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Involvement of Nucleic Acid Synthesis in Cell Killing Mechanisms of Topoisomerase Poisons[1]

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ABSTRACT

The primary cytotoxic mechanism of camptothecin has been proposed to involve an interaction between the replication machinery and the camptothecin-mediated topoisomerase I-DNA cleavable complex (Y. H. Hsiang, M. G. Lihou, and L. F. Liu, Cancer Res., *49:* 5077-5082, 1989). In the present study, we show that killing of V79 cells by the topoisomerase II poisons 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) and etoposide may involve ongoing RNA synthesis in addition to ongoing DNA synthesis. V79 cells synchronized by mitotic shake-off were treated with topoisomerase poisons in the presence of inhibitors of nucleic acid synthesis. S-Phase V79 cells were more sensitive to the topoisomerase I poison camptothecin and the topoisomerase II poison *m*-AMSA than G_1-phase cells. The greater sensitivity of S-phase cells to killing by *m*-AMSA and camptothecin was abolished during cotreatment, but not posttreatment, with aphidicolin, suggesting that ongoing DNA synthesis is involved in cell killing by both topoisomerase I and II poisons. Cotreatment with transcription inhibitors, such as 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole or cordycepin, partially protected cells from the cytotoxic effects of *m*-AMSA but had no effect on camptothecin-mediated cytotoxicity. These results suggest that ongoing RNA transcription may be involved in cell killing by topoisomerase II poisons but not topoisomerase I poisons. Cotreatment with camptothecin reduced *m*-AMSA-mediated cytotoxicity in G_1-phase V79 cells, suggesting a possible antagonism between topoisomerase I and II poisons. This antagonistic effect between topoisomerase I and II poisons could be explained by the strong inhibitory effect of camptothecin on RNA transcription.

INTRODUCTION

A number of antineoplastic drugs have been shown to "poison" DNA topoisomerases in a specific manner and therefore have been termed DNA topoisomerase poisons. These drugs appear to trap an enzymatic reaction intermediate that is transformed into enzyme-linked DNA breaks when denatured by SDS[3] or alkali (reviewed in Refs. 1 and 2). Different from most other forms of covalent modification of DNA, cleavable complexes (using purified topoisomerases) can be readily reversed by a number of nonchemical treatments; they dissociate rapidly following dilution of drug, brief heating to 65°C, or treatment with high salt (3-5). Studies in cultured mammalian cells have provided strong evidence that topoisomerase poisons also induce reversible complexes on chromosomal DNA (6-8), which appear to mediate cell killing (reviewed in Ref. 2). Despite the reversible nature of this type of "DNA damage," brief treatments with topoisomerase poisons are highly lethal. It is possible that lethal damage results from a small fraction of cleavable complexes that either do not dissociate upon drug removal or are transformed into irreversible lesions by cellular processes (9, 10).

DNA synthesis could be one such process that transforms camptothecin-mediated topoisomerase I cleavable complexes into lethal DNA lesions. This has been suggested from the observation that S-phase cells are about 1000-fold more sensitive to killing by pulse treatments with camptothecin than cells in other phases of the cycle (11). More recently it has been shown that camptothecin is virtually nonlethal to cells in which DNA synthesis is inhibited by aphidicolin (9, 12). Although only cells actively engaged in DNA synthesis are killed by camptothecin pulse-treatments, similar numbers of camptothecin-mediated protein-linked strand breaks have been found in aphidicolin-treated S-phase cells as compared with control S-phase cells, and in S-phase as compared with G_1-phase cells (9, 12, 13).

Analysis of SV40 viral replication products in an *in vitro* system has suggested a possible mechanism for the DNA-synthesis-dependent cell killing by camptothecin (9). In the presence of both camptothecin and topoisomerase I, irreversible enzyme-linked breaks were formed in a replication-dependent reaction, in which replication forks were also arrested (9). A working model to explain this result has been proposed in which an interaction between moving replication forks and topoisomerase I cleavable complexes results in irreversible fork arrest and the conversion of reversible cleavable complexes into irreversible enzyme-linked DNA-strand breaks (1).

Here we present studies in Chinese hamster cells that explore the involvement of ongoing DNA and RNA synthesis in cell killing by drugs that poison the type II topoisomerase. In these studies, nucleic acid synthesis inhibitors were added to cells 15 min prior to, or at the same time as, the addition of the topoisomerase poisons and remained in the medium during the 30-min exposures to the topoisomerase poisons. These brief periods of DNA or RNA synthesis inhibition were sufficient to render V79 cells significantly resistant to killing by *m*-AMSA or VP-16.

