Camptothecin Induces Protein-linked DNA Breaks via Mammalian DNA Topoisomerase I*

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Camptothecin, a cytotoxic drug, is a strong inhibitor of nucleic acid synthesis in mammalian cells and a potent inducer of strand breaks in chromosomal DNA. Neither the equilibrium dialysis nor the unwinding measurement indicates any interaction between camptothecin and purified DNA. However, camptothecin induces extensive single strand DNA breaks in reactions containing purified mammalian DNA topoisomerase I. DNA breakage in vitro is immediate and reversible. Analyses of camptothecin-induced DNA breaks show that topoisomerase I is covalently linked to the 3' end of the broken DNA. In addition, camptothecin inhibits the catalytic activity of mammalian DNA topoisomerase I. We propose that camptothecin blocks the rejoicing step of the breakage-reunion reaction of mammalian DNA topoisomerase I. This blockage results in the accumulation of a cleavable complex which resembles the transient intermediate proposed for eukaryotic DNA topoisomerase I. The inhibition of nucleic acid synthesis and the induction of DNA strand breaks observed in vivo may be related to the formation of this drug-induced cleavable complex.

Camptothecin (Fig. 1) is a cytotoxic alkaloid isolated from Camptotheca acuminata (family Nyssaceae), a tree indigenous to China (reviewed in Ref. 1). Camptothecin has strong antitumor activity against a wide range of experimental tumors (2). It also inhibits both DNA and RNA synthesis in mammalian cells. The inhibition of RNA synthesis results in shortened RNA chains and is rapidly reversible upon drug removal (3). The inhibition of DNA synthesis, on the other hand, is only partially reversible upon drug removal (4, 5). Camptothecin is a much stronger inhibitor of DNA synthesis than RNA synthesis in human lymphocytes stimulated by phytohemagglutinin (2). However, in studies using either purified DNA polymerase or RNA polymerase, the inhibitory effect of camptothecin was not observed (4). Another prominent effect of camptothecin is the rapid and reversible fragmentation of cellular DNA in cultured mammalian cells (6). Studies of camptothecin analogs have suggested a correlation between suppression of tumor growth and the ability to cause fragmentation of DNA (reviewed in Ref. 1). Interestingly, camptothecin by itself does not cleave purified DNA. The cellular target(s) of camptothecin which is responsible for DNA fragmentation and inhibition of nucleic acid synthesis is still unknown.

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*The abbreviations used are: m-AMSA, 4′-(9-acridinylamino)-methanesulfon-m-anisidide; SDS, sodium dodecyl sulfate; VM-26, 4′-demethylpipodophyllotoxin thymidylide-β-D-glucoside; bp, base pairs.


**Fig. 1. Chemical structure of camptothecin (lactone form).**

There has been increasing evidence that mammalian DNA topoisomerase II is a common target for a number of antineoplastic agents (7–11). The acridine derivative, m-AMSA,1 and the epipodophyllotoxin, VM-26, are two representative antitumor drugs that affect the breakage-reunion reaction of mammalian DNA topoisomerase II. Both m-AMSA and VM-26 induce reversible DNA fragmentation and protein-DNA "cross-links" in cultured cells (10, 12, 13). In vitro and in vivo studies have suggested that these drugs block the rejoicing reaction of mammalian DNA topoisomerase II by stabilizing a cleavable complex (7–10). Treatment of the drug-induced cleavable complex with protein denaturants results in DNA strand breaks and the covalent linkage of a topoisomerase subunit to each 5' phosphoryl end of the broken DNA (7–10). Removal of the drugs results in an apparent repair of DNA strand breaks presumably via the continuation of the normal rejoicing reaction (7–10). Preliminary studies of the effect of camptothecin on L1210 cells and SV40 infected monkey cells have shown that camptothecin induces protein-linked, single-stranded DNA breaks on cellular and SV40 DNA. To determine if topoisomerase II is the target of camptothecin, we carried out in vitro studies using purified DNA topoisomerases. Surprisingly, camptothecin showed no effect on mammalian DNA topoisomerase II but exhibited strong inhibitory effects on mammalian DNA topoisomerase I.

