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Collateral Sensitivity to the Bisdioxopiperazine Dexrazoxane (ICRF-187) in Etoposide (VP-16)-Resistant Human Leukemia K562 Cells

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ABSTRACT. Etoposide (VP-16)-resistant K562 cells (K/VP.5) were 26-fold resistant to VP-16, due in part to a reduction in DNA topoisomerase II (topoisomerase II) protein levels. Compared with parental K562 cells, VP-16-resistant K/VP.5 cells were found to be 3.4-fold more sensitive to the effects of dexrazoxane (ICRF-187), a topoisomerase II inhibitor that does not stabilize topoisomerase II–DNA covalent complexes. In contrast, K/VP.5 cells were 4.0-fold cross-resistant to merbarone and showed no cross-resistance to fostriecin, two other topoisomerase II inhibitors that do not stabilize topoisomerase II–DNA covalent complexes. Preincubation with ICRF-187 resulted in greater inhibition of subsequent VP-16-induced topoisomerase II–DNA covalent complexes in K/VP.5 cells than in K562 cells. Conversely, preincubation with merbarone resulted in less inhibition of VP-16-induced topoisomerase II–DNA covalent complexes in K/VP.5 cells than in parental K562 cells. Preincubation with fostriecin had little effect on VP-16-induced topoisomerase II–DNA covalent complex formation in either cell line. The onset rates for ICRF-187 inhibition of VP-16-induced topoisomerase II–DNA complex formation were similar in sensitive and resistant cells. In addition, ICRF-187 had a comparable concentration-dependent inhibitory effect on the topoisomerase II catalytic activities of K562 and K/VP.5 cells. Together, our results indicate that collateral sensitivity to ICRF-187 in K/VP.5 cells is due to decreased topoisomerase II protein levels rather than to an alteration in topoisomerase II activity. Furthermore, results suggest that ICRF-187, merbarone, and fostriecin have different mechanisms of action that can be studied effectively in K/VP.5 and K562 cells. BIOCHEM PHARMACOL 52;4:635–642, 1996.

KEY WORDS. topoisomerase II; etoposide; ICRF-187; bisdioxopiperazines; K562 cells

Topoisomerase II[‖] is a DNA-binding protein responsible for transient cleavage of DNA and passage of intact DNA double strands through formed DNA breaks to relieve the torsional stress that occurs during replication and transcription [1–3]. Topoisomerase II is also important for recombination and efficient daughter DNA strand separation during mitosis [1]. Topoisomerase II is a target for a number of clinically effective antineoplastic agents including etoposide (VP-16), teniposide, doxorubicin, and m-AMSA [4–7]. Exposure of cells to these topoisomerase II poisons results in the accumulation of DNA strand breaks due to the stabilization of a covalent complex formed between the enzyme and DNA [7, 8]. Acquired drug resistance to topoisomerase II poisons is often correlated with a decrease in topoisomerase II–DNA covalent complexes related to a reduction in enzyme levels [9–13], a decrease in drug accumulation [14–18], and/or a change in topoisomerase II function [19–25].

Our laboratory has isolated and characterized a stable 26-fold VP-16-resistant K562 cell line (K/VP.5) that exhibits both quantitative and qualitative changes in topoisomerase II but does not manifest alterations in VP-16 membrane transport [13, 24]. Compared with parental K562 cells, K/VP.5 cells contain reduced levels of both topoisomerase II mRNA and protein and exhibit decreased stability of VP-16-induced topoisomerase II–DNA complexes [13, 24]. There is also a difference in the posttranslational modification of the enzyme as phosphorylation of topoisomerase II is reduced in resistant cells [25]. Topoisomerase II hypophosphorylation may account for the reduced stability of VP-16-induced topoisomerase II–DNA

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‖ *Abbreviations:* topoisomerase II, DNA topoisomerase II (M_r 170,000 isoform); VP-16 (etoposide, 4′-demethyl-epipodophyllotoxin-9-(4,6-O-ethylidene-β-D-glucopyranoside); ICRF-187 (dexrazoxane), (+)-1,2-bis(3,5-dioxopiperazinyl-yl)propane; m-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; and DMEM, Dulbecco's modified Eagle's medium.

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covalent complexes in K/VP.5 cells compared with K562 cells [24–26]. Together, the reduced protein levels and hypophosphorylation of topoisomerase II may account for the resistance phenotype in K/VP.5 cells in the presence of drugs known to exert their action through stabilization of topoisomerase II–DNA covalent complexes.

