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Pharmacology

Molecular and cytotoxic effects of camptothecin, a topoisomerase I inhibitor, on trypanosomes and *Leishmania*

(*Trypanosoma brucei*/*Trypanosoma cruzi*/*Leishmania donovani*/chemotherapy/kinetoplast DNA)

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ABSTRACT **Parasites pose a threat to the health and lives of many millions of human beings. Among the pathogenic protozoa, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania donovani* are hemoflagellates that cause particularly serious diseases (sleeping sickness, Chagas disease, and leishmaniasis, respectively). The drugs currently available to treat these infections are limited by marginal efficacy, severe toxicity, and spreading drug resistance. Camptothecin is an established antitumor drug and a well-characterized inhibitor of eukaryotic DNA topoisomerase I. When trypanosomes or leishmania are treated with camptothecin and then lysed with SDS, both nuclear and mitochondrial DNA are cleaved and covalently linked to protein. This is consistent with the existence of drug-sensitive topoisomerase I activity in both compartments. Camptothecin also inhibits the incorporation of [^{3}H]thymidine in these parasites. These molecular effects are cytotoxic to cells *in vitro*, with EC_{50} values for *T. brucei*, *T. cruzi*, and *L. donovani*, of 1.5, 1.6, and 3.2 μM, respectively. For these parasites, camptothecin is an important lead for much-needed new chemotherapy, as well as a valuable tool for studying topoisomerase I activity.**

Parasites cause severely debilitating and fatal illness in millions of people throughout the world (1). Among these organisms are the flagellated protozoa, which include the trypanosomes and leishmania. African trypanosomes (*Trypanosoma brucei* sp.) cause sleeping sickness, a meningoencephalitis that is ultimately lethal if not treated (2). In large areas of South and Central America, *Trypanosoma cruzi* causes Chagas' disease, characterized by cardiomyopathy and massive dilatation of the esophagus and colon (3). Leishmaniasis presents a spectrum of disease, ranging from self-limited cutaneous ulcers, to erosive and disfiguring mucocutaneous disease, to lethal visceral infections (4). There are no effective drugs for Chagas' disease; the treatment of African trypanosomiasis and leishmaniasis typically involves the lengthy, parenteral administration of toxic agents (e.g., trivalent arsenicals, pentamidine, pentavalent antimonials); and resistant organisms are appearing. The need for new molecular targets on which to base future treatment strategies is clear and immediate.

In searching for such strategies, the DNA topoisomerases are attractive candidate targets. These enzymes have gained prominence not only because they are essential for the orderly synthesis of nucleic acids but also because they are the molecular site of action for numerous clinically important antibacterial and antitumor agents (e.g., the fluoroquinolones, etoposide, camptothecin). Topoisomerases mediate the topological manipulations of DNA required by cells during DNA replication and transcription (reviewed in refs. 5–7). Two classes of enzymes are defined, based on their catalytic mechanism: type I topoisomerases make single-stranded breaks in DNA, whereas type II topoisomerases make double-stranded breaks. Both types may be inhibited by compounds that trap the enzyme in midreaction as a protein–DNA complex, termed a "cleavable complex" (8, 9). When rapidly denatured (e.g., by SDS or alkali), cleavable complexes yield covalently linked DNA–protein adducts. In cells, the collision of DNA tracking machinery (involved in replication and transcription) with cleavable complexes is cytotoxic (10–12).

Topoisomerases I and II have been studied in pathogenic hemoflagellates and closely related organisms. Topoisomerase II has been purified (13–16), and several topoisomerase II genes have been sequenced (17–19). In previous studies, we found that topoisomerase II inhibitors promote extensive cleavage of nuclear and mitochondrial DNA in African trypanosomes, that some classical antitrypanosomal drugs are topoisomerase inhibitors, and that topoisomerase II inhibition is lethal to trypanosomes (20–23). Topoisomerase I has been purified from *T. cruzi* (24), *Leishmania donovani* (25), and *Crithidia fasciculata* (a related parasite of insects). The *Crithidia* enzyme immunolocalizes to the nucleus, but not the mitochondrion (26).

The mitochondrial DNA of trypanosomes and leishmania (termed kinetoplast DNA; kDNA) is a highly characteristic feature that is clearly visible in Giemsa-stained cells. As visualized by electron microscopy, purified kDNA is a massive network containing thousands of minicircles (≈1 kb each) and several dozen maxicircles (≈25 kb each) that are topologically interlocked with one another (see refs. 27–29 for reviews of kDNA). Manipulation of this massive and intricate structure during replication and transcription obviously entails numerous topological interconversions. Not surprisingly, trypanosome mitochondria contain large amounts of topoisomerase II (20), and a type II topoisomerase that immunolocalizes only to the mitochondrion has been isolated (30). However, efforts to purify a mitochondrial topoisomerase I in kinetoplastids have not succeeded, leading to the belief that kinetoplast-specific topoisomerases may be exclusively type II enzymes (26).