EXPERIMENTAL PROCEDURES

Enzymes, Nucleic Acids, and Antitumor Drugs—DNA topoisomerase I was purified to homogeneity from HeLa cells and calf thymus glands using published procedures (14). DNA topoisomerase II from calf thymus was purified to homogeneity as described previously (15). Plasmid pBR322 dimer was purified by phenol deproteinization of clarified lysates followed by CaCl2/ethidium isopycnic centrifugation and gel filtration on an A-50m column. m-AMSA (NSC 249992) was

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obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. VM-26 was a gift from Bristol-Myers Co. The lactone form of camptothecin (NSC 94900), which is about 10-fold more potent than sodium camptothecin (NSC 100880) (16), was either obtained from National Cancer Institute or prepared by acidification of the sodium camptothecin (17). Briefly, 52 mg (0.13 mmol) of sodium camptothecin was dissolved in 10 ml of water. 10 ml of 0.01 N HCl was then added with stirring. A fluffy yellow precipitate formed as the pH dropped below 3. Camptothecin lactone was isolated by filtration as a pale green solid and was dried in vacuo to yield 30 mg (64%). All experiments were carried out using camptothecin lactone. Drugs were dissolved in dimethyl sulfoxide at 10 mM concentration and kept frozen (−20 °C) in aliquots.

Preparation of End-labeled pBR322 DNA—The procedure for end labeling of pBR322 DNA has been described previously (18). Briefly, pBR322 dimer was digested with EcoRI and dephosphorylated with alkaline phosphatase (18). Linearized pBR322 DNA was then either labeled at its 5' ends with the large fragment of Escherichia coli DNA polymerase I and [α-32P]dATP or labeled at its 5' ends with T4 polynucleotide kinase and [γ-32P]ATP (18). Unincorporated triphosphates were removed by two cycles of ethanol precipitation in the presence of 2.5 M ammonium acetate. For gel mapping experiments, the labeled DNA was further digested with HindIII restriction endonuclease to remove a small fragment (31 bp) containing one labeled end.

Topoisomerase Activity Assays—Topoisomerase I relaxation assays were done as described previously (14). Topoisomerase II catalytic activity was monitored by using the P4 unknotting assay (19).

Topoisomerase Cleavage Assays—Reaction mixtures (20 pl each) containing 40 mM Tris, pH 7.5, 100 mM KCl, 10 mM MgCl2, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg/ml bovine serum albumin, 50 ng of 32P-labeled DNA (or 20 μg/ml of unlabeled dimeric pBR322 DNA), 20 ng of calf thymus DNA topoisomerase I or II (or 70 ng of enzyme if unlabeled pBR322 DNA is used), and drugs were incubated at 37 °C for 30 min. The reactions were terminated by the addition of 2 μl of 5% SDS. Unless indicated, reaction mixtures were treated with 150 μg/ml proteinase K for another h at 37 °C.

Gel Electrophoresis—For mapping double strand breaks on DNA, samples were electrophoresed using a 1% agarose gel in TBE buffer (0.089 M Tris-borate, 0.002 M EDTA, pH 8.0) (20). For mapping single strand breaks, DNA samples (22 μl final volume) were denatured with 10 μl of 0.45 M NaOH, 50 mM EDTA, 15% sucrose, and 0.25 mg/ml bromocresol green. DNA samples were then analyzed using either a 1% agarose gel in TBE buffer or a 1% alkaline agarose gel in 30 mM NaOH and 1 mM EDTA.

Unwinding Measurements—Plasmid pBR322 DNA was linearized with the same restriction endonuclease and then ligated with T4 DNA ligase in the presence of different drugs. The ligation reaction (20 μl each) was done under conditions identical to those described for topoisomerase assay cleavages (see above) except that ATP (1 mM) was added. The temperature of the ligation reactions was carefully controlled and the reactions were terminated by the addition of 5 μl of a prewarmed stop solution (5% sarkosyl, 25% sucrose, 50 mM EDTA, and 0.05 mg/ml bromphenol blue). Gel electrophoresis was performed in the cold room (4 °C) using a 1.0% agarose gel and TBE electrophoresis buffer supplemented with 5 mM MgCl2.

