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Topoisomerase Inhibitors Can Selectively Interfere with Different Stages of Simian Virus 40 DNA Replication

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I have found that antineoplastic drugs which are known to be inhibitors of mammalian DNA topoisomerases have pronounced and selective effects on simian virus 40 DNA replication. Ellipticine, 4′-(9-acridinylamino)methanesulfon-*m*-aniside, and Adriamycin blocked decatenation of newly replicated simian virus 40 daughter chromosomes in vivo. The arrested decatenation intermediates produced by these drugs contained single-strand DNA breaks. Ellipticine in particular produced these catenated dimers rapidly and efficiently. Removal of the drug resulted in rapid reversal of the block and completion of decatenation. The demonstration that these drugs interfere with decatenation suggests that they may exert their cytotoxic and antineoplastic effects by preventing the separation of newly replicated cellular chromosomes. Camptothecin rapidly breaks replication forks in growing Cairns structures. It is likely that the target of camptothecin is the "swivel" topoisomerase required for DNA replication and that it is located at or very near the replication fork in vivo. Evidence is presented that many of the broken Cairns structures are in fact half-completed sister chromatid exchanges. One pathway for the resolution of these structures is completion of the sister chromatid exchange to produce a circular head-to-tail dimer.

DNA topoisomerases are now recognized as important targets for cancer chemotherapy (for reviews, see references 20 and 32). A number of cytotoxic drugs which were found to have antineoplastic properties have recently been shown to be topoisomerase inhibitors (3, 12, 15, 25, 26, 31, 33). These drugs typically interfere with the breakage-reunion cycles of topoisomerases to produce single- and double-strand DNA breaks. Recent studies have shown a correlation between cytotoxicity and levels of DNA strand breakage caused by these drugs (6, 7, 21). It is also possible that these drugs can exert their cytotoxic and antineoplastic effects through interference with reactions that require topoisomerases, such as replication fork progression, transcription, and separation (decatenation) of newly replicated daughter chromosomes. The work reported here demonstrates that topoisomerase inhibitors can selectively and reversibly interfere either with replication forks or with the decatenation process in simian virus 40 (SV40) DNA replication. Improved understanding of the modes of action of this class of drugs should aid in the rational design of a second generation of antineoplastic topoisomerase inhibitors.

MATERIALS AND METHODS

Cell culture and virus infection. African green monkey kidney cells (CV-1) were grown in Eagle minimal essential medium (Gibco) supplemented with 14 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.2) and 4 mM $NaHCO_3$. Cells were infected with SV40 strain 777 at a multiplicity of 10 PFU/cell, and experiments were carried out 36 h after infection at the peak of SV40 DNA replication.

Radiolabeling and preparation of viral DNA. Replicating SV40 DNA was pulse-labeled with [*methyl*-^{3}H]thymidine as indicated, and viral DNA was extracted by the method of Hirt (10). The Hirt supernatant was treated with proteinase K (0.1 mg/ml) at 37°C overnight or at 45°C for 4 h. The DNA was then extracted with chloroform-isopropanol (24:1) and precipitated with 3 volumes of 95% ethanol either at −70°C for 15 min or at −20°C overnight. The DNA pellet was dried briefly under vacuum (2 to 5 min) and then taken up in gel loading buffer.

Drugs. Ellipticine (Sigma Chemical Co.) was dissolved in 0.01 N HCl at a concentration of 1.0 mg/ml. Adriamycin (Adria Laboratories) was dissolved in deionized water at 2 mg/ml. 4′-(9-Acridinylamino)methanesulfon-*m*-aniside (m-AMSA) was obtained from the National Cancer Institute (NCI), Division of Cancer Treatment, Drug Synthesis and Chemistry Branch (NSC 249992), and was dissolved in dimethyl sulfoxide at 3.96 mg/ml. Camptothecin was obtained from the NCI Division of Cancer Treatment, Natural Products Branch (NSC 94600), and was dissolved in dimethyl sulfoxide at 3.48 mg/ml.

Electrophoretic analysis and fluorography. Both neutral and alkaline agarose gel electrophoresis was carried out as described by Sundin and Varshavsky (23). Neutral agarose gel electrophoresis was done in 0.8% agarose on horizontal gels submerged in running buffer composed of 80 mM Tris hydrochloride (pH 7.5), 5 mM sodium acetate, 1.0 mM sodium EDTA, and 0.1% sodium dodecyl sulfate. Running times and loading slot widths varied. The voltage was maintained at 1.0 V/cm. For two-dimensional neutral-alkaline gels, samples were loaded in duplicate with one lane being cut out and soaked in alkaline running buffer (30 mM NaOH, 2.0 mM EDTA, 1.0 mM EGTA). After equilibration in alkaline buffer, this strip was cast into a horizontal slab of 1.5% agarose prepared in the same buffer and run submerged at 2.33 V/cm. Running times varied as indicated. All gels were fixed and permeated with 2,5-diphenyloxazole (PPO) by immersion with gentle shaking for 4 h in glacial acetic acid containing 20 g of PPO per liter (18). The PPO was precipitated in the gel by immersion of the fixed gel in running tap water for 4 to 6 h. The gels were then dried under vacuum at 60°C, folded in Saran Wrap, and placed against Kodak X-Omat AR film at −70°C for exposure.