The following studies were designed to determine whether inhibition of type I topoisomerases might have molecular and cytotoxic effects on hemoflagellate parasites. For these studies, we chose camptothecin, a chemotherapeutic agent that inhibits topoisomerase I from eukaryotes but not from prokaryotes (9). To our knowledge, camptothecin has not previously been tested against topoisomerase I from kinetoplastids. We now demonstrate that camptothecin treatment of intact parasites yields protein–DNA adducts and that the drug is lethal to trypanosomes and leishmania. We also find that camptothecin traps minicircle DNA–protein complexes, providing evidence for the existence of a mitochondrial topoisomerase I in kinetoplastids.

MATERIALS AND METHODS

Drug Solutions. 20(*S*)-Camptothecin lactone, kindly provided by Leroy Liu (University of Medicine and Dentistry of

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Abbreviations: kDNA, kinetoplast DNA; DMSO, dimethyl sulfoxide.

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New Jersey, Piscataway, NJ), was stored in a desiccator at −20°C. Immediately prior to use, stock solutions and serial dilutions were made in dimethyl sulfoxide (DMSO; 99+%; Aldrich). The final concentration of DMSO in cell suspensions was constant within each experiment and did not exceed 1%. DMSO controls did not differ from controls without solvent.

Cultivation of Parasites. All studies were done with cells in the exponential phase of growth. *Trypanosoma equiperdum* (BoTat 24; African trypanosomes closely related to *T. brucei*) were isolated from rat blood as described (20). All other parasites were maintained in long-term axenic cultures. Bloodstream-form *T. brucei* (MiTat 1.2, strain 427) were grown at 37°C in Hepes-buffered Iscove's modified Dulbecco's medium without phenol red (Mediatech, Herndon, VA) and supplemented with glutamate, hypoxanthine, cysteine, thymidine, sodium pyruvate, mercaptoethanol, and bathocuproinedisulfonate (Aldrich) as described (31) and with 10% Serum Plus (Baxter) and 10% heat-inactivated fetal bovine serum (GIBCO/BRL).

T. cruzi epimastigotes (Silvio X-10 clone; kindly provided by Steven Nickell, Johns Hopkins School of Hygiene and Public Health, Baltimore) were maintained at 28°C in Hepes-buffered RPMI 1640 medium without phenol red (GIBCO/BRL), supplemented with 10% heat-inactivated fetal bovine serum, 4.9 g of thiopeptone per liter (Becton Dickinson), and 1 mg of hemin per liter (Sigma) (32). *T. cruzi* cultures contain various proportions of epimastigote, trypomastigote, amastigote, and staphylomastigote forms; however, in our experiments, >50% of cells were epimastigotes.

L. donovani promastigotes (MHOM/SD/62/1S-CL2D; kindly provided by Dennis Dwyer, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) were maintained at 26°C in Hepes-buffered M199 medium without phenol red (GIBCO/BRL) and supplemented with 10% heat-inactivated fetal bovine serum.

Assay for Cleavable Complexes. For *T. brucei* or *T. cruzi*, cells in medium (≈3 × 10^6 cells per ml) were radiolabeled with [^{3}H]thymidine (340 μCi/ml; 1 Ci = 37 GBq; 3 hr), washed three times with medium, resuspended at ≈3 × 10^6 cells per ml, and incubated for 30 min with DMSO or camptothecin. The cells were lysed with an equal volume of 2.5% SDS/0.8 mg of sheared calf thymus DNA per ml/10 mM EDTA. Covalent DNA–protein complexes were precipitated with KCl and counted (21). To measure total incorporation of [^{3}H]thymidine, aliquots of untreated cells were transferred to Whatman 3MM filters; the DNA was precipitated with trichloroacetic acid, washed, and counted.

L. donovani (6 × 10^6 cells per ml; 26°C) were treated for 30 min with no drug or with 50 μM camptothecin and lysed with SDS as described above. The lysate was divided and treated with or without proteinase K (1.7 mg/ml; 1 hr; 50°C) before phenol extraction. Samples not digested with proteinase K developed a substantial interface between the aqueous and phenol layers. DNA in the aqueous layers of the extractions was precipitated with ethanol, subjected to electrophoresis through agarose, stained with ethidium bromide, and visualized by transillumination as described (20).

