Intercalation into DNA Is Not Required for Inhibition of Topoisomerase I by Indolocarbazole Antitumor Agents

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ABSTRACT

The DNA-intercalating antitumor drug NB-506 is a potent topoisomerase poison currently undergoing phase I/II clinical trials. It contains a planar indolocarbazole chromophore substituted with a glucose residue. Up until now, it was thought that intercalation of the drug into DNA was essential for the stabilization of topoisomerase I-DNA complexes. However, the present study, we show that a regio-isomeric form of NB-506 has lost its capacity to intercalate into DNA, but remains an extremely potent topoisomerase I poison. The new analogue contains two hydroxyl groups at positions 2,10 instead of positions 1,11 in NB-506. The relocation of the two OH groups reduces considerably the strength of binding to DNA and prevents the drug from intercalating into the DNA double helix. However, the topoisomerase I inhibition capacity of the new analogue remains very high. The two drug isomers are equally potent at maintaining the integrity of the topoisomerase I-DNA covalent complexes, but stimulate cleavage at different sites on DNA. NB-506 stabilizes topoisomerase I preferentially at sites having a pyrimidine (T or C) and a G on the 5’ and 3’ sides of the cleaved bond, respectively. The 2,10-isomer induces topoisomerase I-mediated cleavage only at TG sites and, thus, behaves exactly as the reference topoisomerase I poison camptothecin. Finally, cytotoxicity measurements performed with a panel of murine and human cancer cell lines reveal that the newly designed drug is considerably (up to 100-fold) more toxic to tumor cells than the parent drug NB-506. We conclude that the DNA-binding and topoisomerase I poisoning activities of NB-506 can be viewed as two separate mechanisms.

INTRODUCTION

NB-506 (Fig. 1) is a promising antitumor agent targeting topoisomerase I (1). This glycosylated indolocarbazole drug derives from the antibiotic BE13793C, isolated from culture supernatants of a Streptoverticillium species (2). NB-506 and related analogues, such as ED-110, are extremely toxic against various tumor cell lines (including cells resistant to vincristine and adriamycin) and are also very potent at inhibiting the growth of human solid tumors in mice (3–6). For these reasons, NB-506 which is the most active compound in the series, was recently advanced into clinical trials. The results of the first Phase I trials are promising and indicate exciting development for this compound in years to come (7, 8).

Like the CPTs3, NB-506 and related indolocarbazole compounds are potent inhibitors of topoisomerase I both in vitro and in cellular systems (9, 10). But, unlike the CPTs, NB-506 binds tightly to DNA even in the absence of topoisomerase I. Studies with indolocarbazoles related to NB-506 (e.g., rebeccamycin analogues) have revealed that the planar indolocarbazole chromophore can intercalate between two consecutive bp in the DNA double helix, thus placing the appended glucose residue into one of the helical grooves, most likely the minor groove. The sugar residue plays an essential role for the interaction with DNA and the recognition of specific sequences, as well as for the interference with topoisomerase I (11–14). Structure-activity relationship studies have revealed that the removal of the sugar reduces considerably the interaction of the drug with DNA and concomitantly decreases the topoisomerase I inhibition properties (15). Indolocarbazole analogues lacking the sugar moiety exhibit very weak topoisomerase I poisoning activities (12). Similarly, analogues bearing bulky groups on the indolocarbazole chromophore (e.g., halogeno-acetyl) or drugs for which the sugar residue is linked to the two indole nitrogens (as with the related drug staurosporine) generally show very weak interaction with DNA and produce minimal effects on topoisomerase I (16, 17). Therefore, these structure-activity relationships studies have lead to dogma that the DNA binding and topoisomerase I inhibition properties of drugs like NB-506 are tightly associated. In other words, it was thought that the intercalation of the drug into DNA was necessary for the drug to act as a topoisomerase I poison. The present study shows that this idea is not valid: intercalation into DNA need not accompany the binding of topoisomerase I inhibitors to DNA.

