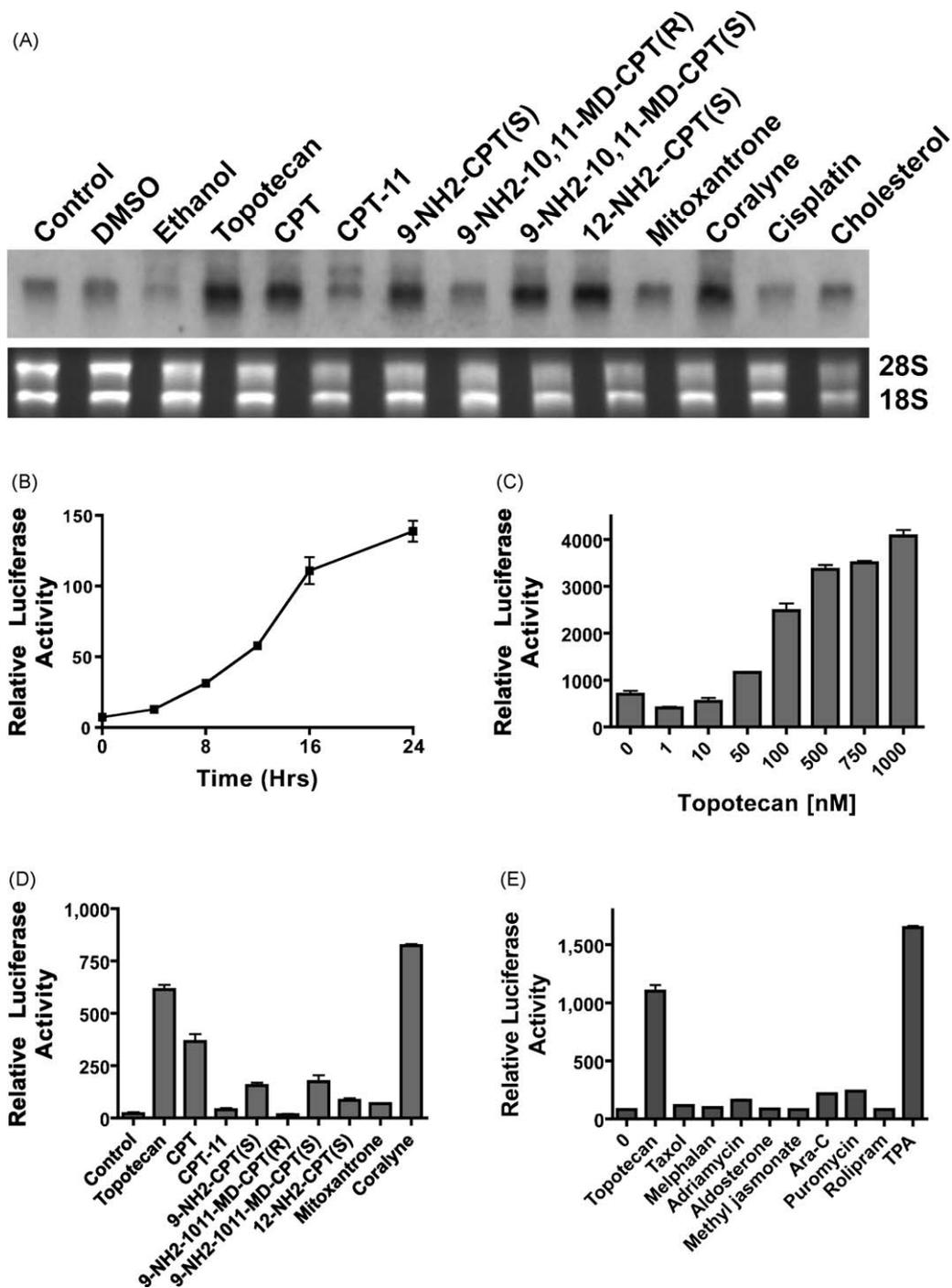


Fig. 2. Induction of PLTP gene expression by anticancer drugs. (A) Northern blot analysis of CPT and its analogues on PLTP gene expression. Except for CPT (10 nM), HepG2 cells were exposed to all other drugs at 1 μ M for 24 h prior to RNA isolation for Northern analysis. (B and C) Transactivation of the PLTP gene promoter activity in a HepG2 subline harboring the luciferase reporter driven by the PLTP gene promoter in a time- and dose-dependent induction by topotecan. In the time-course analysis, cells were treated with 1 μ M topotecan at the indicated times, in the dose-response study, cells were treated with topotecan at the indicated doses for 24 h. (D and E) Transactivation of the PLTP gene promoter activity was recapitulated with CPT and its analogues (D) as in Fig. 2A, and by various other drugs (E). Cells were exposed to 1 μ M of drug, except for CPT (10 nM) and TPA (30 ng/ml) for 24 h. All reporter assays were performed in triplicate and results are means \pm s.e.m.