Recently, topoisomerase II-targeted agents including merbarone [27], fostriecin [28], aclarubicin [29], and the bisdioxopiperazines [30–32] have been reported to inhibit topoisomerase II catalytic activity without stabilizing topoisomerase II–DNA covalent complexes and without causing DNA strand breakage. The bisdioxopiperazine ICRF-187 inhibits chromosomal condensation and segregation during anaphase [33], while merbarone is known to cause a G_2/M blockade [34, 35]. Although the mechanism of action of these topoisomerase II inhibitors has yet to be clearly elucidated, the bisdioxopiperazine derivatives appear to exert their effects by inducing a conformational change in topoisomerase II that results in the enzyme being trapped in an inactive closed protein clamp form [32]. The stabilization of this closed clamp structure inhibits the intrinsic ATPase activity of topoisomerase II and prevents recycling of the enzyme. Since ICRF-187 and other bisdioxopiperazines are considered catalytic inhibitors of topoisomerase II rather than poisons that stabilize enzyme–DNA covalent intermediates, Wasserman and Wang [36] recently suggested that bisdioxopiperazine-mediated topoisomerase II inhibitory activity should result in greater cytotoxicity in cells that contain decreased levels of topoisomerase II protein.

The present study focuses on the effects of non-topoisomerase II–DNA complex stabilizing inhibitors in K562 cells and in the VP-16-resistant subline, K/VP.5, that contains reduced levels of topoisomerase II protein [13, 24]. Consistent with the recently reported effects of bisdioxopiperazines in yeast [37], we found that the activity of ICRF-187 in these cell lines was inversely related to topoisomerase II levels. Conversely, the effect of merbarone was directly related to the level of topoisomerase II. Our results indicate that ICRF-187 and merbarone likely exert their effects through different topoisomerase II inhibitory mechanisms.

MATERIALS AND METHODS

Cells

Human K562 cells and VP-16-resistant K/VP.5 cells [24] were grown in suspension in DMEM containing 7% iron-supplemented newborn bovine serum (Hyclone, Logan, UT) and 2 mM L-glutamine (GIBCO/BRL, Grand Island, NY). Exponentially growing cells (3–7 × 10^5 cells/mL) were used for all experiments.

Drugs and Chemicals

Etoposide (VP-16) was obtained from the Bristol–Myers Squibb Co. (Wallingford, CT) and prepared as a concentrated stock solution by dissolving in 100% DMSO. [U-^{3}H]VP-16 was obtained from Moravek Biochemicals (Brea, CA). The bisdioxopiperazine ICRF-187, merbarone, and fostriecin also were prepared as stock solutions in DMSO. Unless specified, the final DMSO concentration in drug-treated and control cells was 0.4 to 0.8%.

Drug-Induced Growth Inhibition

K562 and K/VP.5 cells were plated in 24-well plates at a concentration of 1 × 10^5 cells/mL and incubated with various concentrations of VP-16, ICRF-187, merbarone, or fostriecin for 48 hr, after which cells were counted on a model ZBF Coulter counter (Coulter Electronics, Hialeah, FL). The extent of growth in drug-treated versus control cells is expressed as percent inhibition of control growth. The 50% growth-inhibitory concentration for each cell line was calculated from replicate concentration–response curves generated from separate experiments.

Topoisomerase II Catalytic Activity

Topoisomerase II-containing extracts of nuclei were prepared from 1–2 × 10^8 K562 and K/VP.5 cells as previously described [24]. The final sodium chloride concentration of the nuclear extracts varied from 0.75 to 0.9 M. *Crithidia fasiculata* was labeled with 8 mCi/mL [*methyl*-^{3}H]thymidine, and kinetoplast mitochondrial DNA (kDNA) was isolated as previously described [38]. Each 30-μL assay contained 50 mM Tris (pH 7.5), 85 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM Na_2EDTA (pH 8.0), 30 mg/mL BSA, 0.4 mM ATP, 1 μg of radiolabeled kDNA and 1 μg of nuclear extract topoisomerase II from K562 or K/VP.5 cells. After incubation for 30 min at 30°, reactions were stopped by the addition of 7.5 μL of a solution containing 50% glycerol, 2.5% SDS, and 0.025 M EDTA (pH 8.0). Tubes were then centrifuged for 15 min at 8000 *g* at 25° following which 20-μL supernatant samples were counted in a liquid scintillation counter in 3.5 mL of Ecolite (ICN Biochemicals, Irvine, CA). Decatenation was quantified subsequent to subtraction of counts found in DMSO controls in the absence of nuclear extract topoisomerase II.