In this study, we present the DNA binding and topoisomerase I poisoning activities of NB-506 (compound 1) and its regio-isomer (compound 2; Fig. 1). The two drugs differ only by the relative positions of the two hydroxyl groups on the indolocarbazole chromophore; the rest of the molecule is absolutely identical. NB-506 has the two OH groups at positions 1,11, whereas they are at positions 2,10 in compound 2. At first sight, this is a minor modification, but the results presented here show unambiguously that the relocation of the two hydroxyl groups has a dramatic effect on the DNA binding capacities of compound 2. Interestingly, complementary biochemical data attest that the newly designed analogue remains a very potent topoisomerase I poison despite its strongly reduced interaction with DNA. In addition, the 2,10-isomer (compound 2) is significantly more cytotoxic that the 1,11-isomer (compound 1) against a panel of tumor cell lines (P388, colon 26, PC-13, HCT-116, DLD-1, and MKN-45). The results provide key information for a better understanding of the rules that govern the recognition of the topoisomerase I-DNA complex by NB-506-type drugs and raise new ideas for the rational design of topoisomerase I-targeted antitumor agents.

MATERIALS AND METHODS

Drugs and Chemicals. The two drugs were provided by Banyu Pharmaceuticals (Tsukuba, Japan). Their chemical synthesis has been reported (18–20). CPT was purchased from Sigma Chemical Co. (La Verpillette, France). The drugs were first dissolved in DMSO at 10 mM and then further diluted with water. The final DMSO concentration never exceeded 0.3% (v/v) in the cleavage reactions. Under these conditions, DMSO, which is also used in the controls, does not affect the topoisomerase activity. The stock solutions of drugs were kept at −20°C and freshly diluted to the desired concentration.
For each series of measurements, 12 samples were placed in a thermostatically controlled cell, the quartz cuvettes. Measurements were made by progressive dilution of drug-DNA complexes in 1 mM sodium cacodylate buffer (pH 6.5). The DNA samples were oriented inside the cuvette was measured with a platinum probe; it was increased over a range of 20–100°C with a heating rate of 1°C/min. The Tm was taken as the midpoint of the hyperchromic transition.

The UVikon 943 spectrophotometer was also used to record the absorption spectra. The reduced DR is defined as follows: 

$$DR = \frac{D_{\text{drug-DNA}} - D_{\text{DNA}+\text{ligand}}}{D_{\text{DNA}} - D_{\text{free ligand}}}$$

where $D$ refers to the reduced dichroism of the drug-DNA complex measured at the absorption maximum of the ligand bound to DNA. The denominator is always negative under the experimental conditions used. The DR is expected to be +1 if the transition moment of the drug chromophore is parallel to the DNA bases, as in the case of complete intercalative binding. For groove binders, the angle between the two helical axes and the long axis of the chromophore lies below 54°, which gives rise to positive dichroism and, thus, to a negative DR value. Under these conditions, the reduced DRs for any given drug-DNA and drug-polynucleotide complexes can be mutually compared with good relative accuracy, independent of the polymer size (24, 25).

To investigate the geometry of drug binding to DNA by ELD, the reduced dichroism $\Delta A/A$ of a ligand-DNA complex measured in the ligand absorption band must be analyzed with respect to the reduced dichroism $\Delta D$ measured for the same DNA or polynucleotide at 260 nm in the absence of drug ($\Delta A/A_{\text{DNA}}$). The reduced DR is defined as follows: 

$$DR = \frac{|(\Delta A/A)_{\text{DNA}+\text{ligand}}| - |(\Delta A/A)_{\text{DNA}}|}{|\Delta A/A_{\text{DNA}}|}$$

The numerator refers to the reduced dichroism of the drug-DNA complex measured at the absorption maximum of the ligand bound to DNA. The denominator is always negative under the experimental conditions used. The DR is expected to be +1 if the transition moment of the drug chromophore is parallel to the DNA bases, as in the case of complete intercalative binding. For groove binders, the angle between the two helical axes and the long axis of the chromophore lies below 54°, which gives rise to positive dichroism and, thus, to a negative DR value. Under these conditions, the reduced DRs for any given drug-DNA and drug-polynucleotide complexes can be mutually compared with good relative accuracy, independent of the polymer size (24, 25).