of PLTP promoter activity. Overexpression of either PKC α or PKC β II dominant negative mutant that inactivates their corresponding enzymatic activities did not affect topotecan-mediated transactivation of the PLTP promoter (Fig. 3B). Inactivation of PKC δ , in contrast, attenuated the transactivation of the PLTP promoter by topotecan, suggesting the potential requirement for specific PKC isozyme in the regulation of PLTP gene expression.

Promoter deletion analysis showed that abolition of two putative sterol-response elements (SREs) as well as an Sp1 site

in the promoter between nucleotides –888 and –639 markedly attenuated topotecan-induced transactivation by approximately 50% (Fig. 4), suggesting that the sterol-response element binding protein (SREBP) might mediate the transactivation of PLTP by topotecan and other topoisomerase I inhibitors.

To determine the induction of PLTP by topotecan *in vivo*, mice were given increasing doses of topotecan and serum was collected for assessing PLTP levels by Western blot analysis. We found significant increase in serum PLTP levels 24 h following a single

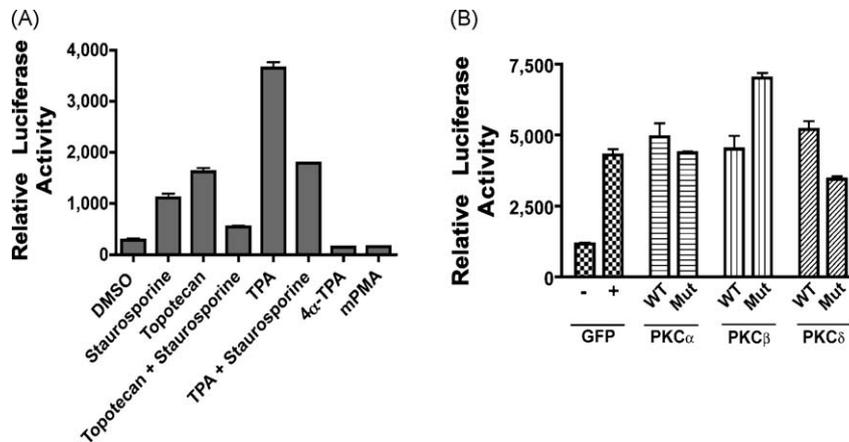


Fig. 3. Characterizing the transcriptional regulation of PLTP gene expression by topotecan and PKC. (A) Transactivation of PLTP gene promoter activity by topotecan (0.5 μ M) and TPA (30 ng/ml) is inhibited by staurosporine (300 nM). Non-PKC activators, 4 α -TPA and mPMA (30 ng/ml each), failed to transactivate PLTP promoter activity. (B) Transactivation of PLTP promoter reporter by topotecan (0.5 μ M) is partially inhibited by a dominant negative mutant of PKC δ , but not PKC α or β II, compared to HepG2 cells transduced with adenovirus expressing GFP that were either untreated (-) or treated (+) with topotecan. In (A) and (B), cells were exposed to drugs for 24 h before harvesting for the reporter assay. Results are in triplicate and error bars indicate means \pm s.e.m.