MATERIALS AND METHODS

Cell Cultures. Monolayer V79 cells (Chinese hamster lung fibroblasts) grown in Dulbecco's minimal essential medium containing penicillin-streptomycin and supplemented with 10% fetal bovine serum (complete medium) were maintained in a humidified atmosphere of 5% CO_2 at 37°C. Under these conditions the population doubling time was 8-9 h.

Drugs. Concentrated stock solutions of *m*-AMSA (16 mM), VP-16 (45 mM), camptothecin (10 mM), DRB (25 mM), and aphidicolin (3 mM) in DMSO and cordycepin (20 mM) in distilled water were stored at −20°C. A concentrated stock of cycloheximide (40 mM) in ethanol was prepared prior to each experiment. The concentration of solvents in all experiments was below 0.3%, which had no discernible effect on cell killing.

Cell Synchrony by Mitotic Shake-off. V79 cells were seeded into T150 flasks (5×10^6/flask) approximately 12-16 h prior to the start of each experiment. On the day of each experiment, loosely attached cells were dislodged by firm tapping of the flasks and were then aspirated.

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[3] The abbreviations used are: SDS, sodium dodecyl sulfate; DRB, 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole; VP-16, etoposide; *m*-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; DMSO, dimethyl sulfoxide.

The monolayer cultures were then rinsed with prewarmed (37°C) complete medium containing 50 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (pH 7.4), and 10 ml of the same medium were added. The cultures were then incubated for 30 min to allow cells to progress into mitosis. Mitotic cells were then detached by firm tapping of the flasks. Mitotic cells were plated into 90-mm dishes and incubated at 37°C to permit progression through the cell cycle.

Cell cycle synchrony was assessed using flow cytometry to quantify DNA content at various times after mitotic shake-off. Cells were fixed with 95% ethanol and refrigerated until the time of analysis when the cell pellet was resuspended in staining buffer and processed according to the method of Vindelov (14).

Clonogenic Survival. Cells were plated into 90-mm dishes at the appropriate densities and exposed to drugs in complete medium at various times following incubation after mitotic shake-off or at 4 h postplating in the case of asynchronous cells. Following drug exposure, dishes were rinsed twice with 10 ml of serum-free medium containing 50 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (pH 7.4). Complete medium was added to the dishes, which were then incubated for 6 d to allow colony formation. Colonies stained with 0.05% crystal violet in methanol were scored only if they contained 50 or more cells.

Potassium-SDS Precipitation Assay. Precipitation of DNA covalently associated with protein was performed exactly as described previously (10).

RESULTS

Active DNA Synthesis Is Required for Cell Killing by the Topoisomerase I Poison Camptothecin. S-Phase cells have previously been shown to be highly sensitive to the cytotoxic effects of camptothecin (11, 13). To test the possibility that active DNA synthesis is an essential component of the cytotoxic mechanism of camptothecin, clonogenic survival was measured in cell populations synchronized by mitotic shake-off. Mitotic cells were reseeded and were then treated with the DNA polymerase inhibitor aphidicolin or DMSO (control) for 15 min prior to and during a subsequent 30-min camptothecin exposure (Fig. 1). In cells treated with camptothecin only, killing increased with progression from G_1- to S-phase and then decreased with progression from S- into G_2-phase. G_1-Phase cells were nearly completely resistant to cell killing by camptothecin (*i.e.*, at 1.5 h following mitotic shake-off, see also Fig. 5). The 97% cell killing at 7 h postmitotic shake-off is probably an underestimate of the true cell killing of a pure S-phase population; the 3% surviving cells are probably contaminating non-S-phase cells that are resistant to camptothecin. When DNA synthesis was inhibited by aphidicolin, camptothecin was completely ineffective in cell killing.

DNA Synthesis Inhibition Protects against Cell Killing by Topoisomerase II Poisons. In contrast to the pronounced S-phase-selective cell killing of camptothecin, *m*-AMSA kills S-phase CHO cells with only about a twofold greater efficiency than cells in other phases of the cycle, and this differential sensitivity was apparent only at lower doses (15). In the present studies, V79 populations synchronized by mitotic shake-off also displayed about a twofold greater sensitivity to *m*-AMSA during S-phase (7 h postmitotic shake-off; see Fig. 1*A*) than during G_1-phase (1.5 h postmitotic shake-off; see Fig. 1*A* and a representative experiment in Fig. 2). The preferential sensitivity of S-phase cells was most pronounced at lower drug concentrations as indicated by the initial steepness of the S-phase survival curve.