Topoisomerase II–DNA Covalent Complexes

Topoisomerase II–DNA covalent complex formation in intact cells was measured as previously described [24]. Mid-log cells (2.0 to 3.0 × 10^5 cells/mL) were labeled for 24 hr with 0.5 μCi/mL [*methyl*-^{3}H]thymidine (0.5 Ci/mmol) and 0.1 μCi/mL [U-^{14}C]leucine (318 mCi/mmol) in DMEM containing 7.5% iron-supplemented calf serum. Cells were then pelleted and resuspended in fresh DMEM/7.5% calf serum and incubated for 1 hr at 37°. Cells were pelleted and resuspended in a pH 7.4 buffer (buffer A) of 115 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM NaH_2PO_4, 25 mM HEPES, and 10 mM glucose at a final concentration of 1.0 × 10^6 cells/mL. Cells were then incubated for different

times with various concentrations of ICRF-187, merbarone, or fostriecin at 37°. After this period, VP-16 (100 μM) was added, and cells were incubated for an additional 15–30 min. Reactions were stopped by adding 1 mL of cell suspension to 10 mL of ice-cold PBS. Cells were then pelleted and lysed, cellular DNA was sheared, and protein–DNA complexes were precipitated with SDS and KCl as described by Zwelling *et al.* [21]. Topoisomerase II–DNA covalent complexes were quantified by scintillation counting, and DNA was normalized to cell number using the coprecipitated ^{14}C-labeled protein as an internal control.

Intracellular Accumulation of VP-16

K562 and K/VP.5 cells were suspended in buffer A at a final concentration of 5 × 10^6 cells/mL. Cells were stirred in specially designed flasks by revolving teflon paddles in a 37° water bath, as described previously [39]. Each cell line was incubated for 5 min with ICRF-187 (500 μM) or DMSO so that the final solvent concentration was 0.1%. [^{3}H]VP-16 (100 μM, 35 dpm/pmol) was then added to all flasks, and cells were incubated for 30 min. Triplicate 1-mL portions of cell suspension from each flask were then injected into 10 vol. of PBS at 0°. Next, cell fractions were separated by centrifugation and washed twice with PBS at 0°. Each washed pellet was drawn up into a plastic pipette tip, extruded onto a polyethylene tare, and dried overnight at 70°. The dried pellets were weighed, placed in a glass scintillation vial, and dissolved in 0.25 mL of 1 M KOH for 90 min at 70°. The digests were neutralized with 0.25 mL of 1 M HCl; 3.5 mL of Ecolite was added, and radioactivity was determined by liquid scintillation counting. Results yielded cellular VP-16 content expressed as nanomoles per gram dry weight. Intracellular water contents of 5.2 and 5.4 mL/g dry pellet weight for K562 and K/VP.5 cells, respectively [24], were determined from the difference between the wet and dry weights of cell pellets minus the [^{14}C]inulin space, as described previously [40]. Molar intracellular drug concentration was then determined from the mole content of cell VP-16 and the intracellular water volume.

RESULTS

Growth Inhibitory Effects of Topoisomerase II Inhibitors

A stable etoposide-resistant K562 subline (K/VP.5) selected by continuous exposure to 0.5 μM VP-16 has been isolated and characterized previously [13, 24–26]. When compared with the parental line, K/VP.5 cells were 26-fold resistant to VP-16 (Fig. 1A; Table 1) and express only one-fifth the level of topoisomerase II protein [13, 24]. K/VP.5 cells were 4.0-fold cross-resistant to merbarone but did not exhibit cross-resistance to fostriecin in a 48-hr growth inhibitory assay (Fig. 1C and D; Table 1). In contrast, K/VP.5 cells were 3.4-fold collaterally sensitive to ICRF-187 (Fig. 1B; Table 1). These results suggest that the mechanisms of action and of resistance differ significantly among these agents. Furthermore, these results suggest that the level of topoisomerase II protein alone is not a direct correlate with sensitivity to these agents.