Analysis of Minicircle DNA. *T. equiperdum* were harvested from rats, suspended in medium, and treated (6 × 10^7 cells per ml; 60 min; 37°C) with 0.63% DMSO or 125 μM camptothecin. (*T. equiperdum* were used in this experiment because they have only one sequence class of minicircles; *T. brucei* have many.) The cells were lysed with an equal volume of 10 mM Tris·HCl, pH 8.0/1 mM EDTA/1% SDS with or without 2 mg of proteinase K per ml, and the lysates were incubated at 55°C for 2 hr prior to phenol extraction. The aqueous phase of the extraction was concentrated with 1-butanol and dialyzed overnight against 10 mM Tris·HCl, pH 8.0/1 mM EDTA, and the DNA was precipitated with ethanol as described in detail (20). DNA was separated by electrophoresis (70 V; 18 hr; 1.5% agarose in buffer containing 1 μg of ethidium bromide per ml/90 mM Tris borate, pH 8.3/2.5 mM EDTA), transferred to GeneScreen, hybridized with ^{32}P-labeled homologous minicircle DNA, washed, and exposed to x-ray film (20).

Cytotoxicity Assay. A modification of the acid phosphatase cytotoxicity assay (33) was used for cultured *T. brucei* and *L. donovani* (details and validation of this assay will be published elsewhere). Briefly, exponentially growing organisms (≈10^5 cells per ml; 199 μl per well) were added to a 96-well microtiter plate containing the drug solutions or DMSO (1 μl per well). Each drug concentration was tested in quadruplicate. Plates were incubated for 24 hr (*T. brucei*; 37°C) or 46 hr (*L. donovani*; 26°C), and acid phosphatase activity in surviving cells was assayed. The production of *p*-nitrophenol from *p*-nitrophenyl phosphate, measured at 405 nm on a microtiter plate reader (Molecular Devices), correlated well with parasite counts. For *T. cruzi*, cells (5 × 10^5 cells per ml; 198 μl per well) were added to a 96-well microtiter plate containing drug solutions (2 μl per well) and incubated for 96 hr at 28°C. Each drug concentration was tested in triplicate. Cell counts for each sample were obtained with a hemocytometer. For all experiments, the data were fit to the equation for the sigmoidal E_{max} model (34).

RESULTS

Camptothecin Promotes Formation of Protein–DNA Adducts and Inhibits DNA Synthesis. To determine whether camptothecin stabilizes cleavable complexes between nuclear DNA and topoisomerase I in African trypanosomes, we used the KSDS precipitation method (21). Bloodstream-form *T. brucei* were labeled with [^{3}H]thymidine, washed, treated for 30 min with DMSO or various concentrations of camptothecin, and then lysed with SDS. The protein-bound DNA was precipitated with KCl, washed, and counted. Only 4% of the DNA in *T. brucei* is mitochondrial (35); hence, a signal of >4% of total incorporated thymidine is consistent with trapping nuclear DNA. In the absence of camptothecin, ≈8% of total DNA was precipitated (Fig. 1). This signal is generated by naturally occurring DNA–topoisomerase cleavable complexes. Camptothecin increased the endogenous level of DNA–protein complexes in a concentration-dependent fashion (Fig. 1). Moreover, lysates digested with proteinase K prior to the addition of KCl (which yield no detectable DNA in the precipitates) established that the DNA was covalently linked

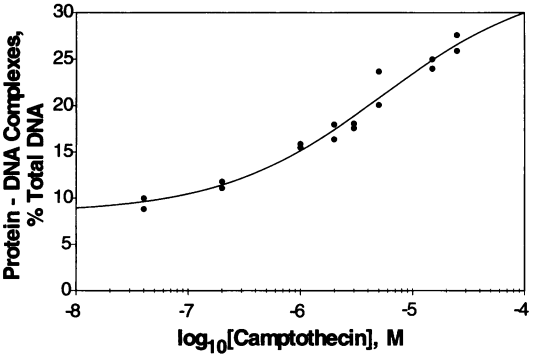

FIG. 1. Camptothecin promotes the *in vivo* formation of cleavable complexes with *T. brucei* nuclear DNA. Exponentially growing bloodstream forms (3 × 10^6 cells per ml) were radiolabeled with [^{3}H]thymidine (340 μCi/ml; 3 hr; 37°C), washed, incubated with DMSO or the indicated concentrations of camptothecin (30 min; 37°C), and lysed with SDS. Covalent DNA–protein complexes were precipitated with KCl and counted (21). The endogenous level of protein–DNA complexes, in DMSO controls, was 8.4% of total DNA. The data were fit by the sigmoidal E_{max} model (34) to obtain an EC_{50} value of 5.14 μM and an apparent maximum of 33.4% (R^2 = 0.97).

to protein. Similar evidence for camptothecin-promoted cleavable complexes was obtained for *T. cruzi* and *L. donovani* (data not shown).