To test whether the preferential sensitivity of S-phase cells to *m*-AMSA requires ongoing DNA synthesis, cells at 7 h postmitotic shake-off were treated with either the DNA polymerase inhibitor aphidicolin or DMSO (control) for 15 min prior to and during a subsequent 30-min exposure to various doses of *m*-AMSA (representative experiment shown in Fig. 3). In the presence of aphidicolin, which reduced DNA synthesis to 5% of the control level within 5 min of addition to cell cultures (data not shown), the sensitivity of S-phase cells to killing by *m*-AMSA was reduced (Fig. 3). Similarly, cycloheximide, which decreased DNA synthesis to approximately 10% of control levels, also reduced the sensitivity of S-phase cells to killing by *m*-AMSA. Similar results were obtained when either aphidicolin or cycloheximide was added at the same time as *m*-AMSA (data not shown). Protection of S-phase cells by aphidicolin or cycloheximide predominated at low doses of *m*-AMSA; in the presence of the inhibitors, a shoulder is added to the initial steep slope of the survival curve, while the final slope remains essentially unchanged but is shifted toward higher drug concentrations. This result suggests that the preferential sensitivity of S-phase cells to *m*-AMSA, relative to G_1-phase cells, is due to active DNA synthesis. Cell killing by VP-16 was also reduced by aphidicolin and cycloheximide cotreatment (data not shown).

The possibility that aphidicolin and cycloheximide can pro-

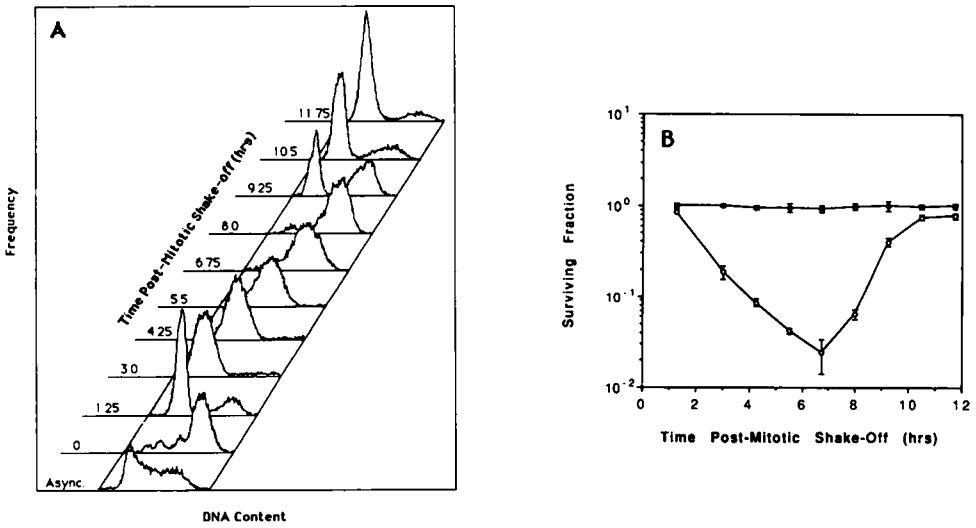

Fig. 1. *A*, frequency histograms for DNA content in V79 cells obtained by mitotic shake-off and sampled for flow cytometric analysis at intervals following reseeding and incubation at 37°C. *B*, aphidicolin cotreatment protects V79 from the lethal effects of camptothecin. At intervals following mitotic shake-off, V79 cells were treated with 3 μM aphidicolin (●) or DMSO (○) for 15 min prior to and during a subsequent 30-min exposure to 10 μM camptothecin. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

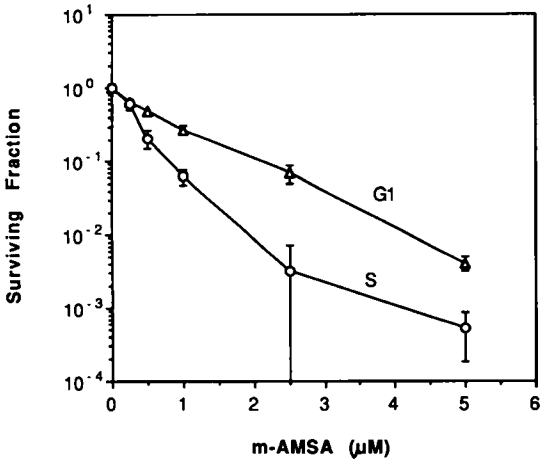

Fig. 2. S-Phase sensitivity to *m*-AMSA. V79 cells were treated with *m*-AMSA for 30 min at either 1.5 h (G_1-phase, Δ) or 7 h (S-phase, O) postmitotic shake-off. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