RESULTS

Analysis by neutral agarose gel electrophoresis. Treatment of SV40-infected CV-1 cells with either ellipticine or

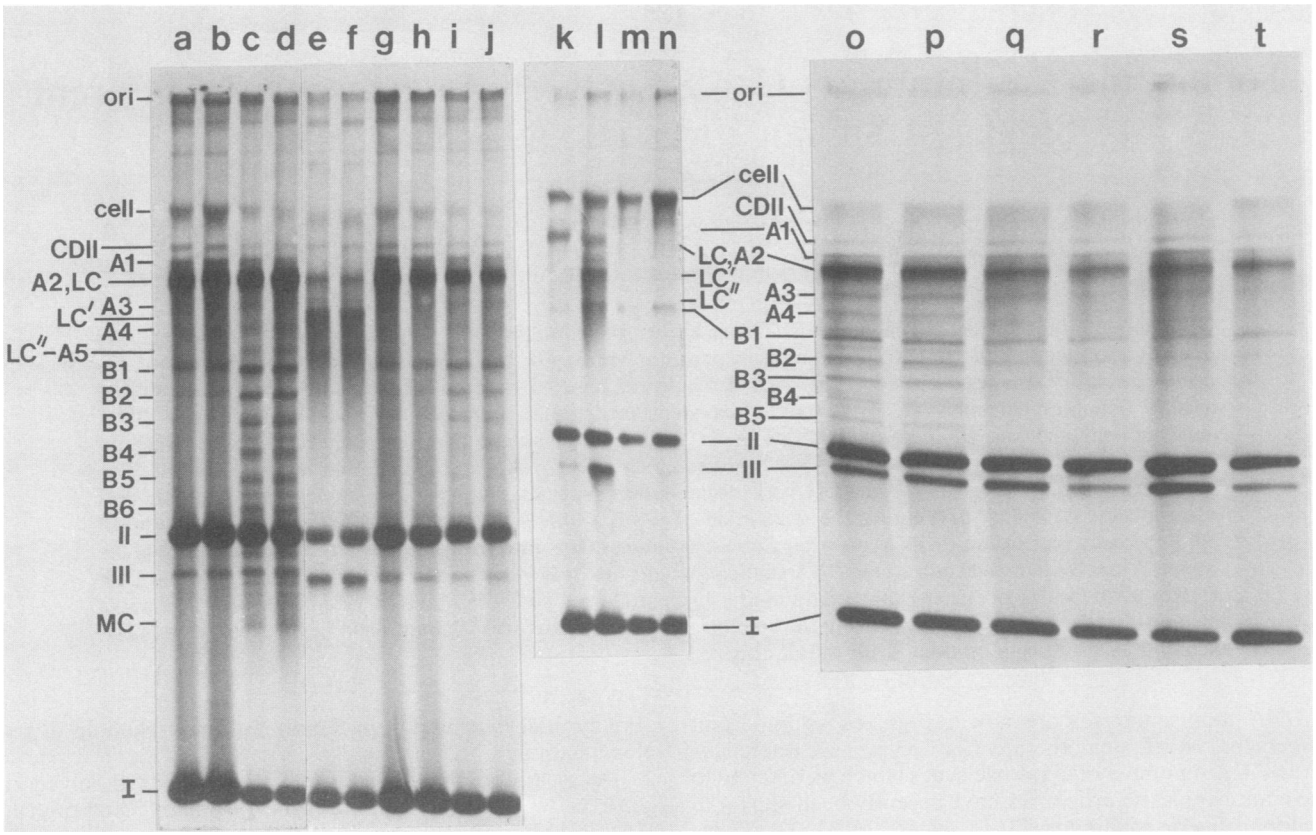

FIG. 1. Comparative effects of topoisomerase inhibitors on SV40 DNA replication as analyzed by one-dimensional neutral agarose gel electrophoresis. Each lane represents the Hirt-extracted SV40 DNA from one 35-mm-diameter plate of infected CV-1 cells. Most experiments were done in duplicate. Electrophoresis was carried out at 1.0 V/cm in 0.8% agarose gels (23) for 43.5 h (lanes a through j) or 33 h (lanes k through t). Lanes: a and b, at 36 h postinfection, [*methyl*-^{3}H]thymidine was added (250 μCi/ml, 62 Ci/mmol) for 30 min, and the viral DNA was extracted by the method of Hirt (10); c and d, labeled and extracted as in a and b, but ellipticine (40 μM) was added 15 min after the start of labeling; e and f, labeled and extracted as in a and b, but camptothecin (40 μM) was added 15 min after the start of labeling; g and h, solvent control for lanes e, f, i, and j, dimethyl sulfoxide added (4 μl/ml) 15 min after the start of labeling; i and j, same as a and b, but m-AMSA (40 μM) was added 15 min after the start of labeling; k through n, pulse-chase experiment with camptothecin: k, pulse-labeling with no drug treatment as in a and b; l, labeled, treated with camptothecin, and extracted as in e and f; m and n, labeled and treated with camptothecin as in l, but chased with drug-free medium containing 20 μM unlabeled thymidine for 15 min before extraction; o and p, labeled with [*methyl*-^{3}H]thymidine (100 μCi/ml, 90 Ci/mmole) for 30 min, Adriamycin (37 μM) added 15 min after the start of labeling; q and r, same as o and p, but 3.7 μM Adriamycin; s and t, same as o and p, but 0.37 μM Adriamycin. Abbreviations: Ori, origin slot; cell, band of cellular DNA; CDII, relaxed circular dimer; A1–A5, catenated dimers with both members relaxed and degree of catenation indicated by the number; B1–B6, catenated dimers with one member of each dimer relaxed and the other superhelical; LC, late Cairns structure; II, form II (relaxed circular) SV40 monomer; III, form III (unit-length linear) SV40; I, form I, superhelical SV40 monomer; MC, band seen at the lower (anode) end of the A-plus-B ladder in ellipticine-treated cells and assumed to represent the maximum level of catenation; LC′ and LC″, altered late cairns structures seen in camptothecin-treated cells.

