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Inhibition of Topoisomerase II by Liriodenine

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ABSTRACT. The cytotoxic oxoaporphine alkaloid liriodenine, isolated from *Cananga odorata*, was found to be a potent inhibitor of topoisomerase II (EC 5.99.1.3) both *in vivo* and *in vitro*. Liriodenine treatment of SV40 (simian virus 40)-infected CV-1 cells caused highly catenated SV40 daughter chromosomes, a signature of topoisomerase II inhibition. Strong catalytic inhibition of topoisomerase II by liriodenine was confirmed by *in vitro* assays with purified human topoisomerase II and kinetoplast DNA. Liriodenine also caused low-level protein–DNA cross-links to pulse-labeled SV40 chromosomes *in vivo*, suggesting that it may be a weak topoisomerase II poison. This was supported by the finding that liriodenine caused topoisomerase II–DNA cross-links in an *in vitro* assay for topoisomerase II poisons. Verapamil did not increase either liriodenine-induced protein–DNA cross-links or catalytic inhibition of topoisomerase II in SV40-infected cells. This indicates that liriodenine is not a substrate for the verapamil-sensitive drug efflux pump in CV-1 cells. BIOCHEM PHARMACOL 54;4:467–473, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. topoisomerase II; antineoplastic; cytotoxic; aporphine; topoisomerase inhibitor; multidrug resistance

Liriodenine (8*H*-benzo[g]-1,3-benzodioxolo[6,5,4-de]-quinolin-8-one), shown in Fig. 1, has been isolated from plant species of many genera [1], often on the basis of bioassay-directed fractionation. The synthesis has also been reported [2]. Liriodenine has a remarkable range of biological activity. It is cytotoxic to human cancer cells [3, 4] and active against gram-positive bacteria, yeasts, and filamentous fungi [5, 6]. Liriodenine is a mutagen [7] and a clastogen, which causes chromosomal aberrations at low doses [8]. We are using an SV40-based§ screen for natural products that disrupt specific steps of mammalian DNA replication [9]. This screen has led us to identify liriodenine as a potent inhibitor of mammalian topoisomerase II. Disruption of topoisomerase II activity is the likely basis for several of the biological activities of liriodenine.

MATERIALS AND METHODS

Cell Culture and Virus Infection

African green monkey cells (CV-1) were maintained in Eagle's minimal essential medium (Gibco) supplemented with 10% calf serum, 14 mM HEPES, pH 7.2, and 4 mM $NaHCO_3$. Confluent cells were infected with plaque-purified SV40 strain 777 at a multiplicity of 10 plaque-forming units per cell.

Drugs

Verapamil (5-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile) and *m*-AMSA were purchased from the Sigma Chemical Co., St. Louis, MO. VP-16 was obtained from the National Cancer Institute, Division of Cancer Treatment. Liriodenine was purified from *Cananga odorata* (J. M. Cassady and N. J. Sun, unpublished data). All drugs were dissolved in DMSO.

In Vivo Assay for Topoisomerase II Inhibition

The *in vivo* assays for topoisomerase inhibition exploit SV40 as a model for mammalian replicons [10]. The virus makes extensive use of host cell enzymes of DNA replication, including cellular topoisomerases. Inhibition of topoisomerase II causes newly replicated SV40 daughter chromosomes to accumulate as highly catenated dimers. SV40 DNA replication intermediates that have been pulse-labeled with [^{3}H]Tdr are selectively extracted by the method of Hirt [11], separated by agarose gel electrophoresis, and visualized by fluorography [12]. Highly catenated SV40 daughter chromosomes (catenated dimers) appear as overlapping ladders of bands, with each band representing a distinct level of catenation. The levels of catenation

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§ *Abbreviations*: DTT, dithiothreitol; GuHCl, guanidinium chloride; ICRF-193, meso-2,3-bis(2,6-doxopiperazine-4-yl)butane; kDNA, kinetoplast DNA; *m*-AMSA, 4′-9-acridinylamino)methanesulfon-*m*-anisidide; MDR, multidrug resistance; SV-40, simian virus 40; Tdr, thymidine; VM-26 (teniposide, NSC-122819), 4′-demethylepipodophylotoxin 9-(4,6-O-2-ethylidene-β-D-glyocpyranoside; and VP-16 (etoposide, NSC-141540), 4′-demethylepipodophyllotoxin 9-[4,6-O-ethylidene-β-D-glucopyranoside.

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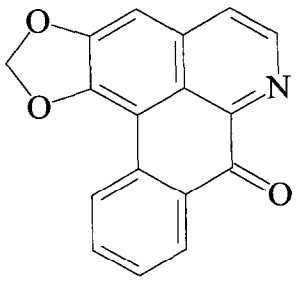

FIG. 1. Liriodenine.

increase with increasing inhibition of topoisomerase II [13, 14].