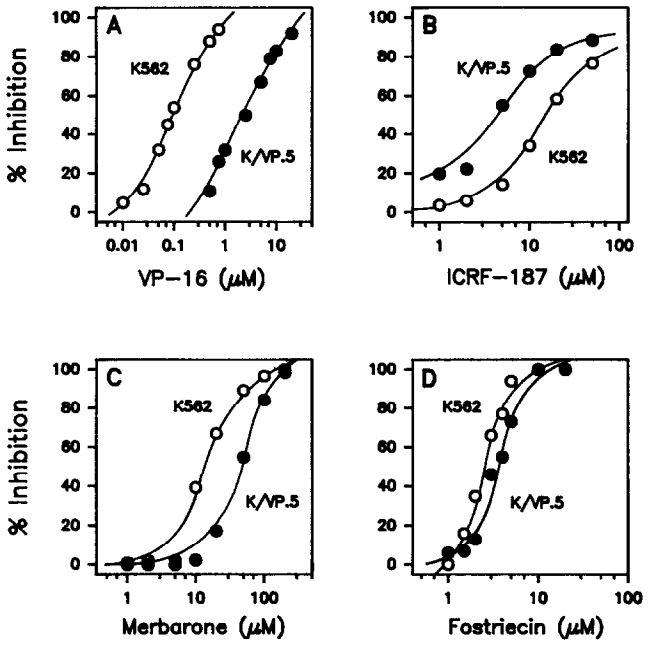

FIG. 1. Growth inhibitory effects of VP-16 (A) and three non-complex-stabilizing topoisomerase II inhibitors, ICRF-187 (B), merbarone (C), and fostriecin (D), in K562 and K/VP.5 cells. K562 and K/VP.5 cells (2 mL at 1 × 10^5 cells/mL) were treated with drug for 48 hr prior to the assessment of growth inhibition. Results shown are representative of at least three experiments performed on separate days.

Inhibition of Topoisomerase II Catalytic Activity by ICRF-187

After normalizing for the difference in topoisomerase II levels in nuclear extracts obtained from K562 and K/VP.5 cells, a similar concentration-dependent inhibition of topoisomerase II catalytic activity by ICRF-187 was observed in both cell lines (Fig. 2). The 50% inhibitory concentrations for topoisomerase II decatenation of kDNA in the presence of ICRF-187 were 21.1 ± 3.1 and 27.2 ± 2.6 μM

TABLE 1. Cross-resistance of K/VP.5 cells to topoisomerase II inhibitors

Agent	IC_{50}* (μM) K562	IC_{50}* (μM) K/VP.5	Relative resistance†
VP-16	0.07 ± 0.01 (5)	1.85 ± 0.34‡ (5)	26.4
ICRF-187	17.1 ± 1.2 (5)	5.1 ± 0.6‡ (5)	0.3
Merbarone	10.4 ± 0.7 (4)	41.3 ± 2.3§ (3)	4.0
Fostriecin	2.5 ± 0.3 (3)	3.2 ± 0.4 (3)	1.3

* Fifty percent inhibitory concentration in a 48-hr growth inhibition assay. Values are means ± SEM; numbers in parentheses = number of experiments performed on different days.

† IC_{50} of K/VP.5 cells divided by that of the parental K562 cell line.

‡ Significantly different from K562 cells ($P < 0.05$); Student's paired *t*-test.

§ Significantly different from K562 cells ($P < 0.05$); Student's unpaired *t*-test.

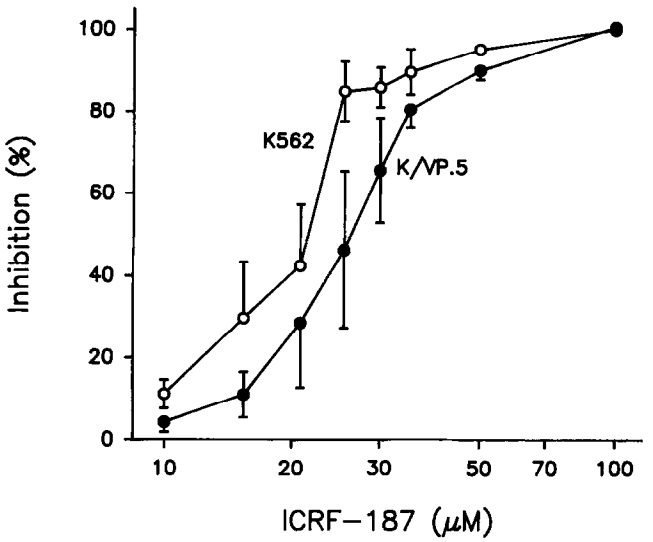

FIG. 2. Inhibition of topoisomerase II catalytic decatenation activity by ICRF-187. Sodium chloride (0.8 M)-extracted topoisomerase II from the nuclei of K562 and K/VP.5 cells was incubated with 1 μg of ^{3}H-labeled kDNA in the presence of 1 mM ATP and 0–100 μM ICRF-187 for 30 min at 30°. Decatenation of kDNA was measured subsequent to centrifugal separation of catenated from decatenated DNA as described in Materials and Methods. To normalize for the 5-fold differences in topoisomerase II protein levels in nuclear extracts from these two cell lines [13, 24], 0.5 and 2.5 μg of nuclear extract protein were used from K562 cells and K/VP.5, respectively. These amounts of nuclear extract proteins from K562 and K/VP.5 cells decatenated 85–95% of kDNA under the experimental conditions used. Inhibition of decatenation is expressed relative to decatenation activity observed in the absence of ICRF-187. Values are means ± SEM for 3–5 experiments. The 50% inhibitory concentrations were 21.1 ± 3.1 and 27.2 ± 2.6 μM ICRF-187 for K562 and K/VP.5 cells, respectively.

for K562 and K/VP.5 cells, respectively. Under identical experimental conditions, we previously demonstrated no difference in VP-16-induced inhibition of topoisomerase II decatenation activity in K562 compared with K/VP.5 cells [24]. Therefore, our results indicate that resistance to VP-16 and the observed collateral sensitivity to ICRF-187 in K/VP.5 cells were not due to an intrinsic change in topoisomerase II catalytic activity.