camptothecin during pulse-labeling with [*methyl*-^{3}H]thymidine resulted in pronounced changes in the patterns of SV40 DNA replication intermediates. Pulse-labeling in the absence of a drug gave a typical pattern when analyzed by high-resolution gel electrophoresis and fluorography (Fig. 1, lanes a and b). The most prominent bands represent form I (superhelical), form II (relaxed circular), and form III (linear) monomeric SV40 DNAs, and the late Cairns structure. A smooth "smear" of growing Cairns structures extended from form I to the late Cairns structure. Also seen in such a pulse-labeling experiment were bands representing cellular DNA (present in uninfected cells), the circular head-to-tail dimer, and members of the A and B catenated dimer families with fewer catenations. As demonstrated by Sundin and Varshavsky (23), the A family is composed of dimers in which both members of each catenated pair are relaxed, the B family is composed of dimers in which one member is relaxed and the other is superhelical, and the C family is composed of dimers in which both members of each pair are superhelical. The addition of 40 μM ellipticine during pulse-labeling resulted in a dramatic enhancement of the A and B dimer bands representing higher degrees of catenation. New bands were also seen on the anode side of form III in the ellipticine-treated samples (Fig. 1, lanes c and d). For both A and B catenated dimers, each unit increase in catenation number produces a band with a higher electrophoretic mobility in this gel system (23, 29). Thus, the new bands resulting from ellipticine treatment were probably highly catenated dimers. The anode end of this ladder was marked by a particularly intense band. This may represent a major kinetic intermediate of definite (highest) catenation number. However, the bands in the ladder became more closely spaced as this point was neared, and it is possible that the heavy band represents unresolved bands of dimers with very high catenation numbers.