**DNA Purification and Labeling.** The plasmid pBS (Stratagene, La Jolla, CA) was isolated from *Escherichia coli* by a standard SDS-sodium hydroxide lysis procedure and purified using Qiagen columns. The 117-mer fragment was prepared by 3'-[32P] end-labeling of the *EcoRI*-PfaI double digest of the pBS plasmid using α-[32P]dATP (Amerham, Buckinghamshire, England) and AMV reverse transcriptase. The digestion products were separated on a 6% polyacrylamide gel under native conditions in TBE-buffered solution (89 mM Tris-borate (pH 8.3) and 1 mM EDTA). After autoradiography, the band of DNA was excised, crushed, and soaked in water overnight at 37°C. This suspension was filtered through a Millipore 0.22-μ filter, and the DNA was precipitated with ethanol. After washing with 70% ethanol and vacuum drying of the precipitate, the labeled DNA was resuspended in 10 mM Tris (adjusted to pH 7.0) containing 10 mM NaCl.

**Footprinting Experiments.** Cleavage reactions by DNase I were performed essentially according to the previously detailed protocols (26). Briefly, reactions were conducted in a total volume of 10 μl. Samples (3 μl) of the 32P-labeled DNA fragment were incubated with 5 μl of the buffer solution containing the desired drug concentration. After a 20-min incubation at 37°C to ensure equilibration of the binding reaction, the digestion was initiated by the addition of 2 μl of DNase I [0.01 unit/ml enzyme in 20 mM NaCl, 2 mM MgCl2, and 2 mM MnCl2 (pH 7.3)]. At the end of the reaction time (typically 4 min at room temperature), the digestion was stopped by freeze-drying. After lyophilization, each sample was resuspended in 4 μl of an 80% formamide solution containing tracking dyes before electrophoresis. A Molecular Dynamics 445Si PhosphorImager was used to collect all data, which were analyzed using the ImageQuant version 4.1 software. Each resolved band on the autoradiograph was assigned to a particular band within the DNA fragment by comparison of its position, relative to sequencing standards.

**DNA Relaxation Experiments.** Supercopiled pKMp27 DNA (0.5 μg) was incubated with 6 units of human topoisomerase I at 37°C for 1 h in a relaxase buffer (50 mM Tris (pH 7.8), 50 mM KCl, 10 mM MgCl2, and 1 mM EDTA) in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/ml. DNA samples were then added to the electrophoresis dye mixture (3 μl) and electrophoresed in a 1% agarose gel without or with ethidium bromide (1 mg/ml), at room temperature for 4 h. Gels were washed and photographed under UV light (27).

**Stimulation of Topoisomerase I-mediated DNA Cleavage.** The plasmid pKM27 (28) was linearized with EcoRI and labeled with α-[32P]dATP in the presence of the Klenow fragment of DNA polymerase I. The labeled DNA was then digested with AviI. The cleavage reaction mixture contained 20 mM Tris-HCl (pH 7.4), 60 mM KCl, 0.5 mM EDTA, 0.5 mM DTT, 2 × 10^6 dpm of α-[32P]-pKM27 DNA, and the indicated drug concentrations.
TOPOISOMERASE INHIBITION BY ANTITUMOR INDOLOCARBAZOLES

The reaction was initiated by the addition of human topoisomerase I (20 units in 200-μl reaction volume) and allowed to proceed for 10 min at 37°C. Reactions were stopped by adding SDS to a final concentration of 0.25% and proteinase K to 250 μg/ml, followed by incubation for 30 min at 50°C. Samples were denatured by the addition of 10 μl denaturing loading buffer consisting of 0.45 M NaOH, 30 mM EDTA, 15% (w/v) sucrose, and 0.1% bromocresol green before loading onto a 1% agarose gel in TBE buffer containing 0.1% SDS. Electrophoresis was conducted at 2 V/cm for 18 h.

