Differential Induction of Topoisomerase I-DNA Cleavage Complexes by the Indenoisoquinoline MJ-III-65 (NSC 706744) and Camptothecin: Base Sequence Analysis and Activity against Camptothecin-Resistant Topoisomerases I

Smitha Antony, Muthusamy Jayaraman, Gary Laco, Glenda Kohlhagen, Kurt W. Kohn, Mark Cushman, and Yves Pommier

Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892-4255 

ABSTRACT

Camptothecin (CPT) and its derivatives target mammalian DNA topoisomerase I (top1) and are among the most effective novel anticancer drugs. However, the activity of CPTs is limited by several factors, including drug inactivation by lactone ring opening, tumor drug resistance, and toxicity in patients. Novel top1 inhibitors are being searched to overcome such limitations and expand the anticancer spectrum of camptothecins. MJ-III-65 (NSC 706744) is among the most promising indenoisoquinolines to date. In this study, we show that MJ-III-65 enhances top1 cleavage complexes by both inhibiting their reversal (religation) more efficiently than CPT and by enhancing their formation. The top1 DNA cleavage complexes induced by MJ-III-65 exhibit a different distribution pattern compared with CPT and exhibit different base sequence preferences immediately around the top1 cleavage sites. Although CPTs have a preference for thymine at the (−1) position and guanine at the (+1) position of the top1-mediated DNA cleavage sites, MJ-III-65 can accommodate different base pairs at the (−1), (+1), or (±2) position, with a preference for a cytosine at the (−1) position on the scissile strand. Another difference with CPT is the activity of MJ-III-65 against CPT-resistant top1 enzymes, implying that the amino acid residue interactions with top1 are different for MJ-III-65 and CPTs. As with CPT, MJ-III-65 is inactive against vaccinia top1. This study shows the specific molecular interactions of MJ-III-65 with top1 and demonstrates that MJ-III-65 is a potentially useful top1 inhibitor that enhances and traps top1 cleavage sites not sensitive to CPTs.

INTRODUCTION

The activity of CPT against a broad spectrum of malignancies validates top1 as an anticancer target (1–5). Although CPTs are very potent and selective top1 inhibitors, they are chemically unstable and are inactivated by lactone E-ring opening (6). Moreover, the rapid reversibility of top1 inhibition upon CPT removal (7) imposes prolonged drug infusions, and CPTs have dose-limiting toxicities. The fact that CPTs are the only class of top1 poisons used in the clinic, to date, prompted us to search for novel top1 inhibitors. Development of novel top1 poisons is also legitimized by the knowledge that drugs from different chemical families, which share a common cellular target, generally exhibit different spectra of anticancer activity (for instance, topoisomerase II or tubulin inhibitors; Refs. 8, 9). After a COMPARE analysis of the National Cancer Institute drug screen database, we identified an indenoisoquinoline (NSC 314622) as a top1 inhibitor (Refs. 9–11; Fig. 1). Subsequent analyses at the biochemical and cellular levels showed that NSC 314622 is a top1 poison producing persistent protein-linked DNA breaks indicative of top1 inhibition (12).

The differential top1 cleavage sequence preference, slower reversibility of cleavage complexes, and chemical stability of NSC 314622 compared with CPT led us to synthesize and investigate a series of novel indenoisoquinolines (13–15), using NSC 314622 as a lead. Substitution of the methyl group at the 3′-position of the parent compound NSC 314622 with an amino alcohol [2-(hydroxyethyl)-aminoalkyl] in MJ-III-65 (NSC 706744; Fig. 1) was found to increase both cytotoxicity in human cancer cell lines and inhibition of top1 (14).

This study was undertaken to gain insight into the molecular interactions between MJ-III-65 and top1. We report a comparison of the DNA sequence selectivity of MJ-III-65 and CPT and the differential trapping of top1 by MJ-III-65 and CPT. We synthesized a 22-mer oligonucleotide with a MJ-III-65-specific site for top1 and found that MJ-III-65 exhibits a different pattern of cleavage from CPT with a preference for a cytosine (C) immediately 5′ from the cleavage site (position −1). We also report that MJ-III-65 remains active against top1 enzymes known to be resistant to CPTs, homoCPTs, and indolocarbazoles (1, 16–20).