Inhibition of VP-16-Induced DNA–Topoisomerase II Covalent Complexes by ICRF-187, Merbarone, and Fostriecin in Intact Cells

Using various concentrations of VP-16, topoisomerase II–DNA complexes were induced to a greater extent in K562 cells than in K/VP.5 cells (Fig. 3) consistent with our previous reports of both qualitative and quantitative changes in topoisomerase II in resistant K/VP.5 cells [24–26]. Previous reports indicate that bisdioxopiperazines and merbarone prevent topoisomerase II–DNA covalent complex formation induced by a variety of topoisomerase II poisons [27, 30, 31, 34, 41]. Therefore, we studied the effects of preincubation with ICRF-187, merbarone, and fostriecin on subsequent VP-16-induced topoisomerase II–DNA covalent complexes in K562 cells and K/VP.5 cells (Figs. 4–7). In these experiments, inhibition of VP-16-induced topoisomerase II–DNA complexes was determined by comparing the fold-increase in VP-16 (100 μM)-induced topoisomerase II–DNA complexes (as in Fig. 3) in the absence and presence of these three inhibitors. Results shown in Fig. 4 indicate that a 5-min preincubation with ICRF-187 (20–100 μM) caused a concentration-dependent inhibition of VP-16 (100 μM)-induced topoisomerase II–DNA complexes in both cell lines and significantly more inhibition of VP-16 activity in K/VP.5 cells than in K562 cells (P = 0.028, paired Student's t-test). These results are consistent with the concept that ICRF-187 inhibits a greater proportion of topoisomerase II molecules in K/VP.5 cells than in K562 cells since K/VP.5 cells contain decreased topoisomerase II protein levels. These results are also consistent with the observed collateral sensitivity of K/VP.5 cells to ICRF-187 (Fig. 1B; Table 1).

To exclude the possibility that ICRF-187-mediated inhibition of VP-16-induced topoisomerase II–DNA complexes was due to a decrease in membrane transport of VP-16, the effect of a 5-min preincubation with ICRF-187 (500 μM) on VP-16 accumulation was examined in both K562 and K/VP.5 cells. After a 30-min incubation with 100 μM VP-16, the intracellular VP-16 concentration in K562 cells was 83.3 ± 0.7 and 83.3 ± 1.5 μM in the absence and presence of ICRF-187 (500 μM), respectively. Similarly,

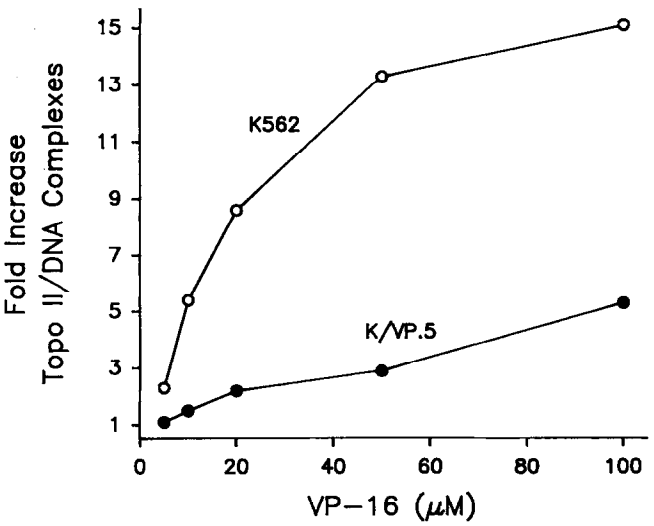

FIG. 3. Formation of topoisomerase II–DNA covalent complexes in K562 and K/VP.5 cells during a 30-min incubation with VP-16. Cells were prelabeled with [*methyl*-^{3}H]thymidine and [U-^{14}C]leucine for 18–24 hr. Cells were incubated for 15 min in the presence of increasing concentrations of VP-16. Potassium chloride–SDS-precipitable complexes were isolated, and the ^{3}H counts were normalized using ^{14}C as an internal standard for cell number as described in Materials and Methods. Results are expressed as fold-increase in VP-16-induced topoisomerase II–DNA complexes relative to complexes isolated from cells incubated in the absence of VP-16.