At 36 hr post-infection, the peak of viral DNA replication, replicating viral DNA was labeled by replacing cell growth medium with serum-free medium containing 250 μCi/mL [*methyl*-^{3}H]Tdr. The infected cells were pulse-labeled for 35 min. When included, verapamil was added 15 min after the start of labeling, and liriodenine and VP-16 were added 20 min after the start of labeling. Labeling and drug treatment were stopped by the removal of the labeling medium and the addition of Hirt lysing fluid. Viral DNA was deproteinized and processed for agarose gel electrophoresis as described [12].

In Vivo *Assay for Topoisomerase Poisons*

Topoisomerase poisons are drugs that stabilize topoisomerase–DNA strand passing intermediates in which the enzyme subunits are attached covalently to the DNA at the site of a DNA strand break. Topoisomerase poisoning can be measured in SV40-infected cells by measuring drug-induced protein cross-links to pulse-labeled SV40 DNA [15]. Pulse-labeling of SV40 DNA, drug exposure, and Hirt extraction were done as described above, except that the Hirt extract supernatant, containing viral DNA, was not deproteinized. Aliquots of the Hirt supernatant were removed and assayed for protein–DNA cross-links (see below).

In Vitro *Assay for Topoisomerase II Inhibition*

The assay measures decatenation of extensively catenated kDNA networks by purified topoisomerase II [16, 17]. Reaction mixtures (20 μL) contained topoisomerase II decatenation assay buffer (50 mM Tris–HCl, pH 8.0, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM DTT, 30 μg BSA/mL), and kDNA (100 ng/reaction). The amount of topoisomerase II was adjusted to give 80–90% decatenation in 30 min at 37°. Reactions were initiated by the addition of topoisomerase II. Liriodenine was dissolved in DMSO and the final concentration of DMSO was kept constant at 5% in all reactions. Decatenation reactions were terminated by the addition of 1/5 vol. of stop buffer/loading dye (5% Sarkosyl, 0.0025% bromophenol blue, 25% glycerol). The sample was electrophoresed in 1% agarose gels with 0.5 μg/mL ethidium bromide in TAE electrophoresis buffer (40 mM Tris acetate, pH 8.0, 1 mM EDTA). The bands of kDNA and free DNA circles were visualized by UV trans-illumination and were quantitated with an IS-1000 digital imaging system (Alpha Innotech Corp., San Leandro, CA). Human topoisomerase II and kDNA were from TopoGen, Columbus, OH.

In Vitro *Assay for Topoisomerase II Poisons*

The assay measures drug-induced cross-linking of purified topoisomerase II to purified ^{3}H-labeled SV40 DNA. Reaction mixtures (30 μL) contained topoisomerase II cleavage buffer (30 mM Tris–HCl, pH 7.6, 3.0 mM ATP, 15 mM 2-mercaptoethanol, 8.0 mM $MgCl_2$, 60 mM NaCl), ^{3}H-labeled SV40 DNA, and the test drug. Drugs were dissolved in DMSO, and a solvent control was included in each experiment. Reactions were initiated by addition of enzyme, and incubation was for 15 min at 37°. The reaction was stopped by the addition of 1/10 vol. of 10% SDS, and protein–DNA cross-linking was determined by a filter assay (see below). Labeled SV40 DNA for the *in vitro* cross-linking assay was prepared by labeling SV40-infected cells for 2 hr ([^{3}H]Tdr, 250 μCi/mL). SV40 DNA was selectively extracted by the method of Hirt [11] and deproteinized by proteinase K digestion (0.1 mg/mL proteinase K, 37°, 10 hr) and chloroform–isopropanol (24:1) extraction [12]. DNA was precipitated with 1/2 vol. of 7.5 M ammonium acetate, pH 7.5, and 2 vol. of 95% ethanol. The DNA pellet was washed two times with 70% ethanol. After brief drying under vacuum, the DNA pellet was dissolved in TE buffer (10 mM Tris–HCl, pH 7.5, 1 mM EDTA), and the concentration of viral DNA was measured by the Hoechst dye assay [18].

Filter Assay for Protein–DNA Cross-links

The filter assay for protein–DNA cross-links has been described [15]. Briefly, aliquots of the Hirt extracts or reaction mixtures from the *in vitro* cross-linking assay were mixed with 0.4 M and 4.0 M GuHCl, and then were filtered through pre-wetted glass fiber filters. In 4.0 M GuHCl, all nucleic acids bind to the filter (equivalent to a trichloroacetic acid precipitation). This gives the value for total labeled DNA in the aliquot. In 0.4 M GuHCl, only proteins and nucleic acids linked to proteins bind to the filter. This gives the fraction of viral DNA linked to protein.