MATERIALS AND METHODS

Drugs, Enzymes, and Chemicals. CPT was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD). The synthesis of MJ-III-65 (NSC 706744) has been described previously (14). Drug stock solutions were made in DMSO at 10 mM for CPT and 5 mM for MJ-III-65. Aliquots were stored at −20°C, and additional dilutions were made in DMSO immediately before use. The final concentration of DMSO in the reaction mixtures did not exceed 10% (v/v).

Recombinant human top1 (wt) and top1 R364H were purified from TN5 insect cells (HighFive; Invitrogen Corp., San Diego, CA) using a Baculovirus construct for the NH2-terminus-truncated human top1 cDNA as described previously (20–23). Top1 N722S was a kind gift from Dr. Lance Stewart (24, 25). Vaccinia topoisomerase was a kind gift from Dr. Stewart Shuman (26). Terminal deoxynucleotidyl transferase, DNA polymerase I (Klenow fragment), deoxynucleotide triphosphate [where N is A (adenosine), C (cytosine), G (guanosine), or T (thymine)], agarose, and polyacrylamide/bis were purchased from Life Technologies, Inc. (Gaithersburg, MD), or New England Biolabs (Beverly, MA). DNA quick spin columns were purchased from Roche Diagnostics Corporation (Indianapolis, IN). [α-32P]dGTP and [α-32P]cordycepin 5′-triphosphate were purchased from DuPont-New England Nuclear (Boston, MA). Oligonucleotides were synthesized by MWG-Biotech (High Point, NC).

Top1 Reactions. The 161-bp fragment from pBluescript SK(−) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with restriction endonucleases PvuII and HindIII in supplied NE buffer 2 (New England Biolabs, Beverly, MA). 50-μl reactions, for 1 h at 37°C. Reaction products were separated by
electrophoresis in 1% agarose gels made in 1× Tris-borate EDTA buffer. The 161-bp fragment was eluted from the gel slice using the Qiaex II kit (Qiagen, Inc., Valencia, CA). The pSK fragment was singly 3′-end labeled by a fill-in reaction. Briefly, linearized pSK (200 ng) was incubated with [α-32P]dGTP in 1× labeling buffer (0.5 mM each dATP, dCTP, and dTTP in 50 mM Tris-HCl (pH 8.0), 100 mM MgCl2, and 50 mM NaCl) in the presence of 0.5 units of the Klenow fragment of DNA polymerase I. Labeled DNA was purified using mini quick spin DNA columns (Roche Diagnostics Corporation).

Single-stranded oligonucleotides were 3′-labeled with [α-32P]-cytidine and terminal deoxynucleotidyl transferase as described previously (27). Labeled mixtures were subsequently centrifuged through mini quick spin Oligo columns (Roche Diagnostics Corporation) to remove the unincorporated nucleotide substrate was 706744) and NSC 314622.

To the reaction mixtures, 3.3 volumes of Maxam Gilbert loading buffer (50 mM sodium acetate, pH 4.5, 7 mM sodium bisulfate, and 1 mM EDTA) by heating the reaction mixtures to 95°C for 5 min, followed by slow cooling to room temperature. For top1 cleavage assays, labeled DNA (~50 fmol/reaction) was incubated with 5 ng of recombinant top1 with or without drug at either 25°C or 0°C in 10 μl of reaction buffer [10 mM Tris-Cl (pH 7.5), 50 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 15 μg/ml BSA, final concentrations]. For reactions with vacinia topoisomerase, 40 ng of the enzyme were used (28). The oligonucleotide substrate was

\[5′ CGTCGCGGGTTTAAGCGATAGTG^* \] GCACAGCCGAAA-TAGGCTATCAC.