DNA Binding Studies by Equilibrium Dialysis—One ml of sonicated calf thymus DNA (2.0 mM nucleotide) in 10 mM Tris-7.5, pH 7.4, 50 mM NaCl or in 20 mM potassium phosphate (pH 7.2) was placed in one well of a two-sided equilibrium dialysis cell. In the other well was placed 1 ml of camptothecin (2-50 μM) in buffer. After shaking at room temperature for 18 h, the fluorescence spectrum of each well was recorded and compared.

RESULTS

Camptothecin Does Not Affect Mammalian DNA Topoisomerase II in Vitro—To investigate whether mammalian DNA topoisomerase II is a target of camptothecin, purified calf thymus DNA topoisomerase II was used in an in vitro DNA cleavage assay (9). While both m-AMSA (Fig. 2, lanes H-L) and VM-26 (Fig. 2, lanes M-Q) induced DNA double strand breaks in this assay, camptothecin failed to induce any detectable cleavage above background (Fig. 2, lanes C-G). To test whether camptothecin can induce topoisomerase II-mediated single strand breaks, the following two experiments were done. First, the same samples were analyzed by alkaline gel electrophoresis to reveal single strand breaks and no increase in drug-induced cleavage was found (data not shown). Second, the same samples were also analyzed by the K-SDS precipitation procedure which was designed to assay protein-linked DNA cleavage (18). Using this assay, both m-AMSA- and VM-26-treated samples showed dose-dependent increases in topoisomerase II-mediated DNA cleavage, while camptothecin did not (data not shown). The possibility that camptothecin might inhibit the catalytic activity of topoisomerase II was also investigated using the P4 unknotting assay (19). Again, no inhibition by camptothecin was observed (Fig. 3, lanes C-G) while strong inhibition by both m-AMSA and VM-26 was quite obvious (Fig. 3, lanes H-L and M-Q, respectively).

Camptothecin Nicks DNA in the Presence of Purified Calf Thymus DNA Topoisomerase I—It is known that many antitumor drugs which induce protein-linked DNA breaks are DNA intercalators (reviewed in Ref. 9). To test whether camptothecin intercalates into DNA, an unwinding measurement was performed using linearized pBR322 DNA and T4 DNA ligase. In this assay, m-AMSA, which is a weak intercalator (7), was used as a positive control (Fig. 4, lanes H-L). VM-26, which neither intercalates into DNA nor binds to DNA (10, 11), was used as a negative control (Fig. 4, lanes M-Q). Similar to VM-26, camptothecin did not unwind DNA to any detectable extent at concentrations up to 125 μM (Fig. 4).
emission), which was unaffected by 2 mM calf thymus and control pRR922 and
and to predict that 32% of the camptothecin would be bound to the
ations, the binding equation of McGhee and von Hippel (21)
side of the dialysis cell.

4, under "Experimental Procedures." Calf thymus
measurement camptothecin binding by this method with similar results. This would result in an easily detectable increase in
fluorescence of camptothecin (370 nm excitation and

DNA (heat-denatured and quickly chilled) was tested for
difference was observed, we can conclude that the drug has
little or no affinity for calf thymus DNA.

The experiments of Fig. 6, lanes D–K). The extent of DNA cleavage depended on the drug concentration (Fig. 6) rather than the time course of incubation (Fig. 7, lanes D–K). (b) The formation of the single strand breaks is reversible. By lowering the temperature of the reaction to 0 °C after the first incubation but prior to the addition of SDS, the extent of cleavage was slightly reduced in a time-dependent fashion (Fig. 7, lanes L–N). In another experiment, the addition of high salt (0.5 M NaCl) to a preincubated reaction dramatically reduced the single strand breaks in a time-dependent fashion (Fig. 8, lanes B–H).