The addition of 40 μM camptothecin during pulse-labeling

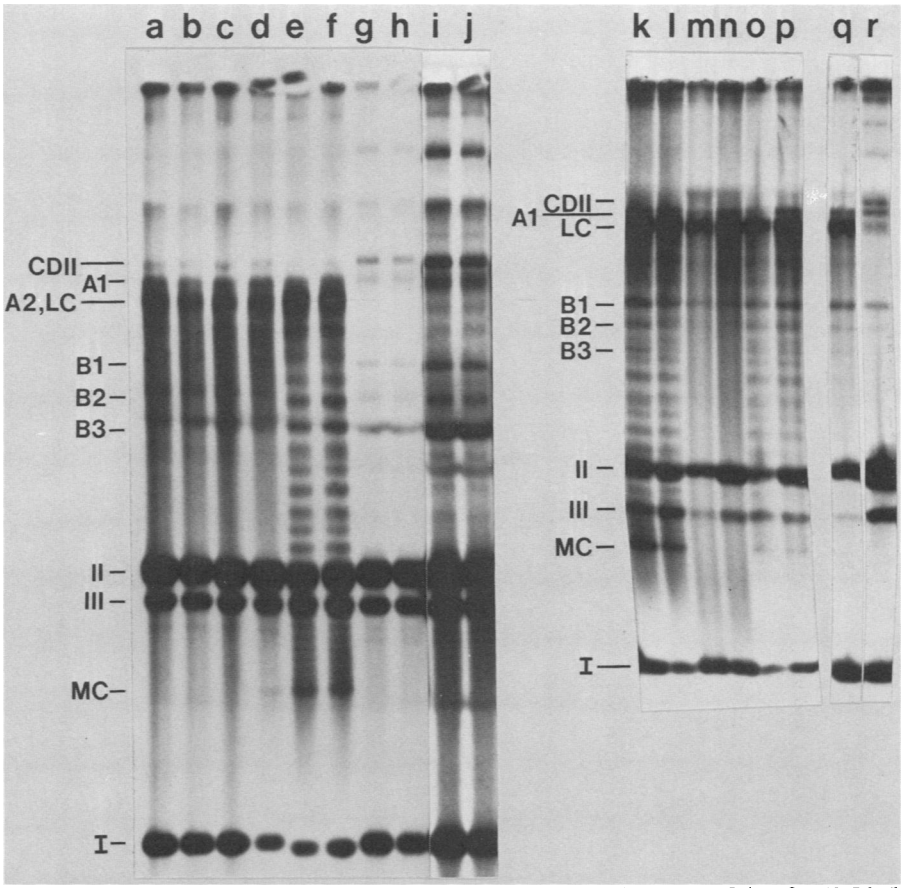

FIG. 2. Pulse-labeling experiments with ellipticine treatments. Electrophoresis was done at 1.0 V/cm for 43.5 h (lanes a through j) or 36 h (lanes k through r). Lanes: a and b, labeled for 30 min with [*methyl*-^{3}H]thymidine (250 μCi/ml, 62 Ci/mmol) at 36 h postinfection and then Hirt extracted; c and d, solvent control for ellipticine, labeled and extracted as in a and b but 10 μl of 0.01 N HCl was added per ml of labeling medium 15 min after the start of labeling; e and f, labeled and extracted as in a and b, but 40 μM ellipticine was added 15 min after the start of labeling; g and h, labeled and treated as in e and f, but label and drug were removed after 30 min of labeling and excess medium without drug or label was added for 1 h before Hirt extraction; i and j, longer fluorographic exposure of g and h; k and l, labeled for 20 min, with ellipticine (40 μM) added 5 min after the start of labeling; m and n, labeled for 20 min and then extracted; o and p, label and 40 μM ellipticine added together for 15 min; q, same as o and p, but followed by a 5-min chase in drug-free medium containing 20 μM unlabeled thymidine; r, same as o and p, but chased for 15 min in drug-free medium with 20 μM unlabeled thymidine. Abbreviations: see Fig. 1 legend.

also resulted in a dramatic but very different distribution of SV40 DNA replication intermediates (Fig. 1, lanes e and f). The normal Cairns smear was replaced by two thick bands located between the late Cairns structure and form II. These bands had the appearance of compressed Cairns smears, with sharp boundaries on the cathode sides and smearing out on the anode sides. The form III monomer band was also markedly enhanced as a result of camptothecin treatment. When the pulse-labeled camptothecin-treated cells were chased with drug-free medium containing unlabeled thymidine, the new bands disappeared, as did much of the form III band (Fig. 1, lanes k through n).

Addition of m-AMSA (Fig. 1, lanes i and j) or Adriamycin (Fig. 1, lanes o and p) during pulse-labeling of SV40 DNA replication intermediates resulted in an enhancement of the A and B catenated dimer bands. This effect was similar to that caused by ellipticine but much weaker. The more highly catenated forms were not seen. Lower concentrations of Adriamycin had no detectable effect (Fig. 1, lanes q through t).

The block to decatenation caused by ellipticine could be reversed by removal of the drug and addition of excess drug-free medium (Fig. 2, lanes a through h). Longer fluorographic exposure of lanes g and h (Fig. 2, lanes i and j) showed that traces of the highly catenated forms remained after 1 h in drug-free medium. Shortening of the interval between addition of label and addition of ellipticine resulted in lower overall labeling and a decrease in the intensity of the form I and MC bands relative to that of the other bands in the ladder (Fig. 2, lanes k through p). Although small traces of the ellipticine-generated ladder lingered during chases in drug-free medium, it is clear that most of these catenated dimers disappeared within a few minutes of the removal of the drugs (Fig. 2, lanes q and r).