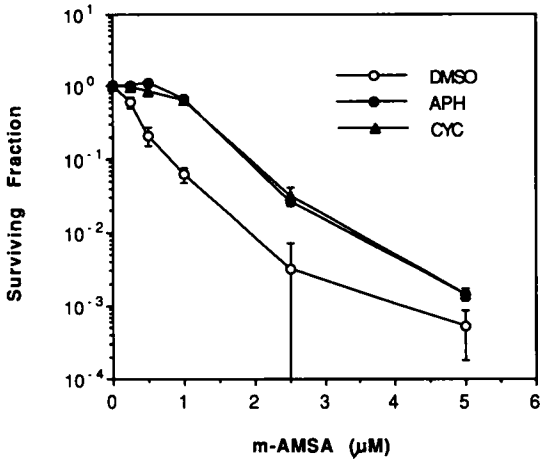

Fig. 3. DNA synthesis inhibition by aphidicolin (*APH*) or cycloheximide (*CYC*) protect S-phase cells from killing by *m*-AMSA. At 7 h following mitotic shake-off, V79 cells were treated with either 3 μM aphidicolin (●), 40 μM cycloheximide (▲), or DMSO (control, O) for 15 min prior to and during a 30-min exposure to *m*-AMSA. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

tect G_1-phase cells from killing by *m*-AMSA was also tested. Cells were treated as described above, but treatments began at 1.5 h (G_1), rather than at 7 h (S) postmitotic shake-off. In these G_1-phase cells, killing by *m*-AMSA was similar whether either inhibitor was present or not (representative experiment shown in Fig. 4). Similarly, killing of G_1-phase cells by VP-16 was not affected by either aphidicolin or cycloheximide (data not shown). The lack of an effect of these inhibitors on G_1-phase cells suggests that in S-phase cells, DNA synthesis inhibition is responsible for protection, rather than some other nonspecific effect such as inhibition of drug uptake. Furthermore, these inhibitors did not affect cleavable complex formation by *m*-AMSA as measured using the potassium-SDS cleavage assay (data not shown). This result is consistent with studies in Chinese hamster DC3F cells, murine mastocytoma cells, L1210 cells, and HL-60 cells that have shown that neither aphidicolin nor cycloheximide affects protein-associated strand-break formation by VP-16 or *m*-AMSA (12, 16, and 17).[4]

DNA Synthesis Inhibitors Must Be Present at the Time of Exposure to Topoisomerase Poisons to Protect against Cell Killing. Conceivably, cells might incur the same amount of lethal DNA damage by topoisomerase poisons in the presence and absence of aphidicolin or cycloheximide. However, reduced cell killing may be observed in inhibitor-treated cells because "*m*-AMSA damage" is repaired during the time required for recovery of normal DNA synthesis levels (several hours in the case of cycloheximide; Ref. 17). Alternatively, less damage might be produced in DNA synthesis inhibitor-treated cells, perhaps because active DNA synthesis is required to transform cleavable complexes into lethal damage. To distinguish between these two possibilities, following a 30-min pulse-treatment with camptothecin or *m*-AMSA, cells were washed free of these drugs and then treated with aphidicolin or cycloheximide. Post-treatment with these inhibitors was totally ineffective in protecting cells from killing by camptothecin or *m*-AMSA (Table 1). This result suggests that these topoisomerase poisons produce DNA-synthesis-dependent DNA damage that is not repaired during a posttreatment period of DNA synthesis inhibition.