To test whether topoisomerase I inhibition blocks DNA synthesis in trypanosomatids, the incorporation of [^{3}H]thymidine into *T. brucei* bloodstream forms, *T. cruzi* epimastigotes, and *L. donovani* promastigotes was monitored in the presence of camptothecin (Fig. 2). A concentration-dependent inhibition of [^{3}H]thymidine incorporation was observed for all three parasites at concentrations that generate cleavable complexes (Fig. 2).

Camptothecin Promotes kDNA Minicircle Linearization. To evaluate the possibility that a drug-sensitive topoisomerase I might be present in the mitochondria of African trypanosomes, we examined the effect of camptothecin treatment on minicircle DNA. *T. equiperdum* were treated with 0.63% DMSO or 125 μM camptothecin (60 min; 37°C) and lysed with SDS. The purified DNA was resolved by agarose gel electrophoresis, blotted, and probed with radiolabeled minicircle DNA (20). By this method, minicircles catenated in kDNA networks remain in the slot, and free minicircles, which are replication intermediates (36), enter the gel. Control cells yield the usual population of free minicircles, consisting largely of monomeric forms that are nicked circles, covalently closed circles, or full-length linearized molecules (Fig. 3, lane 1). Minicircle DNA from camptothecin-treated cells is clearly different (lane 2). First, there is an increase in the total mass of free minicircle DNA from camptothecin-treated cells (compare lanes 1 and 2; each lane contains DNA from the same number of cells). This indicates that minicircle DNA was released from networks into the free population. Second, there is a dramatic increase in the population of linearized minicircles. If the cell lysate is not digested with proteinase K prior to phenol extraction, the linearized minicircles are selectively lost from the aqueous phase of the extraction (lane 3). This is fully consistent with the notion that these molecules are covalently linked to protein and that they were generated from camptothecin-stabilized complexes between mitochondrial topoisomerase I and minicircle DNA *in vivo*.

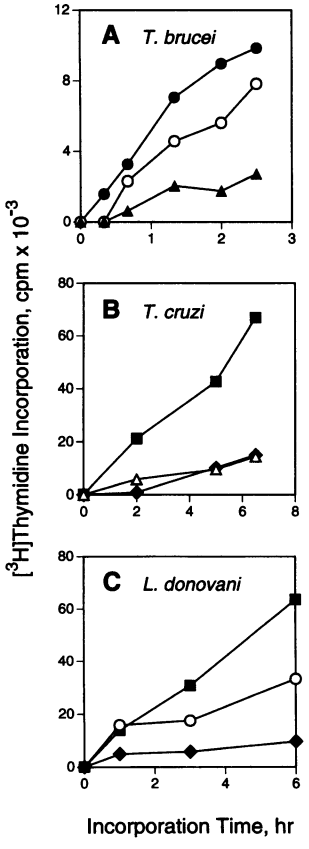

FIG. 2. Camptothecin inhibits DNA synthesis in parasites. (*A*) At time 0, [^{3}H]thymidine (400 μCi/ml) and 0.17% DMSO (●), 1 μM camptothecin (○), or 10 μM camptothecin (▲) were added to *T. brucei* cultures (2 × 10^6 cells per ml; 37°C). At the indicated times, 100-μl aliquots were withdrawn and processed to determine incorporation of label into acid-precipitable DNA. (*B*) Incorporation of [^{3}H]thymidine (90 μCi/ml) into the DNA of *T. cruzi* epimastigotes (2 × 10^6 cells per ml; 28°C) was monitored in the presence of 0.5% DMSO (■), 10 μM camptothecin (△), or 100 μM camptothecin (◆) as described in *A*; 50-μl aliquots were analyzed. (*C*) Incorporation of [^{3}H]thymidine (500 μCi/ml) into the DNA of *L. donovani* promastigotes (3 × 10^6 cells per ml; 26°C) was monitored in the presence of 0.5 DMSO (■), 1 μM camptothecin (○), or 100 μM camptothecin (◆) as described in *A*; 50-μl aliquots were assayed.