Two-dimensional neutral-alkaline gel analysis of SV40 DNA replication intermediates. Alkaline denaturation of nicked catenated dimers produces full-length linear single-stranded DNAs. When analyzed by alkaline gel electrophoresis, these migrate with the full-length linear strands arising from denaturation of form III. Two-dimensional neutral-alkaline gels display these dimers as a horizontal row of spots extending from the late Cairns structure toward the anode in the first dimension and at the level of full-length linear DNA in the alkaline second dimension. When the SV40 DNA replication intermediates produced by ellipticine were analyzed in this way (Fig. 3B), the A and B dimer bands increased relative to

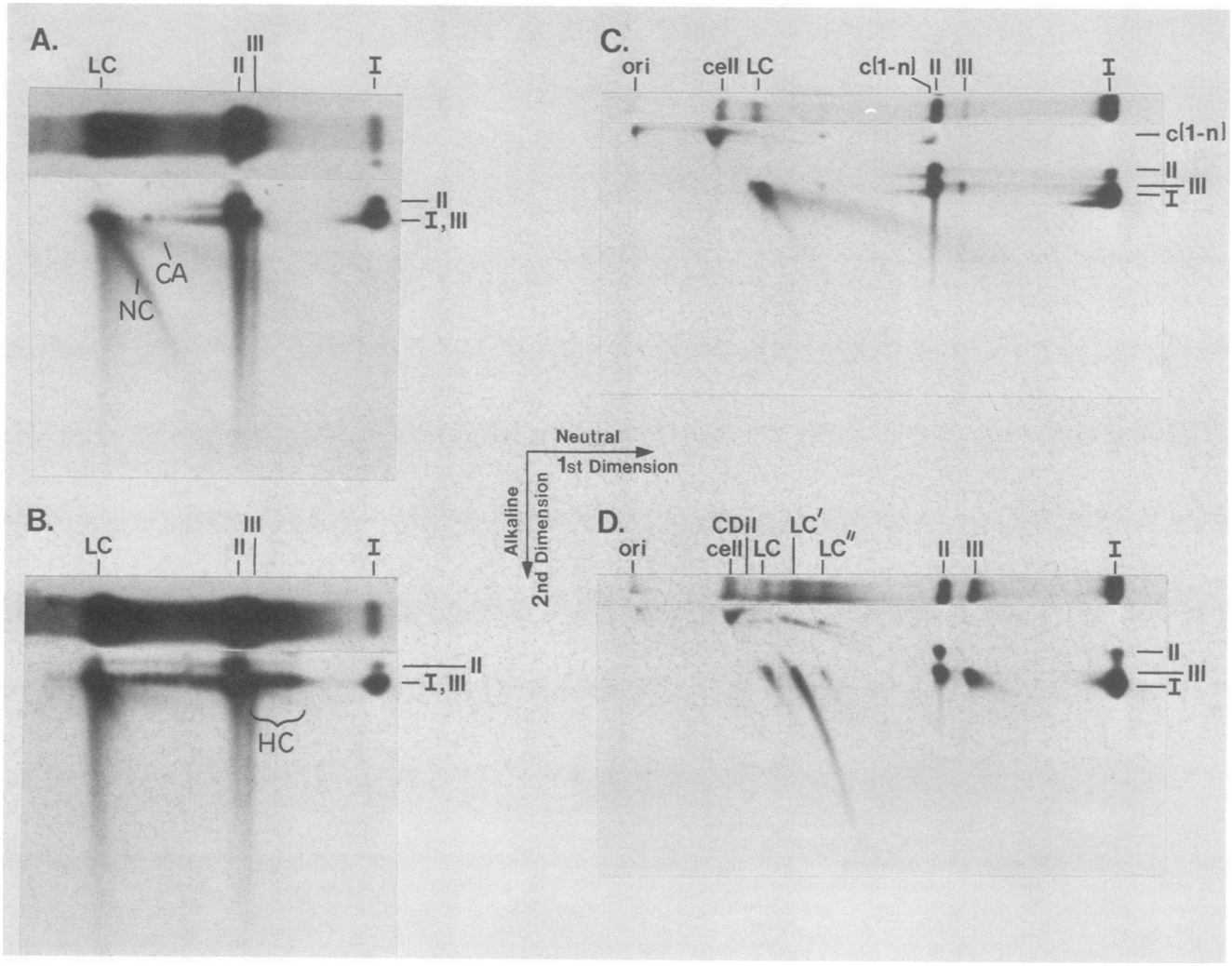

FIG. 3. Two-dimensional neutral-alkaline agarose gel electrophoresis of SV40 DNA replication intermediates following ellipticine or camptothecin treatment. (A) Control for ellipticine treatment. SV40-infected cells were labeled with [*methyl*-^{3}H]thymidine for 30 min and then extracted. The first-dimension neutral gel electrophoresis was carried out for 12 h (top). The first-dimension lane was then cut out, equilibrated in alkaline running buffer, and cast into an alkaline agarose slab gel as described in the text. Second-dimensional alkaline electrophoresis was carried out at 2.0 V/cm for 6 h. (B) Same as A, but 40 μM ellipticine was added 15 min after the start of labeling. (C and D) Camptothecin experiment and control; first dimension run for 33 h, second dimension run at 2.33 V/cm for 6.5 h. (C) Labeled and extracted as in panel A. (D) Same as C, but 40 μM camptothecin added 15 min after the start of labeling. Abbreviations: CA, Cairns arc; NC, arc of nicked Cairns intermediates; HC, highly catenated dimers; other abbreviations, see Fig. 1 legend.

those in the control (Fig. 3A), and this line extended into the region between form III and form I. The fact that alkaline denaturation produced full-length linear molecules from these structures confirmed that they were highly catenated dimers and were not produced by periodic blockage of the growing Cairns structures.