Topoisomerase I Is Covalently Linked to the 3'-Ends of Broken DNA Strands—It has been shown that calf thymus DNA topoisomerase I can be trapped on DNA by protein denaturant treatment (22). The trapped topoisomerase I is covalently linked to the 3'-phosphoryl ends of the broken DNA strands (22). It has been proposed that this trapped topoisomerase I-DNA complex may be related to the putative transient intermediate of the topoisomerase I relaxation re-

FIG. 4. Camptothecin does not unwind DNA. The unwinding measurement was done as described under "Experimental Procedures." Lane A, DNA control, no enzyme, no drug. Lane B, no drug. Lanes C–G, 0.2, 1.0, 5.0, 25, and 125 μM camptothecin, respectively. Lanes H–L, 0.2, 1.0, 5.0, 25, and 125 μM m-AMSA, respectively. Lanes M–Q, 0.2, 1.0, 5.0, 25, and 125 μM VM-126, respectively. Lanes R, S, and T, no enzyme but contained 125 μM of camptothecin, m-AMSA, and VM-26, respectively.

FIG. 5. Nicking of plasmid DNA by camptothecin in the presence of calf thymus DNA topoisomerase I. Reaction conditions were the same as described for the topoisomerase cleavage assay under "Experimental Procedures." Calf thymus DNA topoisomerase I (70 ng) and pBR322 DNA dimer (20 μg/ml) were used. Lane A, control pBR322 DNA. Lane B, no drug. Lanes C–G, 0.2, 1.0, 5.0, 25, and 125 μM camptothecin, respectively.

4, lanes C–G). The possibility that camptothecin might bind to DNA was also tested by equilibrium dialysis. The intense fluorescence of camptothecin (370 nm excitation and 448 nm emission), which was unaffected by 2 mM calf thymus DNA (data not shown) was used to monitor the drug concentration on both sides of the dialysis cell. In all cases (from 1 to 50 μM of camptothecin), the fluorescence spectra and intensities were identical on both the DNA side and the camptothecin side of the dialysis cell. At these DNA and drug concentrations, the binding equation of McGhee and von Hippel (21) predicts that 32% of the camptothecin would be bound to the DNA if the binding affinity was 500 and the site size was 2 bp. This would result in an easily detectable increase in fluorescence intensity on the DNA side of the cell. Since no difference was observed, we can conclude that the drug has little or no affinity for calf thymus DNA. The experiments were repeated using both Tris and phosphate buffers and in the presence of 10 mM MgCl₂. In addition, single-stranded DNA (heat-denatured and quickly chilled) was tested for camptothecin binding by this method with similar results.

Since DNA topoisomerase I is also known to break and rejoin DNA (22), the possibility that DNA topoisomerase I may mediate camptothecin-induced DNA damage was also investigated. Purified calf thymus DNA topoisomerase I was used in an in vitro reaction containing closed circular pBR322 DNA and camptothecin. With increasing concentrations of camptothecin, closed circular pBR322 DNA (form I) was converted to nicked circular DNA (form II) (Fig. 5, lanes C–G). This apparent nicking activity of camptothecin required DNA topoisomerase I since no nicking was observed in its absence (data not shown).

The nicking activity of camptothecin in the presence of calf thymus DNA topoisomerase I was further studied using end-labeled pBR322 DNA (see "Experimental Procedures"). To reveal single strand breaks, the reaction products were denatured with alkali prior to gel electrophoresis in neutral TBE buffer. In the presence of calf thymus DNA topoisomerase I and camptothecin, end-labeled pBR322 DNA was fragmented to smaller pieces which migrated faster in the gel (Fig. 6, lanes C–G). On the other hand, neither m-AMSA nor VM-26 induced any single strand breaks in the presence of calf thymus DNA topoisomerase I (Fig. 6, lanes H–L and M–Q, respectively). Furthermore, camptothecin alone did not induce any single strand breaks (Fig. 6, lane R). It thus appears that DNA topoisomerase I is specifically required for camptothecin-induced DNA damage. If one topoisomerase I molecule was bound to each nick on DNA, we calculated that approximately 10% of DNA topoisomerase I molecules in the reaction mixture are trapped on DNA in the presence of 1 μM of camptothecin.