The caret indicates the top1 cleavage site and the asterisk the 3′-end label. Reactions were stopped by adding SDS (0.5% final concentration). For reversal experiments, the SDS stop was preceded by the addition of NaCl to a final concentration of 0.35 M followed by incubation for varying time points (min) at either 25°C or 0°C.

To the reaction mixtures, 3.3 volumes of Maxam Gilbert loading buffer [80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromphenol blue (pH 8.0)] was added. Aliquots were separated in 16% (for pSK DNA and 51-bp nucleotide) or 20% (for 22- and 24-bp oligonucleotides) denaturing polyacrylamide gels (7 M urea) in 1× Tris-borate EDTA (45 mM Tris, 45 mM boric acid, and 1 mM EDTA) for 2 h at 40 V/cm at 50°C.

Imaging and quantitation were performed using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

### RESULTS

**MJ-III-65 Induces top1-Mediated DNA Cleavage Complexes with a Different Cleavage Pattern from CPT.** Induction of DNA cleavage complexes in the presence of top1 was tested in the PvuII/HindIII fragment of pBluescript SK(−) phagemid DNA (pSK; Fig. 2A; Ref. 13). MJ-III-65 and CPT induced DNA cleavage complexes at several similar sites with differences in their relative intensities (sites 70, 92, 97, and 119 in Fig. 2B). MJ-III-65 and CPT induced DNA cleavage complexes at several similar sites with differences in their relative intensities (sites 70, 92, 97, and 119 in Fig. 2B).

The rate of the forward reaction is denoted as k1, and r is the fraction of the intact DNA cleaved. The second reaction reglates the ends of the broken DNA (after strand passage). The rate of this reverse reaction is denoted as k2.

At steady state,

\[k_1 (1 - r) = k_2 r\]

\[k_1 = \frac{k_2 r}{(1 - r)}\]

where \(r_0\) is the fraction of the cleaved DNA at time \(t = 0\) (time when NaCl is added), and \(r\) is the fraction of cleavage product at the plateau (Fig. 4B).

\[\ln \frac{r}{r_0} = -k_2 t\]

\[\ln \frac{r_0}{r} = -k_2 \frac{t_1/2}{t_1/2}\]

where \((T_{1/2})\) is the time taken for half of the cleaved product to reverse and was calculated from the semilog plot of the percentage of cleavage product remaining after salt reversal (see Fig. 4A).

The relaxation rate \(k_2\) was calculated as:

\[k_2 = \frac{\ln 2}{t_{1/2}}\]

**RESULTS**

**MJ-III-65 Induces top1-Mediated DNA Cleavage Complexes with a Different Cleavage Pattern from CPT.** Induction of DNA cleavage complexes in the presence of top1 was tested in the PvuII/HindIII fragment of pBluescript SK(−) phagemid DNA (pSK; Fig. 2A; Ref. 13). MJ-III-65 and CPT induced DNA cleavage complexes at several similar sites with differences in their relative intensities (sites 70, 92, 97, and 119 in Fig. 2A). Some cleavage sites, however, were unique to MJ-III-65 (sites 44 and 62) or CPT (site 37). This differential DNA cleavage pattern was additionally exemplified in a 51-bp oligonucleotide (Fig. 2B) corresponding to the region 25–77 (Fig. 2C) from within the pSK DNA. Fig. 2B shows the differential cleavage site preferences of CPT at site 37 and MJ-III-65 at sites 44 and 62. Increasing drug concentration increased the top1-mediated DNA cleavage complexes trapped by CPT at site 37. However, MJ-III-65
DNA was reacted with Top1 in the absence of drug (Top1) or in the presence of CPT corresponding to the region 56 to the 3'-end of the breaks. The PvuII fragment with the cleavage at sites 37, 44, and 62 indicated. Top1 (data not shown) is linked positions by Top1 in the DNA fragment analyzed.