Camptothecin Is Cytotoxic to Kinetoplastids. To determine whether formation of cleavable complexes and inhibition of nucleic acid biosynthesis are cytotoxic to kinetoplastid parasites, we developed a cytotoxicity assay for *T. brucei* and *L. donovani* that is simple, rapid, quantitative, and nonradioactive. This assay is based on the acid phosphatase-mediated production of *p*-nitrophenol (33), which can be monitored in a microtiter plate reader. Dose–response curves yielded EC_{50} values with standard deviations of <10% of the means, and R^2 values for the fitted curves that were typically >0.95. Furthermore, the assay results agree well with those obtained from direct cell counts. We found that camptothecin is lethal to *T. brucei* and *L. donovani*, with EC_{50} values of 1.5 and 3.2 μM, respectively (Fig. 4). These parasites were completely eliminated at high concentrations of camptothecin. Camptothecin is also cytotoxic for *T. cruzi* (Fig. 4) with an EC_{50} value of 1.6 μM, as determined from direct cell counts. For *T. cruzi*, a population of residual organisms persisted in the cell lysis debris, a common occurrence after drug treatment of this pleiomorphic parasite (37). The persistent organisms may represent a subpopulation that is inherently resistant to camptothecin or, more likely, the residual cells may be at a developmental stage that is transiently insensitive to the drug.

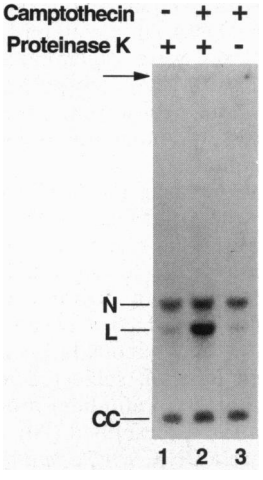

FIG. 3. Camptothecin induces cleavage of kDNA minicircles. *T. equiperdum* were harvested from rats, resuspended in medium, and treated (6 × 10^7 cells per ml; 60 min; 37°C) with 0.63% DMSO (lane 1) or 125 μM camptothecin (lanes 2 and 3). The SDS lysates were incubated with proteinase K (lanes 1 and 2) or without proteinase K (lane 3) prior to phenol extraction. The DNA was partially purified prior to separation by electrophoresis (1.5% agarose in buffer containing 1 μg of ethidium bromide per ml) (20). The DNA was transferred to GeneScreen, hybridized with ^{32}P-labeled homologous minicircle DNA, washed, and exposed to x-ray film. Each lane contains DNA from 3 × 10^7 cells. Nicked/gapped minicircles (N), linearized minicircles (L), covalently closed circular minicircles (CC), and the slot (arrow) are indicated.

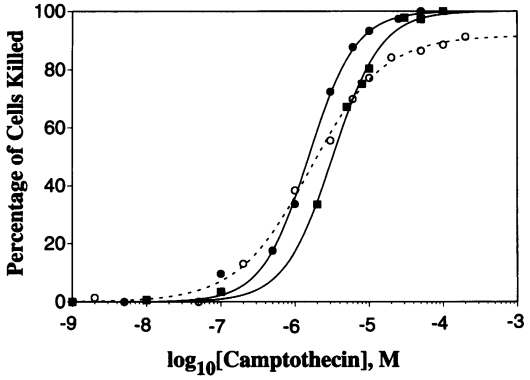

FIG. 4. Camptothecin kills kinetoplastid parasites in culture. *T. brucei* (●) and *L. donovani* (■) were assayed by a modification of the acid phosphatase method (33). Each drug concentration was tested in quadruplicate. For *T. cruzi* (○), each drug concentration was tested in triplicate, and cells were counted directly. Data were fit to the equation for the sigmoidal E_{max} model (34), generating EC_{50} values of 1.5 μM (*T. brucei*; $R^2 = 0.994$), 1.6 μM (*T. cruzi*; $R^2 = 0.997$), and 3.2 μM (*L. donovani*; $R^2 = 0.998$).

To test whether the killing mechanism might involve movement of DNA replication machinery, we treated *T. brucei* with aphidicolin (1 μM; a concentration that completely inhibits [^{3}H]thymidine incorporation in trypanosomes) during a 1-hr exposure to 10 μM camptothecin. The camptothecin cytotoxicity was reduced from 48%, in the absence of aphidicolin, to 15% in its presence, indicating that inhibition of DNA polymerase activity is protective.