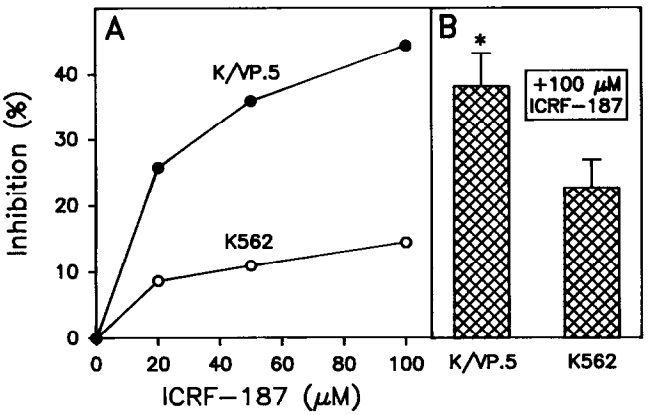

FIG. 4. Inhibition of VP-16-induced topoisomerase II–DNA covalent complexes in K562 and K/VP.5 cells following a 5-min preincubation with ICRF-187. (A) K562 and K/VP.5 cells were incubated for 5 min with the indicated concentrations of ICRF-187 and then incubated for an additional 30 min in the presence of 100 μM VP-16. Data shown are from a single representative experiment out of four experiments run using similar ICRF-187 concentrations and that gave similar results. (B) Inhibitory effects of 100 μM ICRF-187 on topoisomerase II–DNA complex formation induced by 100 μM VP-16. The inhibitory effects of ICRF-187 on VP-16 activity in both cell lines were determined by comparing the fold-increase in VP-16-induced topoisomerase II–DNA complex formation (as in Fig. 3) in the absence and presence of ICF-187. Bars represent the mean ± SEM from five separate experiments performed on different days. Key: (*) the inhibitory effect of ICRF-187 in K/VP.5 cells was significantly different from the ICRF-187 effect in K562 cells ($P < 0.05$, paired Student's *t*-test).

ICRF-187 had no effect on VP-16 accumulation in K/VP.5 cells where the VP-16 concentration was found to be 96.0 ± 1.7 and 96.7 ± 0.3 μM in the absence and presence of ICRF-187, respectively. Besides confirming that ICRF-187 does not inhibit VP-16 uptake, these results indicate that VP-16 does not concentrate in cells and is consistent with our previously published finding that K/VP.5 cells accumulate slightly greater levels of VP-16 compared with K562 cells [24].

Pretreatment of K562 cells and K/VP.5 cells with ICRF-187 (200 μM) for various times prior to the addition of 100 μM VP-16 progressively inhibited formation of VP-16-stabilized topoisomerase II–DNA complexes in both cell lines (Fig. 5). Again, ICRF-187 caused more inhibition of VP-16 activity in K/VP.5 cells than in K562 cells. By linear regression, the rate of onset of the ICRF-187 effect was not significantly different in resistant compared with sensitive cells (four experiments, $P = 0.69$; paired Student's *t*-test). In separate experiments, pretreatment of K/VP.5 cells with ICRF-187 (500 μM) prior to addition of 100 μM VP-16 resulted in a time-dependent inhibition of VP-16 action that reached a plateau at 80% inhibition within 1 hr (Fig. 6). Under these experimental conditions, increasing ICRF-187 concentrations to as high as 1 mM did not further inhibit VP-16-induced topoisomerase II–DNA complex formation (not shown), indicating that ICRF-187 cannot completely inhibit VP-16 action. In contrast, a 15-min preincubation with 1 mM merbarone resulted in 97% inhibition of VP-16 (100 μM)-induced topoisomerase II–DNA complexes in K/P.5 cells (not shown).

The effect of a 15-min preincubation with merbarone (50–600 μM) on VP-16-induced topoisomerase II–DNA complexes in both K562 and K/VP.5 cells is shown in Fig. 7A. In contrast to the effects seen with ICRF-187 (Figs. 4 and 5), however, there was more merbarone-mediated inhibition of VP-16 activity in K562 cells than in K/VP.5 cells. Fostriecin (5–100 μM) was without effect on VP-16-induced topoisomerase II–DNA covalent complex formation in either cell line (Fig. 7B). Together, these results suggest that these non-classic topoisomerase II inhibitors exert their effects through diverse mechanisms.