The side of the gels indicate the migration positions of DNA fragments cleaved at these positions by Top1 in the DNA fragment analyzed. Lane G, purine ladder after formic acid sequencing of the control DNA. Numbers to indicate above the sequence. B, a 51-bp oligonucleotide (sequence shown in C) corresponding to the region encompassing positions 25–77 (as boxed in A) of the pSK DNA was generated and subjected to the same treatments as in A with the indicated concentrations (μM) of drugs. Lane G+A, purine ladder after formic acid sequencing of the control DNA. Numbers to the side of the gels indicate the migration positions of DNA fragments cleaved at these positions by Top1 in the DNA fragment analyzed. C, sequence of the 51-bp oligonucleotide with the cleavage at sites 37, 44, and 62 indicated. Top1 (data not shown) is linked to the 3'-end of the breaks. The underlined sequence corresponds to an oligonucleotide corresponding to the region 56–77 (22 bp) that was used for subsequent studies.

showed a preference for site 44 at lower doses and site 62 at higher doses. These results suggest that MJ-III-65 is a Top1 poison with DNA cleavage patterns exhibiting similarities and differences from those of CPT.

To further understand the molecular mechanisms leading to Top1 trapping by MJ-III-65 and differential DNA sequence preferences for MJ-III-65 and CPT, a 22-bp oligonucleotide corresponding to the region 56–77 bp within the pSK DNA was generated (underlined sequence in Figs. 2C and 3A). This oligonucleotide contains a single MJ-III-65-induced Top1-mediated DNA cleavage site (Fig. 3B, site 62). No cleavage was detectable when the oligonucleotide was 3'-end labeled on the scissile strand (data not shown). Increasing MJ-III-65 concentrations showed increased DNA cleavage; however, CPT did not induce any significant cleavage at this site. This site has the bases C and A on the upper strand at the (−1) and (+1) positions, respectively (Fig. 3A).

MJ-III-65 Inhibits Top1-Mediated DNA Religation More Efficiently Than CPT and Enhances the Formation of Top1 Cleavage Complexes. CPT is known to enhance Top1 cleavage by inhibiting DNA religation (29–33). Increasing the salt concentration can reverse the Top1 cleavage complexes, and this method has been used to compare the molecular interactions between CPT derivatives and Top1 cleavage complexes (31, 34). Fig. 4A and Table 1 show a comparison of the Top1 cleavage complexes induced by MJ-III-65 and CPT. Because CPT is inactive on the MJ-III-65-specific oligonucleotide (Fig. 3B), the comparison for CPT was made using the oligonucleotide containing a high-affinity site for Top1 and CPT (35–37). The reversal rate for MJ-III-65-mediated DNA cleavage complexes at 25°C was about four times slower than for CPT. This was also found to be the case in pSK DNA (Fig. 5) where salt reversal of Top1 cleavage complexes was much slower for MJ-III-65 (sites 44 and 62; Fig. 2A) than for CPT (sites 70 and 119). Site 92, which is targeted by both drugs, showed similar reversal. These results indicate that MJ-III-65-induced Top1-mediated DNA cleavage complexes are more stable than those of CPT. Their reversibility is consistent with the reversible trapping of Top1 cleavage complexes by MJ-III-65.