Although cycloheximide and aphidicolin protected cells from killing by *m*-AMSA equally well, cycloheximide only partially protected cells from killing by camptothecin, while aphidicolin afforded full protection (Table 1). Even with cycloheximide pretreatments as long as 1 h, protection against cell killing by

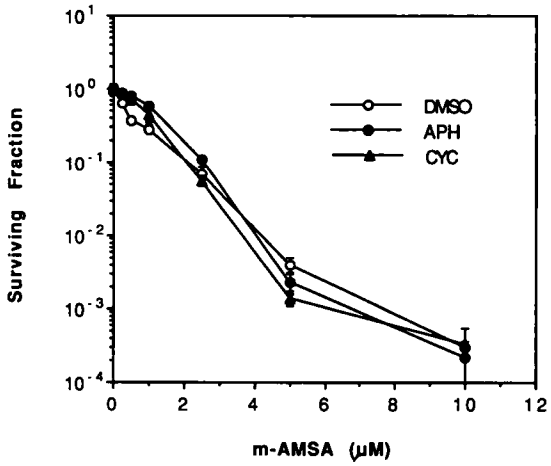

Fig. 4. DNA synthesis inhibition by aphidicolin (*APH*) or cycloheximide (*CYC*) do not protect G_1-phase V79 cells from killing by *m*-AMSA. At 1.5 h following mitotic shake-off, V79 cells were treated with either 3 μM aphidicolin (●), 40 μM cycloheximide (▲), or DMSO (control, O) for 15 min prior to and during a 30-min exposure to *m*-AMSA. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

Table 1 *Effect of pre- and posttreatment with 3 μM aphidicolin or 40 μM cycloheximide on killing of S-phase cells by 5 μM camptothecin or 0.5 μM m-AMSA*

Treatments were begun 7 h after plating of mitotic cells.

15-min pre-treatment	+	30-min co-treatment	30-min treatment	+	45-min post-treatment	Surviving fraction ± SE
DMSO[a]		DMSO				1.00 ± 0.04
DMSO		*m*-AMSA				0.18 ± 0.02
CYC[b]		*m*-AMSA				1.00 ± 0.08
APH		*m*-AMSA				0.98 ± 0.02
DMSO		CAMP				0.04 ± 0.01
CYC		CAMP				0.31 ± 0.02
APH		CAMP				0.93 ± 0.10
			DMSO		CYC	1.14 ± 0.01
			DMSO		APH	1.07 ± 0.02
			CAMP		CYC	0.03 ± 0.01
			CAMP		APH	0.03 ± 0.02
			m-AMSA		CYC	0.17 ± 0.01
			m-AMSA		APH	0.21 ± 0.04

[a] 0.1% DMSO.
[b] CYC, cycloheximide; APH, aphidicolin; CAMP, camptothecin.

[4] S. H. Kaufman, personal communication.

camptothecin was incomplete (not shown). The reason for this result is unknown.

RNA Synthesis Inhibition Protects against Cell Killing by Topoisomerase II Poisons but Not by the Topoisomerase I Poison Camptothecin. To study the role of RNA synthesis in the mechanism of *m*-AMSA cytotoxicity, the topoisomerase I poison camptothecin was used to inhibit transcription. Camptothecin is a potent inhibitor of RNA synthesis (reviewed in Ref. 18); however, it was without lethal effects in G_1-phase V79 cell populations (see Figs. 1 and 5; Ref. 11). In the absence of camptothecin, 2.5 μM *m*-AMSA killed 98% of the cells. In the presence of the highest concentration of camptothecin, 15 min prior to and during a 30-min *m*-AMSA treatment, cell killing by *m*-AMSA was reduced approximately 10-fold (Fig. 5). Camptothecin provided a similar level of protection against cell killing by VP-16 (data not shown).

The transcription inhibitors cordycepin and DRB were also tested for their ability to protect V79 cells from *m*-AMSA. Cordycepin appears to inhibit RNA synthesis by causing termination of the nascent RNA chain (19). In G_1-phase cells treated with cordycepin, which reduced RNA synthesis to 25% of control levels, twice as much *m*-AMSA was required to produce the same amount of killing as when RNA synthesis was uninhibited (Fig. 6*A*). A similar result was obtained when transcription was inhibited by DRB (Fig. 7*A*), which inhibits heterogeneous nuclear RNA synthesis and, to a lesser extent, nucleolar RNA synthesis (20). Similarly, killing of G_1-phase cells by VP-16 was reduced about twofold in the presence of cordycepin or DRB. Transcription inhibition appeared to protect G_1-phase cells more than S-phase cells from *m*-AMSA (Figs. 6*B* and 7*B*) or VP-16 (data not shown); however, in S-phase cells, the protective effect of transcription inhibition may be less discernible due to the predominance of DNA synthesis-dependent cell killing. Below 1% survival, the slopes of the S-phase survival curves are reduced (Figs. 6*B* and 7*B*). This reduced rate of cell killing is probably due to a small number of contaminating cells from other phases of the cell cycle that are resistant to *m*-AMSA.