Neutral-alkaline gel electrophoretic analysis of SV40 DNA replication intermediates produced by camptothecin treatment (Fig. 3D) showed the disappearance of the Cairns arc seen in the control (Fig. 3C) and the appearance of four new arcs. The late Cairns structure remained, as did a trace of the arc of nicked Cairns structures. Both the form III and relaxed circular dimer bands were enhanced with respect to the untreated control. The band of C family catenated dimers was also absent in the camptothecin-treated sample. The four new arcs were identical to the aberrant replication intermediates seen by Gourlie and Pigiet (9) when they analyzed polyomavirus in vitro DNA replication intermediates with this gel system. These authors were able to show by electron microscopy (8) that these arcs (designated LC′ and LC″ in Fig. 3D) are formed by Cairns structures with broken replication forks.

DISCUSSION

Antineoplastic topoisomerase inhibitors can rapidly produce abnormal patterns of SV40 DNA replication intermediates. These abnormal patterns are due to arrest of decatenation with ellipticine, m-AMSA, and Adriamycin and to the breaking of active replication forks with camptothecin. Highly catenated SV40 dimers have been reported to be produced by hypertonic shock of infected cells (23, 24), and breaking of replication forks has been found as an artifact of in vitro polyomavirus DNA replication (8, 9). Both of these earlier studies used the high-resolution one- and two-dimensional gel systems used in this study. The work

reported here demonstrates that these same aberrant DNA replication patterns can be produced in vivo by the action of topoisomerase inhibitors. This finding has implications both for the molecular biology of DNA replication in mammalian cells and for the mechanism of action of this class of antitumor drugs.

Ellipticine, m-AMSA, and Adriamycin are all known inhibitors of type II topoisomerases (3, 15, 21, 25, 26). Ellipticine has also been reported to inhibit type I topoisomerases (3). These drugs block the decatenation of newly replicated SV40 daughter chromosomes and lead to an accumulation of catenated dimers containing single-strand DNA breaks. Sundin and Varshavsky (24) found that hypertonic shock produced accumulations of the same A and B family dimers. The increases in the levels of these dimers caused by hypertonic shock are comparable to the increases caused by treatment with 40 μM m-AMSA or Adriamycin but much less than those produced by 40 μM ellipticine. Hypertonic shock also causes an accumulation of C family catenated dimers. Apparently, the drugs, unlike hypertonic shock, can only act by interfering in the breakage-reunion cycle of the decatenating topoisomerase. Compared with m-AMSA and Adriamycin, ellipticine caused much greater accumulation of catenated dimers and also trapped dimers with very high levels of catenation. Band counting was complicated by form II and form III DNAs as well as the close spacing of the bands at the anode end of the ladder. However, a conservative counting of the A family bands indicates that the most highly catenated dimers have at least 25 intertwinings. The relatively weak effects of m-AMSA and Adriamycin may be due either to differences in cellular transport or to kinetics at the level of the topoisomerase. Recent studies have shown that the cytotoxicity of these drugs correlates with the number of DNA strand breaks produced in mammalian cells (6, 7, 21). As shown here for SV40, the strand breaks were associated with blocked decatenation reactions. Strand breaks produced in cellular DNA by ellipticine have been shown to be rapidly repaired after removal of the drug (19). In SV40, removal of the drug led to rapid completion of decatenation.

Although this is the first demonstration that type II topoisomerase inhibitors can interfere with decatenation in vivo, it is not surprising that they have this ability. Sundin and Varshavsky first provided evidence that such a topoisomerase was required for separation of newly replicated SV40 daughter chromosomes (23, 24). These authors suggested that eucaryotic chromosomes were under similar topological constraints and would also require "decatenation." There is now a body of evidence from genetic studies with yeasts which supports this idea. When temperature-sensitive topoisomerase II mutants of yeast are shifted to the nonpermissive temperature, the cells are unable to separate cellular chromosomes (2, 11, 27, 28). Under the same conditions, circular plasmids of yeast accumulate as catenated dimers (2).