DISCUSSION

Camptothecin, obtained in alcoholic extracts of *Camptotheca acuminata* trees, is an antitumor agent with an unusual heterocyclic structure (Fig. 5). Its structure was elucidated in the mid-1960s (38), and the first total synthesis of optically active 20(*S*)-camptothecin was reported in 1975 (39). In 1985, the discovery that camptothecin stabilizes cleavable complexes in the cell, between topoisomerase I and DNA, provided a satisfying explanation for the well-characterized ability of the drug to generate protein-linked breaks in DNA (40). The specificity of camptothecin for topoisomerase I was clearly demonstrated in studies with yeast cells that have disruptions in the *TOP1* gene (41), and it has proven a valuable tool for investigating the role of topoisomerase I in nucleic acid metabolism of eukaryotic cells (42, 43). Camptothecin is selectively toxic for malignant cells in culture, it inhibits entirely the growth of human cancer xenographs in nude mice, and it overcomes MDR1-mediated resistance, properties that account for its high therapeutic index (44–46). In clinical trials, the principal toxicity of orally administered therapeutic doses up to 8.7 mg/m^2, given daily for several months, is diarrhea (47). The importance of camptothecin is reflected in the wide array of analogs that have been synthesized (48–51), five of which are currently in clinical trials (52, 53).

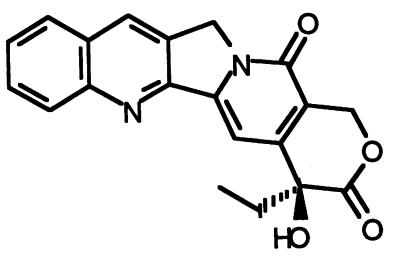

FIG. 5. 20(*S*)-Camptothecin is an antitumor alkaloid with an unusual pentacyclic structure.

We find that camptothecin promotes the formation of nuclear DNA–protein adducts in trypanosomes and leishmania. This provides evidence that these pathogenic hemoflagellates, which are among the most ancient of the eukaryotes (54–56), have camptothecin-sensitive topoisomerase I activity. Furthermore, and of obvious importance for chemotherapy, trypanosomes and leishmania are permeable to camptothecin. This contrasts with a number of other eukaryotes, including *C. fasciculata* (A.L.B., unpublished observation) and yeast (41), which are unaffected by camptothecin concentrations of 100 μM or more. Not surprisingly, cleavable complexes *in vivo* are accompanied by inhibition of DNA synthesis (Fig. 2).

More unexpected was the finding that camptothecin also promotes the formation of mitochondrial DNA–protein adducts. Minicircle DNA from trypanosomes treated with camptothecin shows a striking increase in the population of linearized, protein-bound, molecules (Fig. 3). These linearized forms derive from kDNA networks, and they may arise if multiple molecules of topoisomerase I bind close to one another on opposite strands of the minicircle or if topoisomerase I binds across from one of the preexisting nicks or gaps present in newly replicated minicircles. Topoisomerase I may also bind to covalently closed minicircles; however, this reaction would yield protein-bound nicked circles, which would remain catenated to the network and trapped in the slot of the gel. Camptothecin-promoted cleavage of minicircle DNA is consistent with the notion that trypanosome mitochondria contain topoisomerase I activity, and that this activity is more akin to eukaryotic than to prokaryotic topoisomerase I.

We cannot be absolutely certain that in trypanosomes the only intracellular target of camptothecin is topoisomerase I. However, support for this view is provided by camptothecin's absolute specificity for topoisomerase I in other systems (41, 57), by its inability to bind to DNA or to inhibit purified DNA or RNA polymerases (40, 58), and by its lack of activity against purified topoisomerase II from *T. brucei* (T.A.S., unpublished observation).

Camptothecin is cytotoxic to *T. brucei*, *T. cruzi*, and *L. donovani in vitro*, with EC_{50} values of 1–3 μM (Fig. 4). These levels are within the range for other antitrypanosomal drugs in our assay (e.g, 0.02 and 22 μM for pentamidine and difluoromethylornithine, respectively). In other cells, camptothecin cytotoxicity is S-phase specific and appears to require a collision between the DNA replication machinery and the drug-trapped, topoisomerase I cleavable complex (10–12). Aphidicolin, an inhibitor of DNA polymerase, partially protects *T. brucei* against camptothecin cytotoxicity. This suggests that a similar mechanism is operative in trypanosomes. The fact that aphidicolin affords incomplete protection may indicate that other DNA tracking processes (e.g., transcription) may also convert the cleavable complex into a cytotoxic lesion.