Reversibility of Camptothecin-induced DNA Damage—Two interesting features of these single strand breaks were noted. (a) The induction of single strand breaks by camptothecin and topoisomerase I was immediate. The amounts of single strand breaks reached a plateau level within 30 s after mixing (Fig. 7, lanes D–K). The extent of DNA cleavage depended on the drug concentration (Fig. 6) rather than the time course of incubation (Fig. 7, lanes D–K). (b) The formation of the single strand breaks is reversible. By lowering the temperature of the reaction to 0 °C after the first incubation but prior to the addition of SDS, the extent of cleavage was slightly reduced in a time-dependent fashion (Fig. 7, lanes L–N). In another experiment, the addition of high salt (0.5 M NaCl) to a preincubated reaction dramatically reduced the single strand breaks in a time-dependent fashion (Fig. 8, lanes B–H).

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FIG. 6. Camptothecin induces site-specific DNA cleavage in the presence of topoisomerase I. 3' end-labeled pBR322 DNA and calf thymus DNA topoisomerase I were used in the topoisomerase cleavage assay as described under "Experimental Procedures." DNA samples were denatured prior to electrophoresis in neutral TBE buffer. Lane A, DNA control, no drug, no enzyme. Lane B, no drug. Lanes C–G, 0.2, 1.0, 5.0, 25, and 125 μM camptothecin, respectively. Lanes H–L, 0.2, 1.0, 5.0, 25, and 125 μM m-AMSA, respectively. Lanes M–Q, 0.2, 1.0, 5.0, 25, and 125 μM VM-26, respectively.
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Fig. 7. Time course and temperature reversal of camptothecin-induced DNA cleavage in the presence of topoisomerase I. 3'-end-labeled pBR322 DNA and calf thymus DNA topoisomerase I were used for the topoisomerase cleavage assay (see "Experimental Procedures"). DNA samples were denatured and analyzed by alkaline gel electrophoresis. Lane A, marker DNA. Lane B, DNA control, no enzyme, no drug. Lane C, no drug, 30-min incubation, respectively, at 37 °C. Lanes D–K, 0.5–1, 2–6, 10, 15, 20, and 30-min incubation at 37 °C, respectively, in the presence of 5 μM camptothecin. Lanes L–N, reactions after a 30-min preincubation at 37 °C were cooled on ice for 10, 20, and 30 min, respectively.

Fig. 8. Reversal of camptothecin-induced DNA cleavage by salt. The DNA cleavage assay was done as described in the legend of Fig. 7 except that the amount of calf thymus DNA topoisomerase I in each assay was doubled (40 ng each). Lane A, DNA control. Lane B, 30-min incubation at 37 °C in the presence of 25 μM camptothecin and 40 ng of topoisomerase I. Lanes C–H, after the preincubation (30 min at 37 °C), the NaCl concentration of the reaction mixtures was adjusted to 0.5 M by adding a 5 M NaCl stock solution. The reactions were terminated at 0.5, 1, 2, 10, 20, and 30 min after the addition of NaCl.