Weaver et al. (30) have shown that the pathway of separation of newly replicated SV40 daughter chromosomes is a function of the DNA sequence at the terminus of replication, the multiplicity of infection, and the osmolarity of the medium. These authors suggested that the pathway involving catenated dimers is a minor one and that it is enhanced by abnormal conditions such as hypertonic shock or inhibition of protein synthesis. The findings that ellipticine acted almost instantly to trap newly replicated SV40 DNAs in various stages of decatenation (Fig. 2, lanes o and p) and that B family dimers made up a major fraction of the trapped intermediates (Fig. 1, lanes c and d) strongly suggest that completion of replication followed by decatenation is a major pathway under physiological conditions. The B family is composed of catenated dimers made up of one relaxed monomer and one superhelical monomer. Since the superhelical monomer must have completed DNA replication, it is likely that both monomers completed replication and that the strand break in one is due to the interruption of a topoisomerase breakage-reunion cycle by members of this class of drugs. Adriamycin (Fig. 1, lanes o and p) and m-AMSA (Fig. 1, lanes i and j) also increased the fraction of B family dimers.

Camptothecin has been reported to be a specific inhibitor of type I topoisomerases (12). Its effect on SV40 DNA replication was the rapid production of Cairns structures with broken replication forks. These structures can be detected at low levels in most SV40 and polyomavirus DNA preparations and seem to be especially prominent in the in vitro polyomavirus DNA replication system of Gourlie and Pigiet (9). It seems likely that the target for camptothecin is the swivel topoisomerase required to release superhelical density caused by replication fork progression. In addition, the rapid production of these broken Cairns structures suggests that the swivel is located at or very near the moving forks. If the swivel were located at the terminus of replication or at random sites in the unreplicated part of the Cairns structure, interruption of its breakage-reunion cycle would be expected to produce an intense arc of nicked Cairns structures (NC in Fig. 3). In fact the intensity of this arc decreased as a result of camptothecin treatment.

It should be noted that a type II topoisomerase has been reported to be part of the "replitase" (16). There is also evidence from work with yeast topoisomerase mutants that a type II topoisomerase can replace type I as a swivel for advancing replication forks (27). Thus, more work will be required to determine whether the in vivo target of camptothecin is a type I or type II enzyme. If it is a type II topoisomerase, it is likely to be very different from the decatenating enzyme targeted by ellipticine, m-AMSA, and Adriamycin.

The Cairns structures with broken replication forks have been referred to as rolling-circle replication intermediates (9). This term implies that these structures can produce linear SV40 multimers by analogy with bacteriophage rolling-circle forms (5). The evidence for rolling circles with multimeric tails in normal lytic infections rests on a few observations of very rare molecules in Hirt extracts of infected cells (1). Other workers who have studied these rolling-circle forms have found them to have only tails of unit length or less (4, 9).

Evidence that Cairns structures with broken replication forks do not become true rolling-circle replication intermediates can also be seen in two-dimensional neutral-alkaline gel patterns (Fig. 3D) (9). The DNA replication intermediates are diagrammed in Fig. 4. A broken replication fork produces structure B, a relaxed circle with a tail (after extraction and deproteinization). Ligation of one of the newly synthesized strands to the broken parental strand produces structure C, which also appears as a rolling circle after deproteinization. Both structures C and D can contribute to the lower LC′ arc in Fig. 3D. Only structure D can contribute to the upper LC′ arc, which after longer exposure extended from form II to LC′ in the first dimension and from form II to the level of the relaxed circular dimer in the second dimension. Both upper and lower LC′ arcs have definite upper bounds. Multimeric linear tails would be