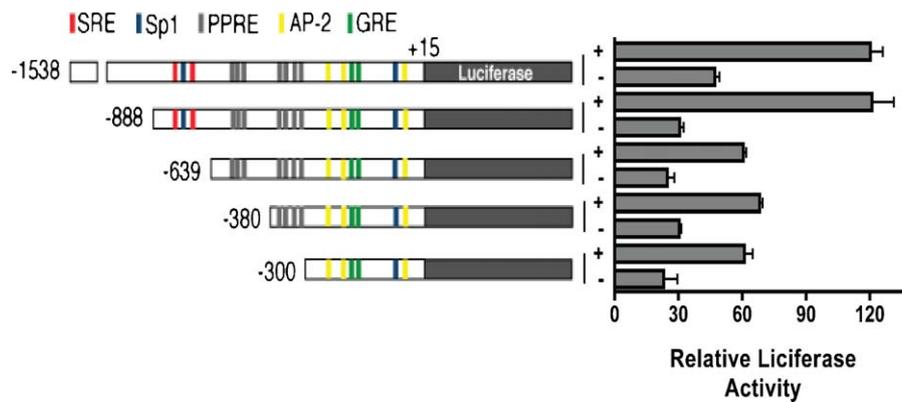


Fig. 4. PLTP promoter deletion analysis. Progressive deletion of a 1.5 kb proximal promoter of the PLTP gene was made as indicated, which showed that deleting the upstream SRE binding sites reduced topotecan (0.5 μ M) transactivation by approximately 50%. All experiments were performed in triplicate and treated with topotecan for 24 h. Error bars indicate s.e.m.

dose of 10 mg/kg of topotecan that was accompanied by an increase in PLTP enzymatic activity (Fig. 5A and B). Since transgenic mice that overexpressed PLTP exhibit marked changes in serum HDL-cholesterol levels [29,30], we examined the serum lipid profile of topotecan treated mice and observed an increase in total serum cholesterol and triglyceride levels at 10 mg/kg of topotecan. Unexpectedly, HDL-cholesterol level was almost abolished in mice treated with 10 mg/kg of topotecan (Fig. 5C). In addition, HepG2 cells treated with 500 nM of topotecan also exhibited increase in PLTP activity in the conditioned media collected 24 h following drug treatment (data not shown).

Since cholesterol and phospholipids are substrates of PLTP, we asked whether topoisomerase I inhibitors might also be substrate for PLTP-mediated transfer into HDL for transport to the liver and subjected to hepatic metabolism. To test this hypothesis, mice were given a dose of [3 H]-CPT and serum was collected from treated animals at various time points to determine the distribution of the radiolabelled drug in lipoproteins following isopycnic centrifugation. Our results showed that CPT was not transferred into lipoproteins by PLTP due to the absence of [3 H]-CPT in the VLDL, LDL and HDL fractions in the serum of treated animals, but was distributed mainly in the albumin fraction, which was found in the bottom fractions, two hours following drug treatment (Fig. 6). In contrast, [3 H]-cholesterol was incorporated and distributed in a time-dependent manner, first in the chylomicron/VLDL fraction,

and then transitioning into the LDL and HDL fractions as time progressed, with a majority of the cholesterol transported by LDL (Fig. 6). These results suggested that topoisomerase I inhibitors are not substrate for PLTP shuttling into HDL for reverse cholesterol transport to the liver.

4. Discussion

Drug resistance in cancer is a complex process associated with the alterations of multiple genetic factors and the transcriptome of the cancer cells. These aberrant changes during tumorigenesis result in the perturbation of biological pathways that may contribute to the drug resistant phenotype. Therefore, understanding how drug resistance emerges in cancer is of paramount importance in improving cancer treatment and outcome. In this report, we identified the transcriptional induction of PLTP gene expression by CPT and its derivatives that are active inhibitor of the topoisomerase I enzyme (Figs. 1 and 2). Induction of PLTP expression was specific to CPT and its family of topoisomerase I poisons, and also restricted to the human liver cancer HepG2 cells. We showed further that topotecan-induced PLTP expression is mediated through the PKC signaling pathway and requires specific PKC isozyme for its transcriptional regulation (Fig. 3). PLTP expression is also induced *in vivo* in mice following treatment with topotecan that is accompanied by a rise in serum triglyceride