The kinetics of formation of MJ-III-65-induced Top1-mediated DNA cleavage complexes was examined in the oligonucleotide containing a single Top1-mediated cleavage site inducible by MJ-III-65 but not by CPT. A, sequence of the 22-bp oligonucleotide corresponding to the region (56–77) of the pSK DNA (underlined in Fig. 2C). The positions relative to the Top1 cleavage site [between positions (−1) and (+1)] are indicated above the sequence. B, the 22-bp fragment was 3'-end labeled on the scissile strand and then reacted with Top1 in the presence of the indicated concentrations (μM) of MJ-III-65 or CPT. Reactions were at 25°C for 20 min and stopped by adding 0.5% SDS. DNA fragments were separated in a 20% denaturing polyacrylamide gel. Lane G+A, purine ladder. Arrow indicates the position of the Top1-mediated DNA cleavage site induced by MJ-III-65 corresponding to the position (62) in the pSK DNA (Fig. 2).
Fig. 4 Kinetics of formation and reversal of MJ-III-65- and CPT-induced top1-mediated DNA cleavage complexes. A, the 22-bp oligonucleotide (Fig. 3A) corresponding to the region 56–77 within the pSK DNA was 3’-end labeled on the scissile strand and then reacted with top1 in the absence of drug (top1) or in the presence of 1 μM MJ-III-65 (MJ-III-65) for 20 min at 25°C. Reactions were stopped with 0.5% SDS before (0) and after reversal. Reactions were salt reversed in 0.35 M NaCl at either 25°C or 0°C for the indicated time periods (min). Time 0 refers to the samples taken immediately before NaCl addition. DNA fragments were separated in 20% denaturing polyacrylamide gels. Similar reactions were carried out using the 22-bp oligonucleotide containing a single CPT-inducible top1-mediated DNA cleavage site in the absence (top1) or presence of 1 μM CPT (CPT). The percentage of top1-mediated cleavage products obtained after reversal for MJ-III-65 (●, □) and CPT (○, △) at 25°C (●, ○) and 0°C (□, △) were quantified and represented graphically. Values are normalized to the drug-induced cleavage product remaining at time 0 taken as 100%. B, using the same oligonucleotides as in A, reactions with top1 in the absence of drug (top1) or presence of either 1 μM MJ-III-65 or 1 μM CPT were carried out at either 0°C or 25°C for the indicated time points (min). Reactions were stopped with 0.5% SDS. DNA fragments were separated in 20% denaturing polyacrylamide gels. The percentage of top1-mediated cleavage products obtained for MJ-III-65 (●, □) and CPT (○, △) at 25°C (●, ○) and 0°C (□, △) were quantified and represented graphically. Quantitation of the kinetic constants is shown in Table 1.

Substitutions at the (−1) but not (+1) and (+2) Affect MJ-III-65-Induced top1-Mediated DNA Cleavage. Poisoning of the top1-DNA cleavage complex by CPT does not occur at all top1 cleavage sites along the DNA chain. This site selectivity exhibited by CPT is dependent on the bases immediately preceding (−1) and following (+1) the cleavage site (29, 32, 33, 37, 38). To identify the base preferences of MJ-III-65, substitutions at the (−1), (+1), and (+2) bases were carried out (Table 2). Substitution of C to T at the (−1) position of the cleavage site on the upper strand created a new CPT-inducible top1-mediated DNA cleavage site (Fig. 6). However, this (−1) change reduced the cleavage efficiency of MJ-III-65 by ~50% (Figs. 6 and 7A). Substitution of the (+1) base from A to G or C or T (Fig. 7A) or the (+2) base from C to a G or A or T (Fig. 7B) on the scissile strand did not significantly affect the cleavage efficiency of MJ-III-65. These results demonstrate different base sequence selectivity for MJ-III-65 and CPT.

Table 1 Cleavage and religation rate constants for MJ-III-65 and CPT

<table>
<thead>
<tr>
<th></th>
<th>MJ-III-65</th>
<th>MJ-III-65</th>
<th>CPT</th>
<th>CPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{1/2}</td>
<td>&gt;180</td>
<td>5.5</td>
<td>61</td>
<td>1.5</td>
</tr>
<tr>
<td>k_{2}</td>
<td>NA</td>
<td>0.126</td>
<td>0.0114</td>
<td>0.462</td>
</tr>
<tr>
<td>k_{1}</td>
<td>NA</td>
<td>0.4865</td>
<td>0.00392</td>
<td>0.276</td>
</tr>
</tbody>
</table>