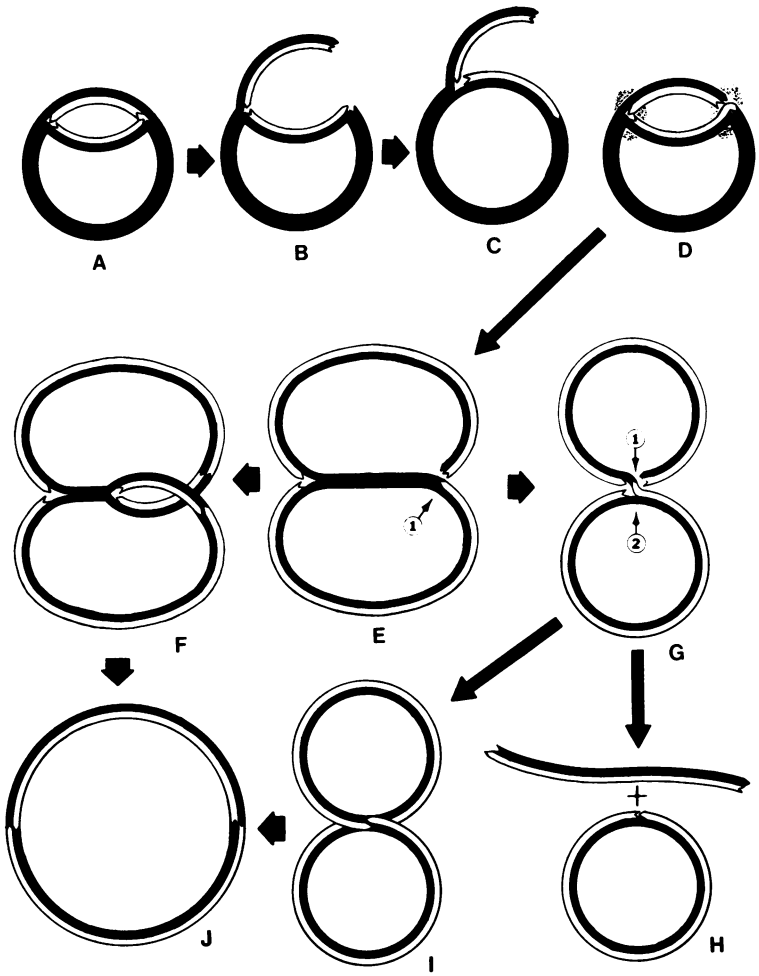

FIG. 4. Pathways for resolution of Cairns structures with broken replication forks. Black lines, parental DNA strands; white lines, newly synthesized DNA. (A) Early Cairns structure. (B) Early Cairns structure with broken replication fork as it appears after extraction and deproteinization. (C) Same as B, but one newly synthesized strand has been ligated to a parental strand at the site of the break. (D) Same as C, but a more realistic diagrammatic representation of the structure in vivo, with intact replication complexes (stippled). (E) Structure resulting from continued movement of the undamaged replication fork (see text for discussion). (F) Structure resulting from completion of SCE and renewed fork progression. (G) Presumed structure of LC′, resulting from continued movement of the undamaged replication fork in structure E. (H) Structures expected from a nick at site 1 in structure G, followed by deproteinization. (I) Structure expected from a nick at site 2 in structure G, followed by ligations (a head-to-tail circular dimer). (J) Circular dimer, unfolded.

expected to produce arcs extending up and back to the first-dimension origin of electrophoresis. However, the lower LC′ arc terminated at the level of full-length linear SV40 DNA (marked by form III and the late Cairns structure in the second dimension), and the upper LC′ arc terminated at the level of linear dimer (marked by the relaxed circular dimer in the second dimension). Thus, at least 95% of the rolling circles complete only one roll. The most likely reason for this is that the broken fork seen in Fig. 4B and C does not disrupt the replication complex at that fork. The break observed after camptothecin treatment was apparently due to interruption of the breakage-reunion cycle of a topoisomerase which is part of the replication complex. Such a break would be a normal transient form of the replication complex and thus would be unlikely to cause distortion or disruption. It might, however, prevent fork progression. If this fork is still intact, it follows that DNA replication will stop when the undamaged fork meets the arrested fork. Such a pathway is shown in Fig. 4D through J. The undamaged fork is allowed to continue its movement, and the fork which has undergone a break and ligation of parental DNA to newly synthesized DNA remains in place. Structures C through E can be viewed as incomplete sister chromatid exchanges (SCEs). Nicking of the remaining intact parental strand at point 1 in structure E, followed by ligation to the other nascent strand and renewed fork movement, would complete the SCE according to the replication bypass (22) or detour (14) models, and a circular head-to-tail dimer would result. However, the intermediates predicted by this pathway were not detected on two-dimensional gels, and it seems likely that structure E is resolved by the pathways leading through structure G (the presumed structure of LC′). Structure G can be resolved by two pathways. A nick a point 1 produces a relaxed circle (form II) plus a double-stranded linear DNA (form III). A nick at point 2 followed by ligations yields the circular head-to-tail dimer (Fig. 4I and J). As seen in Fig. 3D, camptothecin treatment produced an increase in both form III and the relaxed circular dimer compared with the untreated control (Fig. 3C). The heavy form III band produced by camptothecin treatment rapidly disappeared during a chase in drug-free medium (Fig. 1, lanes m and n), suggesting that it may still be

held in a nearly completed replication complex and thus seen as form III only after extraction. It is likely that the structure represented by Fig. 4B can also complete replication with similar options for final resolution. The evidence in Fig. 3 suggests that the pathway leading to Fig. 4J, the circular dimer, is a minor one and that most rolling-circle forms are resolved by the pathway leading to Fig. 4H. It is worth noting that the topoisomerase inhibitors used in this study are intercalating agents, and intercalating agents can be potent inducers of SCE (13, 17).

The finding that topoisomerase inhibitors can selectively interfere with different steps in SV40 DNA replication provides new tools for the analysis of replication and recombination in mammalian chromosomes. In addition, the SV40 DNA replication system may itself be a useful tool for the study and evaluation of new topoisomerase inhibitors designed for use as antineoplastic drugs.

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