RESULTS

Catalytic Inhibition of Topoisomerase II In Vivo

Topoisomerase II is required for the separation of newly replicated chromosomes in eukaryotic cells [19]. This is true of cellular chromosomes as well as plasmid or viral chromosomes. Newly replicated SV40 daughter chromosomes are topologically linked (catenated), and topoisomerase II separates them (decatenation) by a double-strand DNA

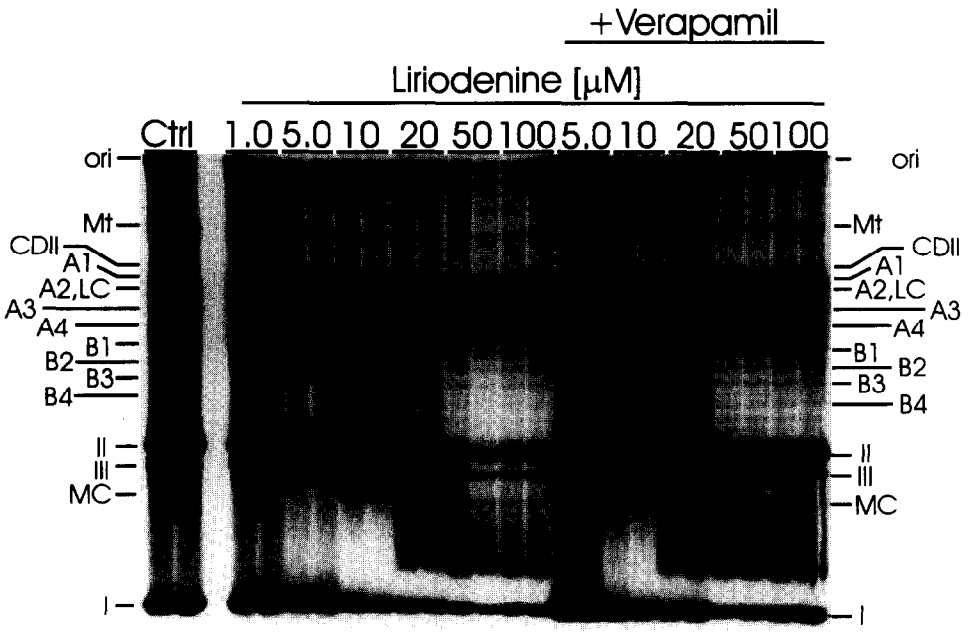

FIG. 2. Catalytic inhibition of topoisomerase II in SV40-infected cells. Abbreviations: Ctrl, controls; ori, origin of electrophoresis; Mt, mitochondrial DNA; I, form I (superhelical circular) SV40 DNA; II, form II (nicked circular) SV40 DNA; III, form III (double-stranded, linear) SV40 DNA; LC, late Cairns structure (a "figure eight" type late replication intermediate); A1–A4, A-family catenated SV40 daughter chromosomes (both daughter chromosomes nicked and catenation linking number indicated); B1–B4, B-family catenated SV40 daughter chromosomes (one nicked and one superhelical, catenation linking number indicated); CDII, circular (head-to-tail) SV40 dimer; and MC, a pseudoband indicating the point at which highly catenated B-family dimers are no longer resolved. The smooth smear of density between form I and the LC band represents growing intermediate Cairns structures ("theta-form" normal replication intermediates). The samples indicated by "+Verapamil" contained 100 μM verapamil.

passing reaction to produce two superhelical form I SV40 genomes. The accumulation of catenated SV40 daughter chromosomes in infected cells is a signature of topoisomerase II inhibition [20]. The catenated SV40 chromosomes occur in three families: A-family (both daughter chromosomes nicked), B-family (one daughter chromosome nicked and one superhelical), and C-family (both daughter chromosomes superhelical). One-dimensional agarose gel electrophoresis not only separates these three types of catenated SV40 dimers from one another, but separates the members of each family on the basis of catenation linking number. Each increase in catenation linking number increases the compactness and electrophoretic mobility of the intermediate. Thus, the A- and B-families of catenated dimers are resolved into ladders of bands in which each band represents a particular level of catenation linkage. The linking number of a particular band can be determined by band counting from the known positions of the A-1 and B-1 dimers. The gel electrophoresis system efficiently separates catenated SV40 daughter chromosomes with low to intermediate levels of catenation, but the separation becomes poor at higher levels of catenation and the catenated dimer bands become more closely spaced. On one-dimensional gel electrophoresis patterns, there is a point where catenated dimers are no longer resolved from one another. This point appears as a pseudo-band and is labeled "MC" in the figures. Increasing inhibition of topoisomerase II causes a progressive increase in catenated SV40 daughter chromosomes relative to other viral replication intermediates and at the same time progressively shifts the catenation linking numbers to higher levels. High concentrations of strong catalytic topoisomerase II inhibitors shift SV40-catenated daughter chromosomes to such high catenation linking levels that many migrate in an unresolved smear found just behind the form I band on one-dimensional gel patterns. This pattern is well established for strong catalytic inhibitors of topoisomerase II such as ICRF-193 and the quinobenoxazines, which have been studied with high resolution one- and two-dimensional gel electrophoresis [13, 14, 21].