Since the diverse inhibitors, camptothecin, DRB, and cordycepin, all protected cells from killing by *m*-AMSA or VP-16, the protection they afford appears to be due to transcription inhibition *per se*. In order to distinguish a possible effect of the inhibitors on cleavable complex formation, the potassium-SDS precipitation assay was used (10). Over the same *m*-AMSA concentration range as used in the cell killing assay, a nearly linear dose-response for cleavable complex formation was observed, and the presence of DRB or cordycepin did not shift the curve (data not shown). The experimental variability was such that a twofold difference in cleavable complex formation would have been statistically detectable. This result is consistent with a previous study in which cordycepin did not affect *m*-AMSA-mediated cleavable complex formation in a murine mastocytoma cell line (16).

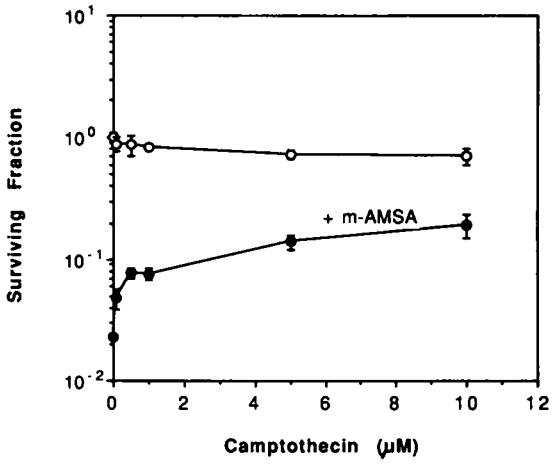

Fig. 5. Transcription inhibition by camptothecin protects G_1-phase V79 cells from killing by *m*-AMSA. Cells at 1.5 h postmitotic shake-off were treated with various doses of camptothecin for 15 min prior to and during a 30-min exposure to either DMSO (control, O) or 2.5 μM *m*-AMSA (●). *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

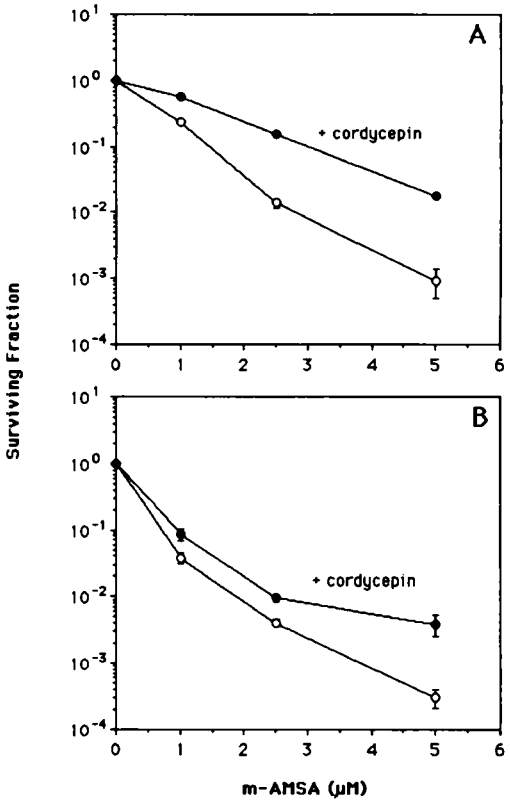

Fig. 6. Transcription inhibition by cordycepin protects G_1 (*A*) and S-phase (*B*) V79 cells from killing by *m*-AMSA. At 1.5 h (*A*) or 7 h (*B*) following mitotic shake-off, cells were treated with 20 μM cordycepin (●) or DMSO (control, O) for 15 min prior to and during a 30-min exposure to *m*-AMSA. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

In contrast to the reduced cell killing by topoisomerase II poisons when RNA synthesis was inhibited, the RNA synthesis inhibitor DRB had no effect on cell killing by the topoisomerase I poison camptothecin (data not shown). This result may not be surprising since camptothecin itself is a strong inhibitor of RNA synthesis (reviewed in Ref. 18).

Complementary Effect of Simultaneous RNA and DNA Synthesis Inhibition in Protecting against Cell Killing by *m*-AMSA. Treatment of an asynchronous population with both aphidicolin and cordycepin produced greater protection against *m*-AMSA cell killing than did either inhibitor alone (Fig. 8). In the presence of both inhibitors, two- to threefold greater concentrations of *m*-AMSA were required to produce the same amount of cell killing as produced when DNA and RNA synthesis were uninhibited. Protection by these two inhibitors appeared to be complementary: aphidicolin preferentially protected against cell killing at lower concentrations of *m*-AMSA, while cordycepin was apparently more effective at higher concentrations of *m*-AMSA.