action (22). To test whether the topoisomerase I-DNA complex induced by camptothecin is related to this putative transient intermediate, the polarity of the DNA strand which is linked to topoisomerase I was determined. We followed the procedure described previously which was designed to determine the polarity of the DNA strand linked to topoisomerase II by using end-labeled DNA (18). If 3'-end-labeled pBR322 DNA was used as the substrate for topoisomerase cleavage and assuming that topoisomerase I is linked to the 3'-end of the broken DNA strand, one would expect topoisomerase I to be linked to the unlabeled DNA strand. In contrast, if 5'-end-labeled DNA was used, one would expect topoisomerase I to be linked to the labeled DNA strand (18). Based on this strategy, two different types of experiments were performed. (a) DNA samples following camptothecin and topoisomerase I treatment were denatured and analyzed by K-SDS precipitation (18). When 3'-end-labeled DNA was used as a substrate, no protein-DNA cross-links were detectable (data not shown). When 5'-end-labeled DNA was used as a substrate, camptothecin clearly produced a dose-dependent increase in protein-DNA cross-links (data not shown). (b) A second type of experiment involves alkaline gel electrophoresis to determine the polarity of the broken DNA strands which are linked to topoisomerase I. As demonstrated previously, protein-linked DNA strands migrate slower than deproteinized DNA strands (18). When 5'-end-labeled pBR322 DNA was treated with camptothecin and topoisomerase I, small single-stranded DNA fragments were produced which migrated slower than their protease-treated counterparts (Fig. 9, compare lanes N and O). When 3'-end-labeled DNA was treated under identical conditions, no such mobility shift was observed (Fig. 9, compare lanes D and E). These results strongly suggest that DNA topoisomerase I is covalently linked to the 3' ends of drug-induced DNA strand breaks.

Camptothecin Specifically Inhibits the Catalytic Activity of DNA Topoisomerase I—The effect of camptothecin on the catalytic activity of calf thymus DNA topoisomerase I was also studied using the relaxation assay. Differing from the cleavage assay, a very low concentration of topoisomerase I was used to monitor the catalytic activity of the enzyme. In the presence of increasing camptothecin, topoisomerase I relaxation activity was inhibited as evidenced by the appearance of monomer form I pBR322 DNA (Fig. 10, lanes C–G). Since camptothecin does not unwind DNA, the appearance

Fig. 9. DNA topoisomerase I was covalently linked to the 3' end of each broken DNA strand. Both 3'-end-labeled (lanes A–I) and 5'-end-labeled DNA (lanes J–S) were used in topoisomerase cleavage assays (see "Experimental Procedures"). DNA samples were denatured and analyzed by alkaline gel electrophoresis. DNA samples in lanes A, C, E, G, I, K, M, O, Q, and S were treated with proteinase K prior to gel electrophoresis. DNA samples in other lanes were not treated with proteinase K. Lanes A, J, and K, control, no enzyme, no drug. Lanes B, C, L, and M, no drug but contained 20 ng of calf thymus DNA topoisomerase I. Lanes D, E, N, and O, 25 μM camptothecin. Lanes F, G, P, and Q, 25 μM m-AMSA. Lanes H, I, l, and S, 25 μM VM-26.

Fig. 10. Camptothecin inhibits the catalytic activity of calf thymus DNA topoisomerase I. Topoisomerase I relaxation assays were done as described (14). 0.2 ng of calf thymus DNA topoisomerase I was used in each assay. Lane A, pBR322 DNA dimer, no enzyme, no drug. Lane B, no drug. Lanes C–G, 0.2, 1.0, 5.0, 25, and 125 μM camptothecin, respectively. Lanes H–L, 0.2, 1.0, 5.0, 25, and 125 μM m-AMSA, respectively. Lanes M–Q, 0.2, 1.0, 5.0, 25, and 125 μM VM-26, respectively. Lanes R, S, and T, no enzyme but contained 125 μM of camptothecin, m-AMSA, and VM-26, respectively.
of monomer form I pBR322 DNA is a strong indication of drug inhibition of enzyme activity. The same experiment was also repeated using m-AMSA and VM-26 to test their possible effect on topoisomerase I relaxation activity. With increasing m-AMSA, form I pBR322 DNA also accumulated (Fig. 10, lanes H–L). Since m-AMSA is known to unwind DNA upon intercalation, the increasing form I pBR322 DNA may be due to bound m-AMSA rather than inhibition of enzyme activity. Indeed, when relaxed pBR322 DNA was used as a substrate, the form I DNA also increased, suggesting that topoisomerase I activity was not inhibited (data not shown). VM-26, which is another topoisomerase II inhibitor, did not inhibit topoisomerase I relaxation activity (Fig. 9, lanes M–Q). These results indicate that DNA topoisomerase I is specifically inhibited by camptothecin but not by topoisomerase II inhibitors such as m-AMSA and VM-26 (7–11).