As seen in Fig. 2, liriodenine caused a concentration-dependent increase in the catenation of newly replicated SV40 daughter chromosomes. The increase in catenated dimers was associated with a concentration-dependent decrease in form I DNA (the product of decatenation). Slight increases in intensity of the B-family dimers were seen even at 1.0 and 5.0 μM liriodenine. Very intense overlapping ladders of highly catenated A- and B-family dimers were caused by exposure of the infected cells to 10 μM liriodenine. The catenation linking numbers of these dimers continued to increase at 20 μM liriodenine and higher concentrations. At the highest concentrations of liriodenine, the catenated SV40 dimers were shifted progressively to high levels of catenation that cannot be resolved on these one-dimensional gels (the MC band and the areas of density ahead of it in Fig. 2).

The electrophoresis pattern was not changed significantly in the presence of 100 μM verapamil. Verapamil is a well-studied inhibitor of the MDR drug efflux pump, P-glycoprotein. This pump is responsible for removing lipophilic compounds from cells, including many structur-

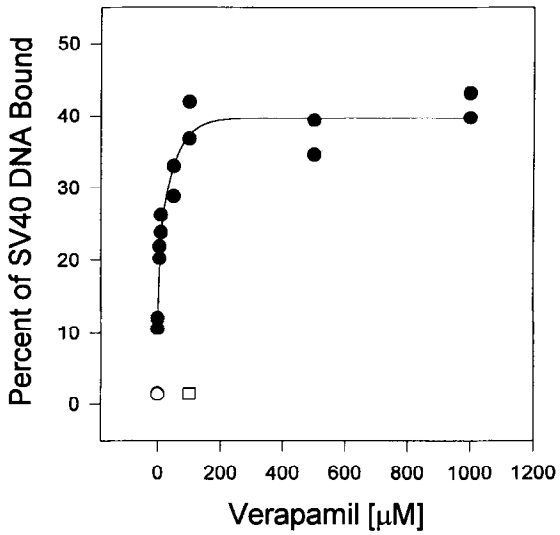

FIG. 3. Effect of verapamil on VP-16-induced topoisomerase II–DNA cross-links in SV40 infected CV-1 cells. Infected cells were labeled with [^{3}H]Tdr for 35 min, with VP-16 added after 20 min of labeling. The level of VP-16 was chosen to cause 10% of the pulse-labeled SV40 DNA to be cross-linked to topoisomerase II. Verapamil was added at the indicated concentrations 5 min before the addition of VP-16. Key: (●) VP-16 (10 μM) with verapamil at the indicated concentrations; (○) no drugs added (background); and (□) verapamil (100 μM) only.

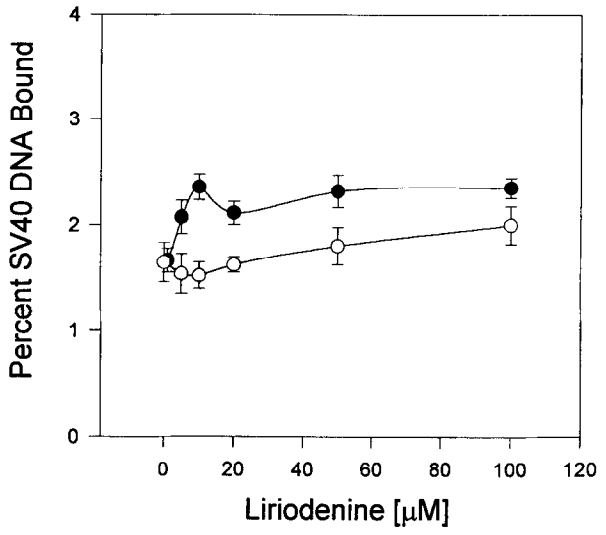

FIG. 4. Liriodenine-induced protein cross-links to [^{3}H]Tdr pulse-labeled viral DNA in SV40-infected CV-1 cells. Key: (●) liriodenine; and (○) liriodenine with 100 μM verapamil. Error bars represent ±SD, N = 4.

ally unrelated anticancer drugs [22, 23]. Cells accumulate much higher intracellular levels of these drugs when the MDR pump is inhibited by verapamil. As seen in Fig. 2, verapamil did not enhance liriodenine's inhibition of SV40 decatenation. The relatively greater label in the B-family dimers with low catenation numbers (B1–B6) and the less pronounced decrease in form I DNA suggest that the intracellular concentration of liriodenine may be slightly lower in the presence of verapamil.