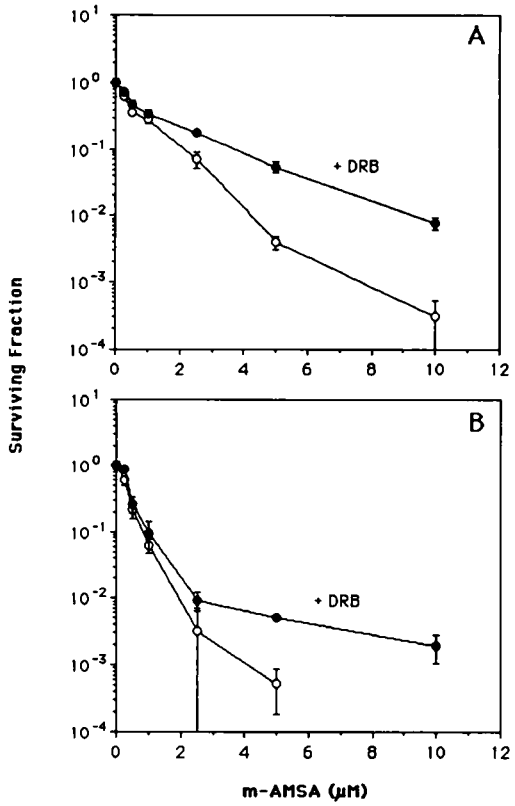

Fig. 7. Transcription inhibition by DRB protects G_1 (*A*) and S-phase (*B*) V79 cells from killing by *m*-AMSA. At 1.5 h (*A*) or 7 h (*B*) following mitotic shake-off, cells were treated with 25 μM DRB (●) or DMSO (control, ○) for 15 min prior to and during a 30-min exposure to *m*-AMSA. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

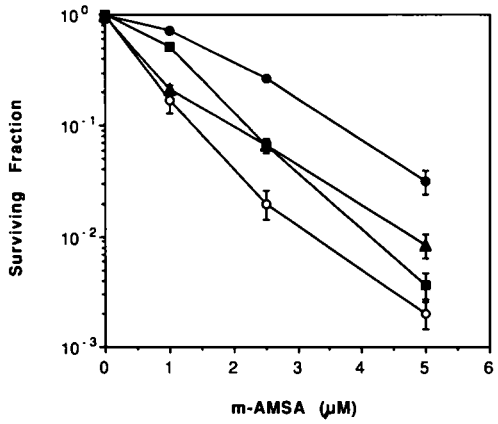

Fig. 8. Combined inhibition of DNA and RNA synthesis protects asynchronous V79 cells from *m*-AMSA cytotoxicity. Asynchronous V79 cells were treated 4 h postplating with DMSO (control, ○), 20 μM cordycepin (▲), 3 μM aphidicolin (■), or 20 μM cordycepin and 3 μM aphidicolin (●) for 15 min prior to and during a 30-min exposure to *m*-AMSA. *Points*, mean clonogenic survival for three independent exposures ± 1 SE.

DISCUSSION

The data presented here demonstrate that ongoing DNA synthesis is an essential component of a cell killing mechanism of the topoisomerase I poison camptothecin, and the topoisomerase II poisons *m*-AMSA and VP-16. In addition, ongoing transcription appears to be an essential component of a cell-killing mechanism of *m*-AMSA and VP-16 but not camptothecin.

The sole cytotoxic target of camptothecin appears to be topoisomerase I, as topoisomerase I purified from camptothecin-resistant mammalian cells has been shown to be resistant to cleavable complex formation (21). Also, topoisomerase I null mutants in *Saccharomyces cerevisiae* are resistant to the cytotoxic effects of camptothecin (22, 23). The observations that camptothecin pulse treatment is practically nonlethal in aphidicolin-treated cells (Fig. 1; Refs. 9 and 12) or in G_1-phase cells (Fig. 5; Ref. 24) have suggested a working model in which the collision of the moving replication fork with the topoisomerase I cleavable complex transforms the normally reversible complex into a lethal DNA lesion (9). Supportive evidence for this model has come from analysis of viral replication intermediates isolated from camptothecin-treated cells. These studies have shown that camptothecin-mediated DNA strand breaks occur preferentially on replicating molecules (25), where they appear to be transformed at replication forks into irreversible DNA breaks (26). Similarly, in a cell-free replication system, when both topoisomerase I and camptothecin were present, DNA synthesis was arrested at the same time that linearized SV40 molecules (broken replication forks) and irreversible cleavable complexes were formed (9). This result is consistent with a mechanism in which the interaction between moving forks and cleavable complexes results in irreversible fork arrest and the production of DNA strand breaks that appear to occur at or near the replication fork. Such breaks, being the equivalent of a double-strand break, would be expected to be highly lethal.