DISCUSSION

Using K562 cells and VP-16-resistant K/VP.5 cells, we have studied the effects of topoisomerase II inhibitors that do not stabilize topoisomerase II–DNA complexes. In K/VP.5 cells, there was 4.0-fold cross-resistance to merbarone, 1.3-fold cross-resistance to fostriecin, and 3.4-fold collateral sensitivity to bisdioxopiperazine ICRF-187. Since the cytotoxicity of various bisdioxopiperazines correlates with topoisomerase II inhibition [42] and since K/VP.5 cells con-

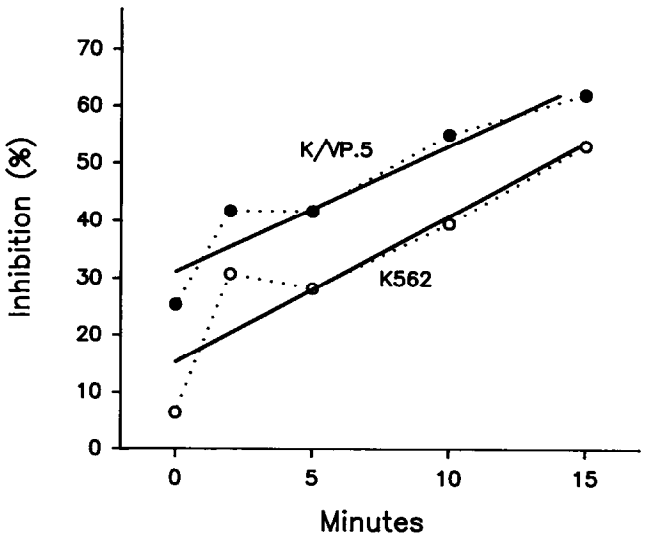

FIG. 5. Onset of ICRF-187 inhibition of VP-16-induced topoisomerase II–DNA covalent complex formation in K562 and K/VP.5 cells. Cells were incubated for 0, 2, 5, 10, or 15 min with 200 μM ICRF-187 followed by a 30-min incubation with 100 μM VP-16. Potassium chloride–SDS-precipitable complexes were isolated, and the ^3H counts were normalized using ^{14}C as an internal standard for cell number as described in Materials and Methods. The experiment shown is representative of four experiments performed on separate days. Averaging results from these four experiments, there was no significant difference in the rate of onset of ICRF-187 inhibition of VP-16 activity (determined by linear regression) in K562 cells compared with K/VP.5 cells ($P = 0.69$; paired Student's *t*-test).

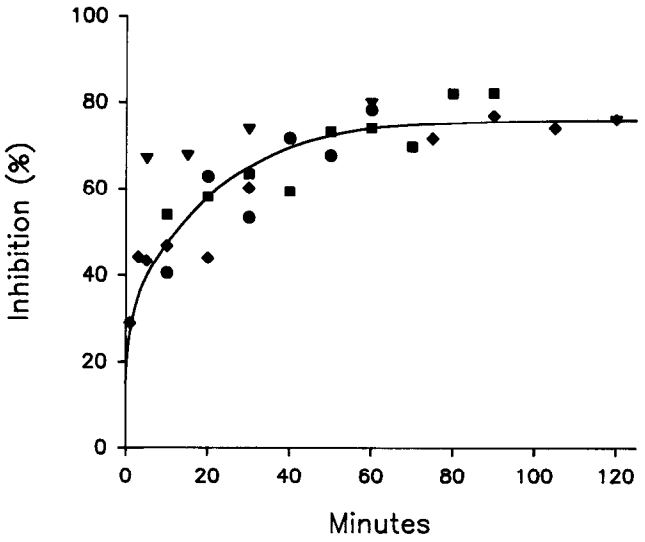

FIG. 6. Time-dependent inhibition by ICRF-187 of VP-16-induced topoisomerase II–DNA covalent complex formation in K/VP.5 cells. Cells were incubated for up to 2 hr with 500 μM ICRF-187 followed by a 30-min incubation with 100 μM VP-16. Potassium chloride–SDS-precipitable complexes were isolated, and the ^{3}H counts were normalized using ^{14}C as an internal standard for cell number as described in Materials and Methods. Results shown are composited from four separate experiments performed on separate days.

tain one-fifth the topoisomerase II content of parental K562 cells, the collateral sensitivity of K/VP.5 cells to ICRF-187 is consistent with the hypothesis that ICRF-187 activity is inversely related to the level of topoisomerase II. These results also suggest that the mechanisms of action of ICRF-187, merbarone, and fostriecin are quite different from one another.

It is believed that bisdioxopiperazines cause a conformational change in topoisomerase II, trapping the enzyme in a closed protein clamp [32]. These agents, therefore, prevent the capture of an intact double-strand of DNA that would normally be transported through a transient gate formed by a topoisomerase II-mediated DNA double-strand break [43]. Also, in the presence of bisdioxopiperazines the closed topoisomerase II clamp is not capable of hydrolyzing ATP, thereby preventing enzyme turnover [32]. These inhibitory effects on topoisomerase II antagonize topoisomerase II–DNA complex formation induced by traditional topoisomerase II poisons such as VP-16, m-AMSA, and daunorubicin [27, 30, 31, 34, 41, 44].