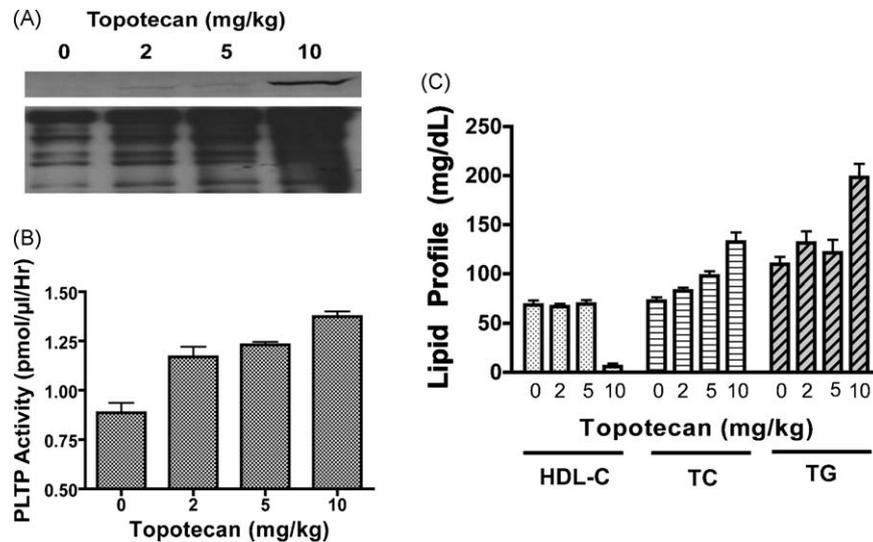


Fig. 5. Effects of topotecan on serum PLTP and lipid profile in mice. (A) Western blot analysis of serum PLTP levels in mice following topotecan treatment (0, 2, 5, and 10 mg/kg). Coomassie stained of a portion of the gel is shown in the lower panel. (B and C) Serum PLTP activity (B) and lipid profile (C) of mice treated with topotecan as in (A). HDL-C, serum HDL cholesterol; TC, total cholesterol; and TG, triglyceride. Where appropriate, experiments were performed in triplicate and results are mean \pm s.e.m.

and cholesterol, and a marked loss of HDL (Fig. 5). In addition, CPT induced increase in PLTP level did not result in the transfer of CPT to HDL.

The profound induction of PLTP expression by CPTs is intriguing, which led us to initially hypothesize that topoisomerase I inhibitors induced their own metabolism via the reverse cholesterol transport pathway. Moreover, it has been shown that cholesterol is a substrate for PLTP [31], and high cholesterol diet increases PLTP activity and PLTP mRNA in mice [32,33]. In addition, crystal structure data of the bacterial permeability increasing (BPI) protein [34,35] and the cholesteryl ester transfer protein (CETP)

[36] showing that proteins in this family, including PLTP, contained intrinsic lipid binding sites that appear to act as carrier proteins or channels that shuttle and redistribute lipids between lipoproteins. These observations raised the possibility that CPTs might be substrates for PLTP-mediated transfer into HDL and subsequently transported to the liver for metabolism via the reverse cholesterol transport pathway. However, our results showed that, unlike cholesterol, CPT was not found in HDL or other lipoproteins, suggesting that CPTs are not substrates for PLTP and thus ruling out the possibility of PLTP-mediated transfer of CPT into HDL for liver metabolism. These observations pose the possibility that the