In contrast to the strong S-phase-specific killing by pulse treatments with camptothecin, cell killing by topoisomerase II poisons is much less dependent on cell cycle phase (15, 27, 28). In the case of the topoisomerase II poison *m*-AMSA, S-phase cells have been shown to be only about twofold more sensitive than cells in other phases, and this sensitivity is most pronounced at lower levels of cell killing (Fig. 2; Ref. 15). One possible explanation for this result is that at low doses of topoisomerase II poisons, only one *m*-AMSA molecule may be intercalated within the DNA bound by a single subunit of each topoisomerase II dimer. Thus, only a single subunit would be trapped by drug while the other would remain in the noncleavable state (2). Alkaline and neutral elution studies have provided evidence that at low doses of the topoisomerase II poisons, teniposide and etoposide, single-strand protein-associated DNA breaks predominate (single-subunit cleavable complexes) (29, 30). Single-strand cleavable complexes have also been shown to predominate at low doses of *m*-AMSA in the purified system (5). In the present studies, the sensitivity of S-phase cells to killing by *m*-AMSA occurs predominantly at low drug doses, which may trap only a single subunit of a topoisomerase II dimer in the cleavable complex. One possible scenario is that sensitivity of S-phase cells to *m*-AMSA is due to single-subunit cleavable complexes, which, similar to topoisomerase I cleavable complexes, may be transformed into lethal lesions by interaction with the DNA synthesis machinery.

Previous studies have demonstrated that cycloheximide or aphidicolin treatment can protect cells from killing by topoisomerase II poisons (16, 17, 31), but in these studies, cells were pretreated with cycloheximide or aphidicolin for 2 to 6 h prior to the addition of the topoisomerase poisons. Resistance was observed after the 2-h pretreatment, although greater protection was afforded by longer pretreatments, which may indicate that mechanisms in addition to DNA synthesis inhibition were operative (16, 17). The authors suggested that cycloheximide inhibits the synthesis of proteins required to process the damage into lethal lesions. In our studies, cycloheximide and aphidi-

colin produced identical protection, even when added at the same time as *m*-AMSA. This result indicates that protection by both cycloheximide and aphidicolin probably result from the same mechanism, inhibition of DNA synthesis.

Because the predominant protection afforded by DNA synthesis inhibition was at low concentrations of *m*-AMSA, cell killing at higher concentrations was proposed to involve other cellular processes. Transcription was shown in the present studies to be one such process; cordycepin, camptothecin, and DRB rendered V79 cells less sensitive to cell killing by the topoisomerase II poison *m*-AMSA or VP-16. More recent studies in HL-60 cells have confirmed our observations that *m*-AMSA and VP-16 are less cytotoxic in cells in which transcription is inhibited by cordycepin, camptothecin, or DRB.[4] In these studies, similar to ours, the transcription inhibitors did not affect cleavable complex formation.

The possible function of topoisomerase II in transcription remains unclear. Topoisomerase II has been localized to the flanking regions of genes, with some cleavage sites occurring within the transcription units (32, 33). Furthermore, the intensity of certain topoisomerase II cleavage sites has been shown to depend on active transcription (32, 33). It is possible that when topoisomerase II is trapped in the cleavable complex within a transcription unit, an interaction with the RNA polymerase complex, or transcription-generated superhelical tension, may transform it into a lethal lesion.

Significant cell killing by *m*-AMSA was observed even when both DNA and RNA synthesis were inhibited. This killing might possibly be due to residual nucleic acid synthesis in the presence of the inhibitors. However, it is also likely that other processes are involved in transforming *m*-AMSA-mediated cleavable complexes into lethal lesions.

Cleavable complexes may be good models for many types of potentially lethal DNA damage, which, if not first transformed into lethal lesions by the nucleic acid synthesis machinery, can be repaired in quiescent cells prior to mitogenic stimulation. The highly reversible nature of the cleavable complex allows analysis of the instantaneous processing of this type of damage, which appears to be the first step in a cell killing pathway.

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