Fig. 2. Absorption titrations for the interaction of compounds 1 and 2 with calf thymus DNA. The figure contains the absorption spectrum of the free drug, intermediate spectra, and final spectra of the drug-DNA complexes, in which the ligands have been sequestered completely by the DNA. To 3 ml of drug solution (20 μM BPE buffer) were added aliquots of a concentrated CT-DNA solution. Spectra are referenced against DNA solutions of exactly the same DNA concentration and were adjusted to a common baseline at 450 nm. The P:D ratio increased from 0 to 7 (top to bottom curves, at 302 nm for compound 1 and 345 nm for compound 2).

RESULTS

Drug-DNA Interaction. Initial attempts to evaluate the drug-DNA interaction were made by titrating measured quantities of a stock solution of calf thymus DNA into a known volume of the drug solution and monitoring the resulting changes in the absorption spectrum of the ligand. As shown in Fig. 2, a significant bathochromic shift (11 nm) and 29% hypochromism are observed when DNA is added to compound 1, whereas, under identical conditions (neutral pH, 16 mM Na+), the absorption spectrum of compound 2 is totally unaffected by the addition of DNA. The absorption maximum of the free drug compound 2 at 347 nm remains unchanged, and the hypochromism is extremely weak (3%), suggesting that the 2,10-isomer has no significant interaction with the DNA double helix. In the same buffer, a DNA binding constant of 2.64 × 10^4 (M^-1) was accurately measured for compound 1 using the intrinsic fluorescence of the indolocarbazole chromophore (14). The affinity constant for compound 2 is too weak to be measured precisely; it must be in the order of ∼10^3 M^-1.

Two alternative procedures were tried to compare the strength of...
the drug-DNA interaction. First, we determined the ability of the test drugs to alter the thermal denaturation profile of DNA. The duplex stabilizing effect ($\Delta T_m$) of compound 1 is relatively weak, but superior to that of compound 2 (Fig. 3a). Second, we used fluorescence spectroscopy to examine the abilities of the test drugs to compete with a DNA-binding ligand for available binding sites. In these experiments, the fluorescent probe used was the intercalating drug ethidium bromide, which is highly fluorescent in the presence of DNA. Only compound 1 significantly reduces the fluorescence of the ethidium-DNA complexes. Almost no quenching was observed with compound 2 (Fig. 3b). The results of the Tm and fluorescence quenching experiments are, thus, totally consistent with the absorption measurements and concur that the 2,10-isomer 2 has minimal, if any, interaction with DNA.

CD and ELD were used to characterize the mode of binding of the drugs to DNA. Addition of calf thymus DNA induces significant changes in the CD spectrum of compound 1. The band at 340 nm decreases as the DNA concentration is raised; meanwhile, the CD in the 310–330-nm region increases significantly (Fig. 4a). In sharp contrast, the CD spectrum of compound 2 is not affected at all by the addition of DNA (Fig. 4b). In the presence of DNA, the variation in CD intensity at 340 nm is very different for the two drugs (Fig. 4c), indicating, thus, that their DNA binding modes strongly differ. The ELD measurements confirmed this belief. The reduced dichroism in the 300–340-nm region, where the indolocarbazole chromophore absorbs the light, reaches 0.45 with compound 1, whereas it does not exceed 0.15 with compound 2 (Fig. 5a). The intensity of the ELD signal is a function of the degree of alignment of the DNA molecules in the electric field. When the drug molecules are fully bound to DNA (i.e., for P:D ratios ≥20; Fig. 5b), the reduced dichroism depends on field strength similarly at 260 nm for the DNA bases and at 340 nm for compound 1 (Fig. 5c). This indicates that the indolocarbazole ring of compound 1 is tilted close to the plane of the DNA bases, consistent with an intercalative mode of binding. The situation strongly differs with compound 2. In this case, the reduced dichroism measured at 350 nm is much less negative than that of DNA. Therefore, on the basis of the CD and ELD experiments, we can firmly conclude that the 2,10-isomer 2 does not intercalate into DNA. Additional ELD experiments with a series of synthetic polynucleotides of different base compositions confirmed that compound 1, but not compound 2, behaves as a typical DNA intercalating agent. With compound 1, the DR
(defined in “Materials and Methods”) was always close to +1 (data not shown; i.e., the expected value for a drug parallel to the DNA bp plane). With compound 2, the DR never exceeded 0.5, whatever the sequence. The ELD data with compound 2 are totally incompatible with an intercalation binding mode.