These studies support the concept that topoisomerase I is a suitable target for antiprotozoal chemotherapy and that camptothecin will be a valuable reagent for studying topoisomerase I in these organisms. In view of the severely limited resources available for development of new antiparasitic drugs (1, 59), the rather broad spectrum of antiparasitic activity of camptothecin, unusual for drugs against kinetoplastid parasites, is especially attractive.

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1. Warren, K. S. (1988) in *The Biology of Parasitism*, eds. Englund, P. T. & Sher, A. (Liss, New York), pp. 3–12.
2. Hajduk, S. L., Englund, P. T. & Smith, D. H. (1990) in *Tropical and Geographical Medicine*, eds. Warren, K. S. & Mahmoud, A. A. F. (McGraw–Hill, New York), pp. 268–281.
3. Nogueira, N. & Coura, J. R. (1990) in *Tropical and Geographical Medicine*, eds. Warren, K. S. & Mahmoud, A. A. F. (McGraw–Hill, New York), pp. 281–296.
4. Neva, F. & Sacks, D. (1990) in *Tropical and Geographical Medicine*, eds. Warren, K. S. & Mahmoud, A. A. F. (McGraw–Hill, New York), pp. 296–308.
5. Vosberg, H.-P. (1985) *Curr. Top. Microbiol. Immunol.* **114,** 19–102.
6. Sutcliffe, J. A., Gootz, T. D. & Barrett, J. F. (1989) *Antimicrob. Agents Chemother.* **33,** 2027–2033.
7. Wang, J. C. (1991) *J. Biol. Chem.* **266,** 6659–6662.
8. Nelson, E. M., Tewey, K. M. & Liu, L. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1361–1365.
9. Schneider, E., Hsiang, Y.-H. & Liu, L. F. (1990) *Adv. Pharmacol.* **21,** 149–183.
10. D'Arpa, P., Beardmore, C. & Liu, L. F. (1990) *Cancer Res.* **50,** 6919–6924.
11. Holm, C., Covey, J. M., Kerrigan, D., Kohn, K. W. & Pommier, Y. (1991) in *DNA Topoisomerases in Cancer*, eds. Potmesil, M. & Kohn, K. W. (Oxford Univ. Press, New York), pp. 161–171.
12. Hsiang, Y.-H., Lihou, M. G. & Liu, L. F. (1989) *Cancer Res.* **49,** 5077–5082.
13. Douc-Rasy, S., Kayser, A., Riou, J.-F. & Riou, G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7152–7156.
14. Chakraborty, A. K. & Majumder, H. K. (1987) *Mol. Biochem. Parasitol.* **26,** 215–224.
15. Shlomai, J., Zadok, A. & Frank, D. (1984) *Adv. Exp. Med. Biol.* **179,** 409–422.
16. Melendy, T. & Ray, D. S. (1989) *J. Biol. Chem.* **264,** 1870–1876.
17. Strauss, P. R. & Wang, J. C. (1990) *Mol. Biochem. Parasitol.* **38,** 141–150.
18. Fragoso, S. P. & Goldenberg, S. (1992) *Mol. Biochem. Parasitol.* **55,** 127–134.
19. Pasion, S. G., Hines, J. C., Aebersold, R. & Ray, D. S. (1992) *Mol. Biochem. Parasitol.* **50,** 57–68.
20. Shapiro, T. A., Klein, V. A. & Englund, P. T. (1989) *J. Biol. Chem.* **264,** 4173–4178.
21. Shapiro, T. A. & Englund, P. T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 950–954.
22. Shapiro, T. A. (1994) *Mol. Cell. Biol.* **14,** 3660–3667.
23. Shapiro, T. A. & Showalter, A. F. (1994) *Mol. Cell. Biol.* **14,** 5891–5897.
24. Riou, G. F., Gabillot, M., Douc-Rassy, S., Kayser, A. & Barrois, M. (1983) *Eur. J. Biochem.* **134,** 479–484.
25. Chakraborty, A. K., Gupta, A. & Majumder, H. K. (1993) *Indian J. Biochem. Biophys.* **30,** 257–263.
26. Melendy, T. & Ray, D. S. (1987) *Mol. Biochem. Parasitol.* **24,** 215–225.
27. Shapiro, T. A. & Englund, P. T. (1995) *Annu. Rev. Microbiol.* **49,** in press.
28. Simpson, L. (1987) *Annu. Rev. Microbiol.* **41,** 363–382.
29. Ray, D. S. (1987) *Plasmid* **17,** 177–190.