T_{1/2}, religation rate half-life determined from the salt reversal experiments (Fig. 4A); k_{2}, religation rate constant determined from T_{1/2}; k_{1}, cleavage rate constant (see "Materials and Methods"). NA, not applicable.
MJ-III-65 Induces top1-Mediated DNA Cleavage in the Presence of CPT-Resistant top1 Enzymes. To further investigate the interactions of MJ-III-65 with top1, two CPT-resistant top1 mutants were investigated: top1 R364H (where arginine 364 is changed to a histidine; Ref. 19) and top1 N722S (where asparagine 722 is changed to a serine; Ref. 16). These mutant top1s were used to test whether MJ-III-65 interaction with top1 involved the same residues as CPT. Fig. 8A–C show that using the mutant top1s, the DNA cleavage efficiency of CPT dropped by over 10-fold while that of MJ-III-65 was reduced 2-fold, indicating that these residues although important are not critical for MJ-III-65-induced top1-mediated DNA cleavage. Thus, MJ-III-65-top1 interactions differs from CPT.

MJ-III-65 Does Not Stabilize Vaccinia top1-Mediated DNA Cleavage Complexes. Vaccinia top1 exhibits a specificity for cleavage at the target sequence 5'-(C/T)CCCT 3' (the arrow indicates the top1 cleavage site; Ref. 39). MJ-III-65 was ineffective in stabilizing DNA-cleavage complexes induced by vaccinia top1 (Fig. 8D). Although MJ-III-65 has a relaxed base preference compared with CPT with respect to the bases pairs flanking the top1 cleavage sites (Fig. 6), as with CPT (26), MJ-III-65 remains inactive against vaccinia top1.

DISCUSSION

Since our first indenoisoquinoline was developed (40), ~100 derivatives have been synthesized with various structural modifications (13–15, 41); our goal being to discover more potent indenoisoquinoline derivatives (14). Among the most promising derivatives, MJ-III-65 (NSC 706744), which has an aminoalkyl substitution, exhibits both antiproliferative activity at submicromolar concentrations (Mid Graph Midpoint for Growth Inhibition [MGM] in

![Fig. 5. Trapping of top1 cleavage complex by MJ-III-65. The pSK 3'-end labeled DNA fragment was the same as in Fig. 2A. Reactions were performed with top1 in the presence of 1 μM CPT or 1 μM MJ-III-65. After incubation at 25°C for 20 min, reactions were reversed with 0.35 M NaCl for the indicated time points before addition of 0.5% SDS. Time 0 refers to the samples taken immediately before NaCl addition. DNA fragments were then separated in a 16% denaturing polyacrylamide gel. The top1-mediated cleavage products obtained upon treatment with CPT at sites 70, 92, and 119 and MJ-III-65 at sites 44, 62, and 92 (Fig. 2A) after salt reversal were quantified and represented graphically. Values are normalized to the drug-induced cleavage product obtained at time 0 taken as 100%.

![Fig. 6. C to T substitution at the (-1) position differentially affects MJ-III-65- and CPT-induced top1-mediated DNA cleavage. The 22-bp fragment (Fig. 3A) and its (-1) substituted oligonucleotide (Table 2) were 3'-end labeled on the scissile strand. C (-1) corresponds to the unmodified DNA (Fig. 3A), whereas T (-1) corresponds to an oligonucleotide where the (-1) bp was substituted to T/A (Table 2). Reactions were performed as in Fig. 3B in the presence of the indicated concentrations (μM) of MJ-III-65 and CPT. Lane G+A, purine ladder. Arrow indicates the top1-mediated DNA cleavage site corresponding to the position (62) in the pSK DNA and in the oligonucleotides shown in Fig. 3A and Table 2.](cancerres.aacrjournals.org)
the NCI cell screen, MGM = 0.21 μM) and potent top1 inhibition (14).