Next, we evaluated the sequence selectivity of the drugs. The results of the footprinting experiments presented in Fig. 6 reflect the spectroscopic binding data described above. As anticipated, no footprint was seen with compound 2 and, on the opposite, the 1,11-isomer protected the sequence 5'-CCAGGGT from cutting by the nuclease. The footprint detected with compound 1 around nucleotide position 70 on the 117-bp DNA fragment is in accordance with previous footprinting studies with rebeccamycin analogues, indicating that binding occurs preferentially at sequences containing CG or TG sites (13). It is worth noting that there is no correspondence between the topoisomerase I cleavage sites stimulated by compound 1 (described below) and the position of the preferential drug binding site on the same DNA fragment. One of the most pronounced topoisomerase I cleavage sites (at position 26) occurs some distance from the main drug binding site at the GC-rich sequence around position 70 (compare Fig. 7 with Fig. 9). This is another indication that the two molecular events, DNA binding and topoisomerase I inhibition, may not be related.

**Topoisomerase I Inhibition.** The topoisomerase I inhibitory properties of the drugs were first examined using a DNA relaxation assay. Negatively supercoiled plasmid pKMp27 was incubated with topoisomerase I in the presence of increasing concentration of the two drugs. The DNA samples were treated with SDS and proteinase K to remove any covalently bound protein and were resolved in a 1% agarose gel without ethidium bromide. As shown in Fig. 7A, supercoiled DNA is fully relaxed by topoisomerase I in the absence of drug (compare lanes DNA and Topo I). In the presence of the two drugs, the intensity of the band corresponding to the nicked form of DNA has increased significantly. This effect, observed with CPT, reflects the stabilization of topoisomerase I-DNA cleavable complexes. Interestingly, we noted that at concentrations >10 μM, compound 1 inhibited the relaxation of DNA by topoisomerase I, whereas the relaxation was still complete with compound 2, even when using a drug concentration as high as 100 μM.

To better differentiate the specific (poisoning) and nonspecific effects, we repeated the experiments using agarose gels prestained with ethidium bromide (Fig. 7B). In this case, the relaxed DNA migrates faster than the supercoiled plasmid because of ethidium-induced DNA unwinding effects. Here again, a marked increase in the intensity of the band corresponding to nicked DNA molecules can be detected with the two drugs. It seems clear that both drugs efficiently stabilize topoisomerase I-DNA complexes despite their distinct DNA
binding capacities. Interestingly, a strong shift in the mobility of supercoiled plasmid (form I) was observed with increasing concentrations of compound 1, but not with compound 2 (Fig. 7B). This is another evidence for their very different DNA binding properties. The effect of compound 1 on the electrophoretic mobility of supercoiled DNA can be attributed to a decrease in plasmid DNA-linking number due to intercalation (the same effect was observed in the absence of the enzyme). These results indicate that compound 1 can behave both as specific topoisomerase I inhibitor, trapping the cleavable complexes and as nonspecific inhibitors of a DNA-processing enzyme acting via DNA binding. In sharp contrast, the 2,10-isomer 2 behaves exclusively as a specific topoisomerase I poison, as it is the case with CPT.

Next, we used a \(^{32}\)P-labeled linear plasmid DNA as a substrate for human topoisomerase I. The 3' end-labeled EcoRI-AvaI restriction fragment of pKM27 was incubated with the enzyme in the presence and absence of the indolocarbazoles, and the resulting DNA cleavage products were analyzed by agarose gel electrophoresis under alkaline conditions. The profiles of DNA cleavage, presented in Fig. 8, are different with the two drugs. One of the cleavage sites (open arrowhead) is much more enhanced in the presence of compound 1 than with compound 2. Conversely, at another site (filled arrowhead), the cleavage is more intense with compound 2 than compound 1. The position of the hydroxyl groups seems to influence the cleavage of DNA by the enzyme. It is important to note, also, that the cutting profiles obtained with the two indolocarbazoles are significantly different from that obtained with CPT.