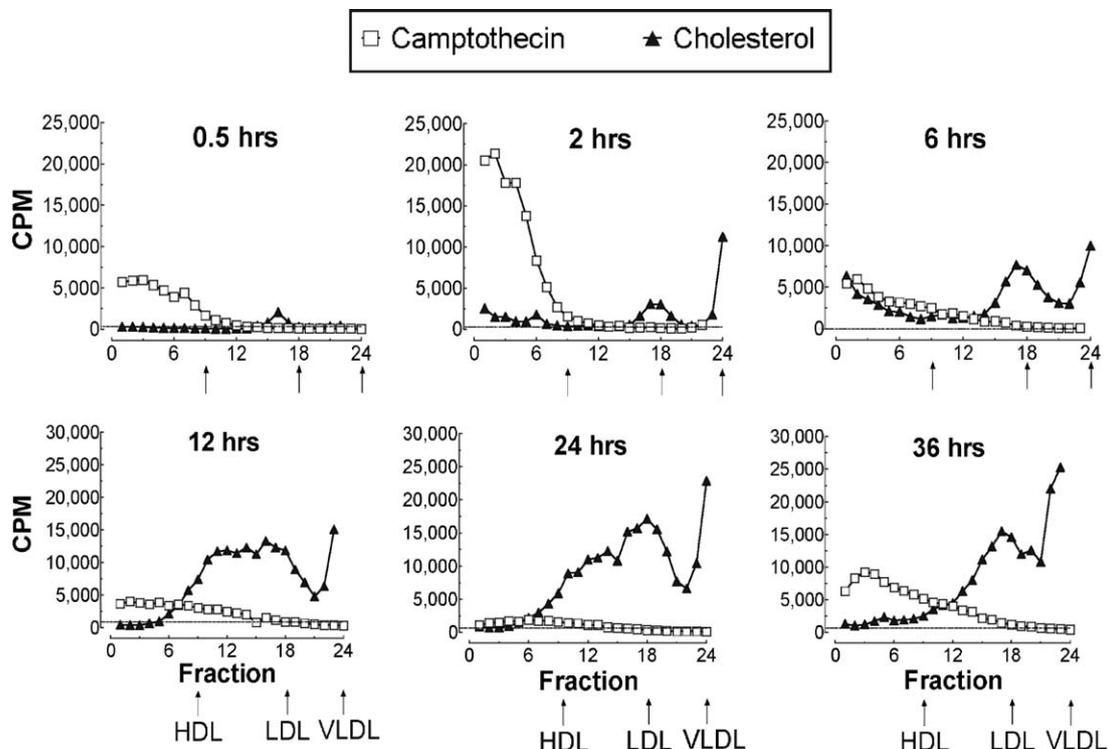


Fig. 6. Distribution of $[^3\text{H}]$ -CPT and $[^3\text{H}]$ -cholesterol in lipoproteins. Mice were given 50 μCi of either $[^3\text{H}]$ -CPT or $[^3\text{H}]$ -cholesterol and serum was collected at the time points indicated and subjected to isopycnic ultracentrifugation to determine the distribution of $[^3\text{H}]$ -CPT or $[^3\text{H}]$ -cholesterol in serum lipoproteins as described in Section 2. The arrows indicate the peak fractions corresponding to VLDL, LDL, and HDL.

induction of PLTP gene expression by CPTs is merely the result of bystander effect following drug exposure, which inadvertently activates the expression of many genes, and some of which including PLTP may have no physiological consequences or whose functional impacts are unknown.

Besides topotecan, it has previously been reported that PLTP expression is induced by hypoxia [37], fenofibrate [38], and the liver X receptor (LXR) agonist, T0901317 [39]. These results suggest that PLTP expression is responsive to stimulation by small pharmacological molecules. While PLTP mRNA is found abundantly in a variety of tissues [40], however, the induction by topotecan is cell-type specific. The underlying reason for this conditional expression pattern is unclear, and we speculate that it may be dependent on the small pharmacological molecules as well as the presence of specific transcriptional regulators in the respective tissues or cell types.

Another curious observation in our study is the unexpected rise in serum cholesterol and triglyceride levels, and a severe depletion of serum HDL in mice treated with a high dose of topotecan. The abrupt rise in serum triglyceride is known to be associated with the onset of acute pancreatitis [41]. Though under-reported and under-appreciated, drug-induced acute pancreatitis are commonly encountered in drug therapy [42]. The etiologic cause of most drug-induced acute pancreatitis and atherosclerosis may be associated with hyperlipidemia, resulting from increased total serum triglyceride and cholesterol levels [43]. The mechanisms of drug-induced hyperlipidemia are unknown. Increase in PLTP activity has been linked to hypertriglyceridemia [44,45], which may trigger the onset of acute pancreatitis [43,46]. It is noteworthy that acute pancreatitis has been reported in cancer patients treated with topoisomerase I inhibitors such as irinotecan [47] and exatecan [48], as well as other chemotherapeutic agents including L-asparaginase [49,50], tamoxifen [51], interferon α [52], and capecitabine [53]. These observations, together with our results, suggest that hypertriglyceridemia induced acute pancreatitis may be correlated with the increase in PLTP, thus suggesting that PLTP may serve as a biomarker for CPTs and other drug-induced hypertriglyceridemia and acute pancreatitis.

In summary, our results showed that CPTs induced PLTP expression may have no functional consequence on drug resistance in cancer, but instead may be associated with drug-induced hyperlipidemia that triggers the onset of acute pancreatitis. Therefore, monitoring the changes in serum PLTP levels or activity may serve as an important biomarker for drug-induced hyperlipidemia and the development of acute pancreatitis. How an increase in PLTP expression causes hyperlipidemia (hypercholesterolemia and hypertriglyceridemia) and lowers HDL levels needs to be further investigated.

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