**DISCUSSION**

It has been demonstrated that a number of antitumor drugs affect the breakage-reunion reaction of mammalian DNA topoisomerase II by stabilizing a cleavable complex which upon treatment with protein-denaturants produces protein-linked DNA breaks (7–10). Our preliminary studies have clearly established that camptothecin affects only DNA topoisomerase I but not DNA topoisomerase II. Since DNA topoisomerase I is known to introduce transient protein-linked single strand breaks, the lack of double strand breaks on SV40 DNA following camptothecin treatment of SV40-infected monkey cells is thus explained. Although our present studies suggest that topoisomerase I is a target of camptothecin, additional biochemical and genetic studies are necessary to support this proposition.

The effect of camptothecin on purified DNA topoisomerase I is specific and pronounced. Even at 0.5 μM, camptothecin induces extensive nicking of DNA in the presence of DNA topoisomerase I. The rapid induction and the reversibility of camptothecin-induced DNA damage is unusual. It is possible that camptothecin may interact with DNA topoisomerase I or a topoisomerase I-DNA complex in a noncovalent manner. Based on our present knowledge of the enzyme mechanism, a simple working model for the drug action is shown schematically in Fig. 11. We assume that there are at least two enzyme intermediates, the noncleavable complex (Fig. 11A) and the cleavable complex (Fig. 11B), at rapid equilibrium. The cleavable complex (Fig. 11B) may be related to the putative transient intermediate in the normal enzyme reaction. Camptothecin may affect this equilibrium in such a way that the equilibrium concentration of drug-altered cleavable complex is greatly increased. Exposure of the cleavable complex to protein-denaturants (e.g. SDS or alkali) results in DNA single strand breaks and the covalent linkage of topoisomerase I to the 3'-phosphoryl end of the breaks through a tyrosine phosphate linkage (22). This drug-altered cleavable complex may be responsible for the inhibition of the relaxation activity of topoisomerase I. The cytotoxic effect and the stimulation of sister chromatid exchanges and chromosome aberrations may all be due to the accumulation of the drug-altered cleavable complex (23).

The biological function(s) of mammalian DNA topoisomerase I has not been established. Its swivel-like enzymatic activity suggests a possible function in DNA replication and RNA transcription (22). In vitro studies have shown that topoisomerase I is required for the elongation phase of adenovirus DNA replication (24). Studies in chicken embryos and Drosophila polytene chromosomes have also suggested that topoisomerase I may be involved in transcription (25, 26). Interference with the swivel mechanism of DNA topoisomerase I by camptothecin may thus lead to the inhibition of both DNA synthesis and RNA synthesis. Indeed, camptothecin has been shown to inhibit the replication of two DNA viruses, adenovirus and vaccinia virus, that replicate in the nucleus and cytoplasm of HeLa cells, respectively (1). The effect of camptothecin on cell cycle traverse has also been analyzed in synchronised cultures of Chinese hamster cells (27). Camptothecin did not block the initiation of DNA synthesis but prevented cells from progressing to mitosis (27).

Such an effect of camptothecin can be explained if topoisomerase I functions as a swivel for the elongation phase of DNA synthesis. The effect of camptothecin on transcription is also quite interesting. It has been shown that camptothecin inhibits the synthesis of ribosomal RNA in HeLa cells to a greater extent than 4–5 S RNA (4). The synthesis of precursor ribosomal RNA in the nucleus is also more sensitive to camptothecin than that of hnRNA (3). This differential effect of camptothecin on ribosomal RNA transcription can be explained by the recent findings that topoisomerase I is enriched in the nucleus (3). Whether the observed inhibition of nucleic acid synthesis and fragmentation of cellular DNA are due to drug interference with the topoisomerase swivel function in vivo remains to be determined. The establishment of camptothecin as a specific inhibitor of topoisomerase I may provide a powerful tool to probe the function(s) of this important nuclear enzyme. Understanding the mechanism of action of camptothecin may also be important in the establishment of topoisomerase I as a useful therapeutic target for cancer chemotherapy.

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