To investigate further the effect of the OH groups on the sequence-specific cleavage of DNA by topoisomerase I, we used the 117-bp fragment from plasmid pBS, as used in the above footprinting experiments. The DNA restriction fragment (\(^{32}\)P-labeled at the 3' end of the EcoRI site) was incubated with the test drug and the enzyme, and the resulting cleavage products were resolved on sequencing gels so as to identify the sequence of the drug-induced topoisomerase I cleavage sites. A typical example of a gel is shown in Fig. 9. The positions of seven cleavage sites were determined with nucleotide resolution (Table 1). An eighth site can be seen at the top of the gel around nucleotide position 105, but it lies beyond the area accessible to densitometric analysis. The weak site 1 at a TA step is specific to CPT. Sites 3, 5, and 6 are only detected with compound 1; they correspond to CG sites. This compound also promotes cleavage at the TG sites, especially at site 2, much more weakly at sites 4 and 7. In sharp contrast, the topoisomerase I cleavage sites stimulated by compound 2 are restricted to the TG sites. The cutting profiles observed with compound 2 are almost identical to those seen

![Fig. 8. Topoisomerase I-mediated cleavage of DNA in the presence of compounds 1 and 2. Purified human topoisomerase I (20 units) was incubated with the EcoRI-AvaI restriction fragment from plasmid pKM27 (3' end \(^{32}\)P-labeled at the EcoRI site) in the absence (Topo I) and the presence of the test ligands. Cleaved DNA fragments were analyzed on a 1% agarose gel containing 0.1% SDS in the TBE buffer. CPT was used at 20 \(\mu\)M.](image)

![Fig. 9. Sequencing of drug-induced topoisomerase I cleavage sites. The 117-bp DNA fragment was subjected to cleavage by human topoisomerase I in the presence of the test drug at 20 or 50 \(\mu\)M. Cleavage products were resolved on an 8% polyacrylamide gel containing 7 M urea. Guanine-specific sequence markers obtained by treatment of the DNA with dimethylsulfate, followed by piperidine, were run in the lane marked G. Topo I refers to the radiolabeled DNA substrate incubated with the enzyme, but with no drug. The position of seven drug-induced topoisomerase I cleavage sites are indicated, and the corresponding sequences are given in Table 1.](image)
The IC₅₀ values are collated in Table 2. In all cases, ase I activities measured at poisoning topoisomerase I. This is consistent with the topoisomerase I, but which interacted strongly with DNA. Nevertheless, all the drugs used in this study, including the two drugs used in the studies with indolocarbazoles. Indeed, recently Fukasawa et al. reported that NB-506 stimulates DNA cleavage by topoisomerase I, but also because they reconcile the results of two previous studies that suggest that topoisomerase I is a very important, if not the essential, target for indolocarbazoles.

**Formation of Protein-DNA Complexes in Cells.** A potassium/SDS precipitation assay was used to investigate the effect of the drugs on the formation of protein-DNA complexes in HCT116 human colon cancer cells. Both drugs enhance the formation of the complexes in a dose-dependent manner (Fig. 10). No significant differences between the two drugs can be detected, suggesting that they are equally potent at poisoning topoisomerase I. This is consistent with the topoisomerase I activities measured *in vitro*.

**Cytotoxicity.** Six cell lines were used to evaluate the cytotoxicity of the drugs. The IC₅₀ values are collated in Table 2. In all cases, compound 2 proved to be more toxic than compound 1. With the two murine cell lines P388 and colon 26, compound 2 is about 10 times more toxic than compound 1. The difference is about the same with the DLD-1 human colon cancer cells. But with HCT116 cells, which also derive from a human colon cancer, the 2,10-isomer 2 is 60 times more toxic than the parent compound. A smaller difference was found when using the human gastric cancer cells MKN-45 and the human lung cancer cells PC-13; however, in both cases, compound 2 is about seven times more toxic than compound 1. The change of the positions of the hydroxyl groups reinforces significantly the cytotoxicity of the drug.