Our results indicate that top1 inhibition by MJ-III-65 exhibits both similarities and differences from CPT (Fig. 2). The similarities could be attributed to a structural overlap (as indicated by the dashed lines in Fig. 1) in the structures of the two heterocyclic top1 inhibitors (4, 42). The difference in the DNA cleavage patterns of CPT and MJ-III-65 is a reflection of the differential base sequence preferences between the two drugs. CPTs have a strict preference for a T (thymine) at the (−1) position and a preference for G (guanine or adenine) at the (+1) position of the top1-mediated DNA cleavage site (Fig. 6; Ref. 37), MJ-III-65 exhibits a relaxed preference at such positions (Figs. 6 and 7). Despite a preference for C (cytosine) at the (−1) position, MJ-III-65 produces top1-mediated DNA cleavage complexes with different bp at positions (−1), (+1) and (+2; Figs. 6 and 7). Such differences between indenoisoquinolines and CPTs may be important in cells because some genes may be more selectively targeted by one compound than the other, which may be translated into selective effects against various tumors. The concurrent use of both indenoisoquinolines and CPTs should reveal a more complete picture of the top1 cleavage/binding sites within the genome, which otherwise would have been restricted by using only one of the compounds.

The top1-mediated DNA cleavage complexes trapped by MJ-III-65 are more stable than those induced by CPT (~4-fold in Figs. 4 and 5 and Table 1), indicating that MJ-III-65 enhances top1 cleavage complexes by inhibiting top1-mediated DNA religation. Additional experiments indicate that after drug removal, the top1-DNA complexes induced by MJ-III-65 in human leukemia cells persist under conditions where CPT-induced top1-DNA complexes reverse completely (data not shown). Moreover, MJ-III-65 enhances the DNA cleavage rate of top1 ~2-fold more than CPT, which suggests that MJ-III-65 enhances top1 cleavage complexes also by inducing top1-mediated DNA cleavage.

From a drug therapeutic viewpoint, the greater stability of the top1 cleavage complexes induced by MJ-III-65 than by CPT might be
advantageous because the rapid reversibility of the CPT-induced cleavage complexes imposes long drug inductions. Also, by contrast to CPT-11 (Irinotecan), which needs to be hydrolyzed to its active metabolite SN-38 (5, 43, 44), MJ-III-65 is a synthetic compound that does not require metabolic activation. MJ-III-65 is also chemically stable, whereas CPTs are inactivated by E-ring opening (45–47).

One of the major clinical complications in cancer therapy is resistance to chemotherapeutic agents. Several studies of CPT-resistant mammalian cells (reviewed in Ref. 1) or yeast cells (48–50) identified point mutations in the Top1 gene, most of which have been shown in vitro to confer enzymatic drug resistance. Examination of the Top1 crystal structure (51) shows that these mutations cluster at the enzyme-DNA interface near tyrosine 723 (1), the residue involved in the covalent linkage of the enzyme to DNA that occurs transiently during the Top1 catalytic cycle and when Top1 cleavage complexes are trapped by anticancer drugs and DNA mutagenic lesions (16). Our studies using CPT-resistant Top1 enzymes (Fig. 8A–C) indicate that MJ-III-65 is effective against CPT-resistant enzymes, implying that the amino acid residue interactions within Top1 are different for Top1 and CPTs. Such differences may have considerable clinical significance for tumors that are refractory to CPT attributable to Top1 mutations because such tumors would remain sensitive to MJ-III-65. Although MJ-III-65 functions in CPT-resistant enzymes, we found that MJ-III-65 is ineffective in stabilizing vaccinia Top1 DNA cleavage complexes (Fig. 8D). Clearly, although being flexible with regard to DNA cleavage and Top1 amino acid residues critical for CPT activity, MJ-III-65 does retain specificity for the human Top1 enzymes.

The chemical stability, persistence of Tops1 DNA cleavage complexes, enhancement of Top1-mediated DNA cleavage, distinct DNA cleavage pattern and base sequence preferences, and ability to induce Top1 DNA cleavage complexes in the presence of CPT-resistant Top1 enzymes make MJ-III-65 a promising non-CPT Top1 inhibitor for therapeutic development and a useful reagent for mapping Top1 cleavage complexes in mammalian genomes.

REFERENCES


Differential Induction of Topoisomerase I-DNA Cleavage Complexes by the Indenoisoquinoline MJ-III-65 (NSC 706744) and Camptothecin: Base Sequence Analysis and Activity against Camptothecin-Resistant Topoisomerases I

Smitha Antony, Muthusamy Jayaraman, Gary Laco, et al.