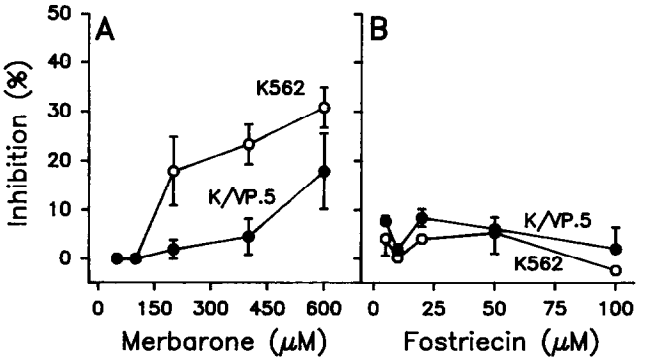

FIG. 7. Percent inhibition of VP-16-induced topoisomerase II–DNA covalent complex formation by (A) merbarone and (B) fostriecin. Cells were incubated for 15 min in the presence of merbarone or fostriecin followed by a 15-min incubation with VP-16. Potassium chloride–SDS-precipitable complexes were isolated, and the ^{3}H counts were normalized using ^{14}C as an internal standard for cell number as described in Materials and Methods. Points are means ± SEM for at least three experiments.

We have demonstrated that, in a concentration-dependent manner, both ICRF-187 and merbarone antagonize VP-16-induced topoisomerase II–DNA covalent complex formation in K562 and K/VP.5 cells. In K/VP.5 cells that contain a reduced topoisomerase II protein level compared with K562 cells, merbarone was less effective in inhibiting VP-16 effects. In contrast, ICRF-187 caused a greater inhibition of VP-16-induced topoisomerase II–DNA complexes in K/VP.5 cells than in the parental K562 cells. Decreased topoisomerase II protein levels in K/VP.5 cells permit more complete inhibition of topoisomerase II by ICRF-187, resulting in greater antagonism of VP-16 effects. These results are consistent with the demonstration in yeast that bisdioxopiperazine cytotoxicity is inversely related to topoisomerase II levels [37].

Although merbarone, like ICRF-187, is a topoisomerase II inhibitor that does not stabilize a covalent intermediate, its effects are directly related to topoisomerase II levels since there is: (1) 4-fold cross-resistance in K/VP.5 cells; and (2) less antagonism of VP-16 activity in K/VP.5 cells that contain less enzyme. Fostriecin caused minimal inhibition of VP-16-induced topoisomerase II–DNA complex formation consistent with the recent report that this agent exerts its cytotoxic effect primarily by acting as a phosphatase inhibitor [45]. In addition, the lack of cross-resistance to fostriecin in K/VP.5 cells is in accord with a recent report in human small-cell lung carcinoma cells that exhibit resistance to topoisomerase II poisons but lack cross-resistance to fostriecin [46]. Overall, our results indicate that mechanistic differences exist between ICRF-187, merbarone, and fostriecin.

As the preincubation time with ICRF-187 was increased, there was progressively greater inhibition of VP-16-induced topoisomerase II–DNA covalent complex formation in both sensitive and resistant cells. The rate of onset of ICRF-187-mediated inhibition of VP-16 activity did not differ between the two cell lines, suggesting that the collateral sensitivity of the K/VP.5 cells is not due to some intrinsic change in topoisomerase II activity but rather is reflective of the difference in topoisomerase II levels found in these cells.

Preincubation of K/VP.5 cells for up to 2 hr with 500 μM ICRF-187 did not inhibit completely the formation of VP-16-induced topoisomerase II–DNA covalent complexes.

These results suggest that there exists a pool of topoisomerase II that is sensitive to VP-16 but inaccessible to ICRF-187. Topoisomerase II poisons can affect both salt-extractable and nuclear matrix-bound topoisomerase II [47], but topoisomerase II inhibitors such as ICRF-187 have only been examined using salt-extractable enzyme. We are currently investigating the possibility that incomplete ICRF-187 inhibition of VP-16-induced topoisomerase II–DNA complexes is due to a reduced or non-existent interaction with nuclear matrix-bound topoisomerase II.

Taken together, results presented here indicate that VP-16-resistant K/VP.5 human leukemia cells are collaterally sensitive to the non-complex-stabilizing topoisomerase II inhibitor ICRF-187. Collateral sensitivity is due, at least in part, to the decrease in topoisomerase II levels that are characteristic of K/VP.5 cells, since ICRF-187 does not seem to differentially affect topoisomerase II catalytic activity or the rate at which ICRF-187 effects are manifest in K562 cells compared with K/VP.5 cells. The differential cross-resistance patterns and dissimilar effects of ICRF-187, merbarone, and fostriecin on VP-16 activity indicate the usefulness of our VP-16-resistant K/VP.5 cell line for the future study of the distinct mechanisms of action of non-classical topoisomerase II inhibitors.

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