**DISCUSSION**

Over the past 5 years, a great diversity of topoisomerase I inhibitors have been discovered and developed, but only a few of them have shown useful antitumor activities (32, 33). The indolocarbazoles represent the only class of non-CPT topoisomerase I poisons that shows potent antitumor effects *in vivo*. There are good reasons to hope that in the near future NB-506, or a closely related congener, will take its place in the arsenal against cancer.

There is absolutely no doubt that the two drugs used in this study, which only differ by the position of the hydroxyl groups, exhibit very distinct DNA-binding properties. NB-506 behaves as a typical DNA intercalating agent and binds reasonably well to double-stranded DNA. In sharp contrast, the 2,10-isomer poorly interacts with DNA, at least in the absence of topoisomerase I. Its affinity for duplex DNA is too weak to be measured, and no sign of intercalation between two consecutive bp was detected by CD or ELD. However, both drugs are potent inhibitors of topoisomerase I. We can, therefore, conclude that intercalation into DNA is not required for the stabilization of topoisomerase I-DNA covalent complexes by indolocarbazoles. This is an important conclusion that will be useful for future drug design in this series of antitumor agents.

Compound 2 has lost the capacity to intercalate into DNA, however, it remains highly cytotoxic, even more cytotoxic than NB-506 against a panel of tumor cells. This does not mean that the DNA-binding capacities of NB-506 are detrimental to the anticancer activity. The intercalation into DNA may well contribute to the biological activity, at least partially. We have previously obtained highly cytotoxic indolocarbazole drugs endowed with modest effects on topoisomerase I, but which interacted strongly with DNA. Nevertheless, all of the studies suggest that topoisomerase I is a very important, if not the essential, target for indolocarbazoles.

The results of the sequencing studies presented in Fig. 9 and Table 1 are important because they reveal unambiguously that the OH groups of NB-506 serve to control the activity of the DNA cleaving enzyme, but also because they reconcile the results of two previous studies with indolocarbazoles. Indeed, recently Fukasawa et al. (34) reported that NB-506 stimulates DNA cleavage by topoisomerase I.

<table>
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<tr>
<th>Compound</th>
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<th>2</th>
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<tr>
<td>P388</td>
<td>0.062</td>
<td>0.005</td>
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<tr>
<td>colon 26</td>
<td>1.2</td>
<td>0.11</td>
</tr>
<tr>
<td>MKN-45</td>
<td>0.39</td>
<td>0.055</td>
</tr>
<tr>
<td>PC-13</td>
<td>0.25</td>
<td>0.036</td>
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<tr>
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<td>2.1</td>
<td>0.58</td>
</tr>
<tr>
<td>HCT-116</td>
<td>0.12</td>
<td>0.002</td>
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preferentially at sites having a C and G on the 5' and 3' sides of the cleaved bond, respectively, whereas sequencing studies with rebeccamycin analogues (which lack the key OH groups on the drug chromophore) showed that topoisomerase I-mediated cleavage occurred predominantly at 5'-TG-3' sites (12). The reason for the slight discrepancy between the two studies is now elucidated. The selectivity for CG or TG sites depends on the absence or presence (and the position) of the two OH groups in the test drugs.

The newly designed NB-506 analogue, compound 2, is a potent topoisomerase I inhibitor with practically no binding to DNA (at least in the absence of topoisomerase I). In this respect, this compound appears very similar to CPT, which also fails to bind to DNA in the absence of the enzyme (but DNA contributes to its effects on topoisomerase I; Ref. 35). The analogy between the two drugs is even more striking if one considers that they both stimulate cleavage by topoisomerase I at the same sites in DNA, with a marked preference for TG steps (Fig. 9 and Table I). It is likely that, despite differences in chemical structures, they interfere similarly with topoisomerase I-DNA covalent complexes. Studies are in progress to try to identify the molecular features of the drug in direct contact with the enzyme.

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REFERENCES


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