Protein–DNA cross-links to pulse-labeled SV40 DNA can be measured by a glass fiber filter assay developed in this laboratory [15]. When the protein–DNA cross-links are caused by drugs known to be topoisomerase poisons, these cross-links can be assumed to be topoisomerase–DNA cross-links, and thus a measure of topoisomerase poisoning. Figure 3 shows that the filter assay can be used to demonstrate the effects of an MDR inhibitor such as verapamil. Here, sufficient VP-16 was added to SV40-infected CV-1 cells to cause about 10% of the pulse-labeled SV40 DNA to be cross-linked to topoisomerase II. Verapamil increased the VP-16-induced topoisomerase II–DNA cross-links up to 4-fold, reflecting an increase in the intracellular concentration of the drug. VP-16 is a well known substrate for the MDR drug efflux pump [24]. Verapamil increases intracellular concentrations of VP-16 in treated cells [25].

Liriodenine caused low-level but measurable protein–DNA cross-links to pulse-labeled SV40 DNA in infected cells (Fig. 4). The concentration–response curve showed a reproducible pattern consisting of a small peak around 10 μM liriodenine followed by a slight decrease. This same pattern was seen in five repeated experiments. Verapamil did not increase liriodenine-induced protein–DNA cross-links, again indicating that liriodenine is not a good substrate for the verapamil-sensitive drug efflux pump in CV-1 cells. The slight decrease in liriodenine-induced protein–DNA cross-links is probably not due to direct verapamil interference with the liriodenine–topoisomerase II interaction since verapamil has no effect on liriodenine's inhibition of topoisomerase II catalyzed decatenation *in vitro* or on liriodenine-induced topoisomerase II–DNA cross-links in the *in vitro* cleavage assay (data not shown). Since liriodenine is not a known topoisomerase II poison, it is not clear from this result alone that the protein–DNA cross-links are due to topoisomerase II.

In Vitro *Assays for Topoisomerase II Inhibition*

Liriodenine was a strong inhibitor of topoisomerase II in a decatenation assay using purified human topoisomerase II and kDNA (Fig. 5). This agrees well with the *in vivo* results showing liriodenine to be a potent inhibitor of SV40 decatenation in infected cells. To test liriodenine as a topoisomerase II poison, the filter assay for protein–DNA cross-links was adapted for use *in vitro* with purified DNA and enzyme. Purified ^{3}H-labeled SV40 DNA alone did not bind significantly to GF/C filters in 0.4 M GuHCl (Fig. 6A). The addition of purified human topoisomerase II resulted in a concentration-dependent increase in filter retention of the labeled DNA, indicating that it is becoming cross-linked to the topoisomerase II as expected. The higher the level of topoisomerase II, the higher the levels of trapped cleavable complexes. Based on this result, we chose a concentration of topoisomerase II that would cause about 18% of the labeled DNA substrate to be cross-linked to the topoisomerase in the absence of any drug. As expected, the

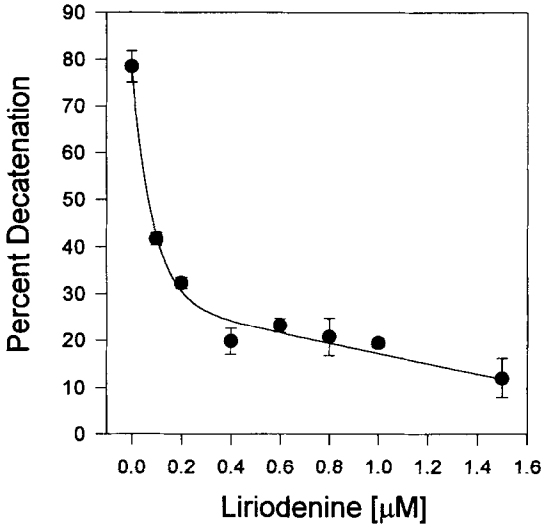

FIG. 5. Catalytic inhibition of human topoisomerase II *in vitro*. Human topoisomerase II was added to the reaction so that 80% of the kDNA was decatenated in the absence of liriodenine. Error bars represent ±SD, N = 4.

topoisomerase II poison *m*-AMSA greatly increased the levels of topoisomerase II–DNA cross-linking for this concentration of topoisomerase II (Fig. 6B). The decrease in *m*-AMSA-induced topoisomerase II–DNA cross-links at higher *m*-AMSA concentrations is also seen *in vivo* [15, 19] and is an example of the well-known self-inhibition of DNA-intercalating topoisomerase II poisons [26]. In this same assay, liriodenine caused low-level topoisomerase II cross-links to the labeled SV40 DNA substrate. The concentration–response curve was similar to that seen for *in vivo* protein–DNA cross-links, with a small peak at low concentrations of liriodenine, followed by a decrease and a slight rise at very high concentrations. This pattern was seen in five replications of the experiment.