30. Melendy, T., Sheline, C. & Ray, D. S. (1988) *Cell* **55,** 1083–1088.
31. Carruthers, V. B. & Cross, G. A. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8818–8821.
32. Nickell, S. P., Gebremichael, A., Hoff, R. & Boyer, M. H. (1987) *J. Immunol.* **138,** 914–921.
33. Martin, A. & Clynes, M. (1991) *In Vitro Cell. Dev. Biol.* **27,** 183–184.
34. Holford, N. H. G. & Sheiner, L. B. (1981) *Clin. Pharmacokinet.* **6,** 429–453.
35. Borst, P., van der Ploeg, M., van Hoek, J. F. M., Tas, J. & James, J. (1982) *Mol. Biochem. Parasitol.* **6,** 13–23.
36. Ryan, K. A., Shapiro, T. A., Rauch, C. A. & Englund, P. T. (1988) *Annu. Rev. Microbiol.* **42,** 339–358.
37. Kierszenbaum, F. (1984) in *Parasitic Diseases: The Chemotherapy*, ed. Mansfield, J. M. (Dekker, New York), pp. 133–151.
38. Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T. & Sim, G. A. (1966) *J. Am. Chem. Soc.* **88,** 3888–3890.
39. Corey, E. J., Crouse, D. N. & Anderson, J. E. (1975) *J. Org. Chem.* **40,** 2140–2141.
40. Hsiang, Y.-H., Hertzberg, R., Hecht, S. & Liu, L. F. (1985) *J. Biol. Chem.* **260,** 14873–14878.
41. Nitiss, J. & Wang, J. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7501–7505.
42. Yang, L., Wold, M. S., Li, J. J., Kelly, T. J. & Liu, L. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 950–954.
43. Snapka, R. M. (1986) *Mol. Cell. Biol.* **6,** 4221–4227.
44. Giovanella, B. C., Hinz, H. R., Kozielski, A. J., Stehlin, J. S., Silber, R. & Potmesil, M. (1991) *Cancer Res.* **51,** 3052–3055.
45. Pantazis, P., Hinz, H. R., Mendoza, J. T., Kozielski, A. J., Williams, L. J., Jr., Stehlin, J. S., Jr., & Giovanella, B. C. (1992) *Cancer Res.* **52,** 3980–3987.
46. Chen, A. Y., Yu, C., Potmesil, M., Wall, M. E., Wani, M. C. & Liu, L. F. (1991) *Cancer Res.* **51,** 6039–6044.
47. Stehlin, J. S., Natelson, E. A., Hinz, H. R., Giovanella, B. C., de Ipolyi, P. D., Fehir, K. M., Trezona, T. P., Vardeman, D. M., Harris, N. J., Marcee, A. K., Kozielski, A. J. & Ruiz-Razura, A. (1995) in *Camptothecins: New Anticancer Agents*, eds. Potmesil, M. & Pinedo, H. (CRC, Boca Raton, FL), pp. 59–65.
48. Cai, J.-C. & Hutchinson, C. R. (1983) in *The Alkaloids*, ed. Brossi, A. (Academic, New York), pp. 101–137.
49. Wall, M. E. & Wani, M. C. (1991) in *DNA Topoisomerases in Cancer*, eds. Potmesil, M. & Kohn, K. W. (Oxford Univ. Press, New York), pp. 93–102.
50. Kingsbury, W. D., Boehm, J. C., Jakas, D. R., Holden, K. G., Hecht, S. M., Gallagher, G., Caranfa, M. J., McCabe, F. L., Faucette, L. F., Johnson, R. K. & Hertzberg, R. P. (1991) *J. Med. Chem.* **34,** 98–107.
51. Crow, R. T. & Crothers, D. M. (1992) *J. Med. Chem.* **35,** 4160–4164.
52. Slichenmyer, W. J., Rowinsky, E. K., Donehower, R. C. & Kaufmann, S. H. (1993) *J. Natl. Cancer Inst.* **85,** 271–291.
53. Costin, D. & Potmesil, M. (1994) *Adv. Pharmacol.* **29,** 51–72.
54. Fernandes, A. P., Nelson, K. & Beverley, S. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11608–11612.
55. Landweber, L. F. & Gilbert, W. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 918–921.
56. Maslov, D. A., Avila, H. A., Lake, J. A. & Simpson, L. (1994) *Nature (London)* **368,** 345–348.
57. Eng, W.-K., Faucette, L., Johnson, R. K. & Sternglanz, R. (1988) *Mol. Pharmacol.* **34,** 755–760.
58. Horwitz, M. S. & Horwitz, S. B. (1971) *Biochem. Biophys. Res. Commun.* **45,** 723–727.
59. Aldhous, P. (1994) *Science* **264,** 1857–1859.