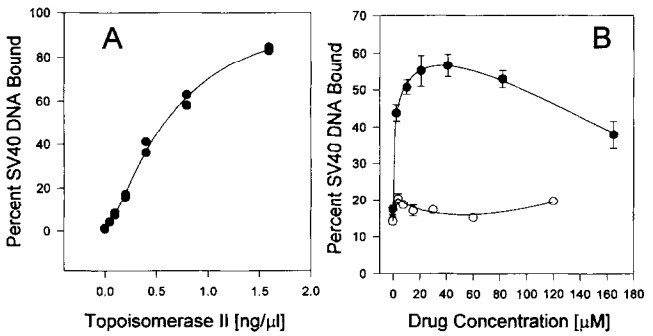

FIG. 6. *In vitro* assay for topoisomerase II–DNA cross-links stabilized by topoisomerase II poisons. (A) Relation between topoisomerase II concentration and cross-links to [^{3}H]Tdr-labeled SV40 DNA in the absence of drugs. (B) Concentration–response curve for topoisomerase II–DNA cross-links due to the topoisomerase II poison *m*-AMSA and liriodenine. Key: (●) *m*-AMSA; and (○) liriodenine at the indicated concentrations. Error bars represent ±SD, N = 4.

DISCUSSION

Our results show that the cytotoxic and antineoplastic drug liriodenine is a potent catalytic inhibitor of topoisomerase II both *in vivo* and *in vitro*. The rapid, efficient inhibition of the decatenation step in SV40 DNA replication indicates that liriodenine rapidly crosses cell membranes and efficiently blocks this topoisomerase II-dependent step in DNA replication. Topoisomerase II has also been shown to be required for the separation of newly replicated cellular chromosomes in eukaryotic cells [27]. Failure to separate the chromosomes results in cell death. Since purely catalytic inhibitors of topoisomerase II can have significant anticancer activity, it is likely that catalytic inhibition of topoisomerase II contributes to the anticancer activity of liriodenine.

The most commonly used antineoplastic topoisomerase II inhibitors (Adriamycin®, VP-16, VM-26, *m*-AMSA) are also topoisomerase poisons. These drugs stabilize topoisomerase II DNA strand passing reaction intermediates at a step in which the topoisomerase subunits are covalently linked to the DNA at the site of a DNA strand break. These intermediates are known as cleavable complexes. Drug-stabilized cleavable complexes are clearly cytotoxic since cells selected for drug resistance often express mutant topoisomerases that are insensitive to cleavable complex-stabilizing drugs. The *in vivo* assay for protein–DNA cross-links suggests that liriodenine may be a weak topoisomerase II poison. This was supported by the results of the *in vitro* assay for drug-induced topoisomerase II–DNA cross-links. The concentration–response for topoisomerase II–DNA cross-links was complex, with an initial steep increase to a small peak, followed by a decline and a slow increase again at higher concentrations. The same pattern was seen both *in vivo* and *in vitro*.

Verapamil significantly increased VP-16-induced topoisomerase II–DNA cleavable complexes in SV40-infected CV-1 cells. This shows that a verapamil-sensitive drug efflux pump for VP-16 is active in CV-1 cells. This pump is likely to be the P-glycoprotein MDR pump for which both verapamil and VP-16 are well known substrates. P-Glycoprotein is normally expressed at high levels in kidney [22], and CV-1 cells are monkey kidney cells. Verapamil increases the cytotoxicity of drugs such as VP-16 by competing for sites on P-glycoprotein. In the case of VP-16, this results in increased intracellular concentrations, elevated levels of VP-16-stabilized topoisomerase II–DNA cleavable complexes, and increased VP-16 cytotoxicity [25]. However, a verapamil-sensitive non-P-glycoprotein drug efflux mechanism has been reported [28]. Whatever the mechanism of the drug efflux pump in CV-1 cells, it is verapamil sensitive, and liriodenine is not a substrate.

Both *in vivo* and *in vitro* assays also indicate that liriodenine is a strong catalytic topoisomerase II inhibitor and a weak topoisomerase II poison with a multi-phasic concentration–response. Targeting of topoisomerase II could be the basis for liriodenine's documented biological activities.

Many topoisomerase II inhibitors have antineoplastic activity. Although liriodenine appears to be a weak topoisomerase II poison, a role for topoisomerase II poisoning in its anticancer activity cannot be ruled out. While some clinically useful antineoplastic drugs are strong topoisomerase II poisons (VP-16, VM-26, *m*-AMSA), some weak topoisomerase II poisons (for instance Adriamycin®) are effective anticancer drugs. Catalytic topoisomerase II inhibitors, which do not stabilize cleavable complexes, can also have good anticancer activity [13, 21]. Topoisomerase II is likely to be the relevant target for the anticancer activity of liriodenine.

Inhibition of topoisomerase II is also the likely basis of liriodenine's clastogenic and mutagenic activity. Topoisomerase II poisons are well known for causing chromosome damage, including illegitimate recombination [29, 30], deletions [31], sister chromatid exchanges [32], and translocations [33, 34]. Topoisomerase II poisons have also been reported to be mutagenic [35, 36].

This study has focused on inhibition of mammalian topoisomerase II by liriodenine, and we have not investigated inhibition of microbial type II topoisomerases. However, inhibitors of mammalian topoisomerase II often have activity against fungal and bacterial topoisomerase II. Bacterial topoisomerase II (DNA gyrase) is the target of a number of very effective antibiotics. On the basis of these considerations, it will not be surprising if liriodenine also has activity against microbial type II topoisomerases.

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References

1. Ginaudeau H, Leboeuf M and Cave A, Aporphine alkaloids. *Lloydia* **38:** 275–338, 1975.
2. Nimgirawath S and Taylor WC, Photochemical synthesis of 8H-benzo[g]-1,3-benzodioxolo-[6,5,4-de]quinolin-8-one (Liriodenine) via 7-methyl-6,7-dihydro-5H-benzo[g]-1,3-benzodioxolo[6,5,4-de]quinoline. *Aust J Chem* **36:** 1061–1065, 1983.
3. Dong XP, Modranodnra IO, Che CT, Fong HS and Farnsworth NR, Kmeriol and other aromatic constituents of *Kmeria duperreana*. *Pharmacol Res* **6:** 637–640, 1989.
4. Warthen D, Gooden EL and Jacobson M, Tumor inhibitors: Liriodenine, a cytotoxic alkaloid from *Annona glabra*. *J Pharm Sci* **58:** 637–638, 1969.
5. Clark AM, Watson ES, Ashfaq MK and Hufford CD, *In vivo* efficacy of antifungal oxoaporphine alkaloids in experimental disseminated candidiasis. *Pharmacol Res* **4:** 495–498, 1987.
6. Hufford CD, Funderburk MJ, Morgan JM and Robertson LW, Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera L.* *J Pharm Sci* **64:** 789–792, 1975.
7. Nozaka T, Watanabe F, Tadaki S, Ishino M, Morimoto I, Kunitomo J, Ishii H and Natori S, Mutagenicity of isoquinoline alkaloid, especially of the aporphine type. *Mutat Res* **240:** 267–279, 1990.
8. Tadaki S, Nozaka T, Yamada S, Ishino M, Morimoto I, Tanaka A and Kunitomo J, Clastogenicity of aporphine alkaloids *in vitro*. *J Pharmacobiodyn* **15:** 501–512, 1992.
9. Snapka RM, Ferrer CA, Sun NJ and Cassady JM, Drug discovery and evaluation with SV40. In: *The SV40 Replicon Model for Analysis of Anticancer Drugs* (Ed. Snapka RM), pp. 127–148. Academic Press, San Diego, 1996.
10. Snapka RM, Papovavirus models for mammalian replicons. In: *The SV40 Replicon Model for Analysis of Anticancer Drugs* (Ed. Snapka RM), pp. 1–3. Academic Press, San Diego, 1996.
11. Hirt B, Selective extraction of polyoma DNA from infected mouse cell cultures. *J Mol Biol* **26:** 365–369, 1967.
12. Snapka RM, Permana PA, Marquit G and Shin C-G, Two-dimensional agarose gel analysis of simian virus 40 DNA replication intermediates. *Methods Companion Methods Enzymol* **3:** 73–82, 1991.
13. Permana PA, Snapka RM, Shen LL, Chu DTW, Clement JJ and Plattner JJ, Quinobenoxazines: A class of novel antitumor quinolones and potent mammalian DNA topoisomerase II catalytic inhibitors. *Biochemistry* **33:** 11333–11339, 1994.
14. Permana PA, Ferrer CA and Snapka RM, Inverse relationship between catenation and superhelicity in newly replicated simian virus 40 daughter chromosomes. *Biochem Biophys Res Commun* **201:** 1510–1517, 1994.
15. Shin C-G, Strayer JM, Wani MA and Snapka RM, Rapid evaluation of topoisomerase inhibitors: Caffeine inhibition of topoisomerases *in vivo*. *Teratogenesis Carcinog Mutagen* **10:** 41–52, 1990.
16. Miller KG, Liu LF and Englund PT, A homogeneous type II DNA topoisomerase from HeLa cell nuclei. *J Biol Chem* **256:** 9334–9339, 1981.
17. Muller MT, Helal K, Soisson S and Spitzner JR, A rapid and quantitative microtiter assay for eukaryotic topoisomerase II. *Nucleic Acids Res* **17:** 9499–9503, 1989.
18. Cesarone CF, Bolognesi C and Santi L, Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Anal Biochem* **100:** 188–197, 1979.
19. Snapka RM and Yamasaki EF, Topoisomerase II and termination of DNA replication. *The SV40 Replicon Model for Analysis of Anticancer Drugs* (Ed. Snapka RM), pp. 65–100. Academic Press, San Diego, 1996.
20. Snapka RM, Topoisomerase inhibitors can selectively interfere with different stages of simian virus 40 DNA replication. *Mol Cell Biol* **6:** 4221–4227, 1986.
21. Roca J, Ishida R, Berger JM, Andoh T and Wang JC, Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc Natl Acad Sci USA* **91:** 1781–1785, 1994.
22. Fisher GA, Lum BL, Hausdorff J and Sikic BI, Pharmacological considerations in the modulation of multidrug resistance. *Eur J Cancer* **32A:** 1082–1088, 1996.
23. Wigler PW, Reversal of multidrug resistance in cancer cells. *J Natl Cancer Inst* **86:** 148–149, 1994.
24. Germann UA, P-glycoprotein—A mediator of multidrug resistance in tumour cells. *Eur J Cancer* **32A:** 927–944, 1996.
25. Yalowich JC and Ross WE, Verapamil-induced augmentation of etoposide accumulation in L1210 cells *in vitro*. *Cancer Res* **45:** 1651–1656, 1985.
26. Zwelling LA, Mayes J, Hinds M, Chan D, Altschuler E, Carroll B, Parker E, Deisseroth K, Radcliffe A, Seligman M, Li L and Farquhar D, Cross-resistance of an amsacrine-resistant human leukemia line to topoisomerase II reactive DNA intercalating agents. Evidence for two topoisomerase II directed drug actions. *Biochemistry* **30:** 4048–4055, 1991.
27. Page BD and Snyder M, Chromosome segregation in yeast. *Annu Rev Microbiol* **47:** 231–261, 1993.
28. Center MS, Non-P-glycoprotein multidrug resistance in cell lines which are defective in the cellular accumulation of drug. *Cytotechnology* **12:** 109–125, 1993.

29. Sperry AO, Blasquez VC and Garrard WT, Dysfunction of chromosomal loop attachment sites: Illegitimate recombination linked to matrix association regions and topoisomerase II. *Proc Natl Acad Sci USA* **86:** 5497–5501, 1989.
30. Bae Y-S, Kawasaki I, Ikeda H and Liu LF, Illegitimate recombination mediated by calf thymus DNA topoisomerase II *in vitro*. *Proc Natl Acad Sci USA* **85:** 2076–2080, 1988.
31. Bae Y-S, Chiba M, Ohira M and Ikeda H, A shuttle vector for analysis of illegitimate recombination in mammalian cells: Effects of DNA topoisomerase inhibitors on deletion frequency. *Gene* **101:** 285–289, 1991.
32. Dillehay LE, Denstman SC and Williams JR, Cell cycle dependence of sister chromatid exchange induction by DNA topoisomerase II inhibitors in Chinese hamster V79 cells. *Cancer Res* **47:** 206–209, 1987.
33. Negrini M, Felix CA, Martin C, Lange BJ, Nakamura T, Canaani E and Croce CM, Potential topoisomerase II DNA-binding sites at the breakpoints of a t(9;11) chromosome translocation in acute myeloid leukemia. *Cancer Res* **53:** 4489–4492, 1993.
34. Pedersen-Bjergaard J and Philip P, Balanced translocations involving chromosome bands 11q23 and 21q22 are highly characteristic of myelodysplasia and leukemia following therapy with cytostatic agents targeting at DNA–topoisomerase II. *Blood* **78:** 1147–1148, 1991.
35. Ferguson LR and Baguley BC, Topoisomerase II enzymes and mutagenicity. *Environ Mol Mutagen* **24:** 245–261, 1994.
36. Pommier Y, Zwelling LA, Kao-Shan C-S, Whang-Peng J and Bradley MO, Correlations between intercalator-induced DNA strand breaks and sister chromatid exchanges, mutations, and cytotoxicity in Chinese hamster cells. *Cancer Res* **45:** 3143–3149, 1985.