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DNA Damage Effects of a Polyamide-CBI Conjugate in SV40 Virions

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ABSTRACT

Polyamides are a class of synthetic molecules that exhibit high-affinity, sequence-specific reversible binding in the DNA minor groove but are incapable of inducing DNA damage. In cell-free systems, polyamides have been shown to regulate gene expression by activation, repression, and antirepression. However, effectiveness in cell culture has met with limited success and seems to be cell-dependent. By combining a polyamide with a moiety of a DNA-alkylating agent of the cyclopropylpyrroloindole (CPI) family, a conjugate molecule [polyamide 1-CBI (1-(chloromethyl)-5-hydroxyl-1,2-dihydro-3*H*-benz[*e*]indole) conjugate] capable of sequence-specific DNA alkylation was shown to exhibit cellular activity (i.e., cell-growth inhibition and cell-cycle arrest) in mammalian cells. These effects, however, occur at concentrations several orders of magnitude higher than those of its parent CPI agent adozelesin. In addition, 1-CBI is able to interact sequence-specifically with viral DNA and inhibit SV40 DNA replication in infected BSC-1 (African green monkey kidney epithelial) cells, albeit at a greatly reduced ability compared with its CPI parent. On the basis of results from previous studies, we tested whether pretreatment of virus with 1-CBI, compared with direct treatment of infected cells, would enhance its cellular activity. Therefore, using SV40 virions as a model system, we examined the ability of this conjugate molecule to penetrate SV40 virions and damage viral DNA. Our results demonstrate that 1-CBI is able to damage encapsidated SV40 DNA. Both DNA replication and virus production are effectively inhibited in a concentration-dependent manner after infection of BSC-1 cells with 1-CBI–pretreated virions. It is surprising that, unlike in mammalian cells, the relative activity of 1-CBI in SV40 virions is comparable with that of the highly cytotoxic CPI agent adozelesin. Because 1-CBI is able to efficiently penetrate virions and damage DNA, these findings may provide the framework for the development of polyamide-based antiviral agents with enhanced sequence-preference capabilities.

Small molecules designed to target predetermined DNA sequences and regulate gene expression or block DNA replication would be a useful tool in biology and perhaps in human medicine. Such agents would offer the potential for modulation of aberrant gene expression and could provide a means to selectively target viral or bacterial pathogens. Polyamides containing pyrrole and imidazole amino acids are synthetic ligands that target specific DNA sequences with affinities comparable with natural DNA binding proteins. DNA recognition depends on aromatic amino acid pairings in the minor groove (Dervan and Edelson, 2003). Under cell-free conditions, sequence-specific hairpin polyamides, which lack DNA-damage capabilities, have been shown to interfere with transcription factor binding (Gottesfeld et al., 1997; Dickinson et al., 1999; Chiang et al., 2000; Dervan and Edelson, 2003) and to inhibit replication of human immunodeficiency virus type I (Dickinson et al., 1998). However, despite these findings, few studies have reported polyamide-induced down-regulation of endogenous gene expression in intact cells, possibly because of their limited uptake into mammalian cells (Belitsky et al., 2002; Best et al., 2003).

A DNA binding conjugate 1-CBI, which combines the DNA sequence-specificity of a polyamide and the alkylating ability of a CPI agent, has been synthesized (Chang and Dervan, 2000) (Fig. 1). This conjugate molecule binds a six–base-pair site, 5′-(W)GG(W)C(W)-3′, and alkylates a single adenine flanking the polyamide binding site in a strand-selective fashion (Chang and Dervan, 2000; Wang et al., 2002). In mammalian cells, 1-CBI has been shown to interact se-

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ABBREVIATIONS: SV40, simian virus 40; 1-CBI, polyamide 1-(chloromethyl)-5-hydroxyl-1,2-dihydro-3*H*-benz[*e*]indole conjugate; CPI, cyclopropylpyrroloindole; pfu, plaque-forming units; MEM, minimal essential medium; MEM-2, minimal essential medium containing 2% calf serum.

quence-specifically with DNA, inhibiting both cell growth and viral replication and inducing cell-cycle perturbations (Wang et al., 2002), although at a much limited capacity compared with its CPI parent.

Nevertheless, the ability of 1-CBI to alkylate DNA and interact with SV40 DNA target sequences (Wang et al., 2002) led us to further study its biological effects in a viral system. In contrast to cells, SV40 virions consisting of capsid proteins and chromatin structure present a dissimilar barrier for drug uptake and DNA interaction. In this study, we assessed the capacity of 1-CBI to penetrate SV40 virions and analyzed the inhibitory effects on SV40 viral DNA replication and virus production after virion pretreatment with this conjugate molecule. The biological activity of 1-CBI was compared with that of the highly cytotoxic CPI agent adozelesin.

Materials and Methods

Reagents and Cell Culture. 1-CBI, a hairpin polyamide-seco-CBI conjugate (Fig. 1), was synthesized as described previously (Chang and Dervan, 2000). Adozelesin was obtained from Pfizer, Inc. (New York, NY). Before use, 1-CBI was diluted in autoclaved water, and adozelesin was diluted in dimethyl sulfoxide. Stock solutions of both agents were stored at −20°C protected from light. Form I SV40 DNA was purchased from Invitrogen (Carlsbad, CA), whereas SV40 large T-antigen [10^7 plaque-forming units (pfu)/ml] was purified from baculovirus-infected High-Five insect cells (Invitrogen) according to a published procedure (Stillman and Gluzman, 1985). BSC-1 cells (African green monkey kidney cells) were grown in minimal essential medium (MEM) supplemented with 8% fetal calf serum and 2% fetal bovine serum and maintained at 37°C in a 5% CO_2/95% air incubator.

SV40 DNA Forms Conversion Assay. Naked SV40 DNA (approximately 150 ng) or encapsidated SV40 virion DNA (approximately 13 μg DNA) was incubated at 37°C with increasing concentrations of 1-CBI (naked, 0–0.2 μM; encapsidated, 0–2 μM) for 4 h. After treatment, SV40 virion was incubated with 1% SDS for 1 h at 37°C to prevent rebinding of 1-CBI to DNA. All samples were then incubated in Tris-EDTA buffer (+ 150 mM NaCl) at 70°C for 2 h to convert alkylated bases to DNA strand breaks. SV40 DNA was purified using a QIAprep Spin Miniprep Kit (QIAGEN, Valencia, CA). Purified DNA was electrophoresed on a 1% agarose gel in 1× Tris-acetate electrophoresis buffer for 5 h, stained with Sybr green (Molecular Probes, Eugene, OR) and quantified using densitometry and ImageQuant program (Amersham Biosciences Inc., Piscataway, NJ).

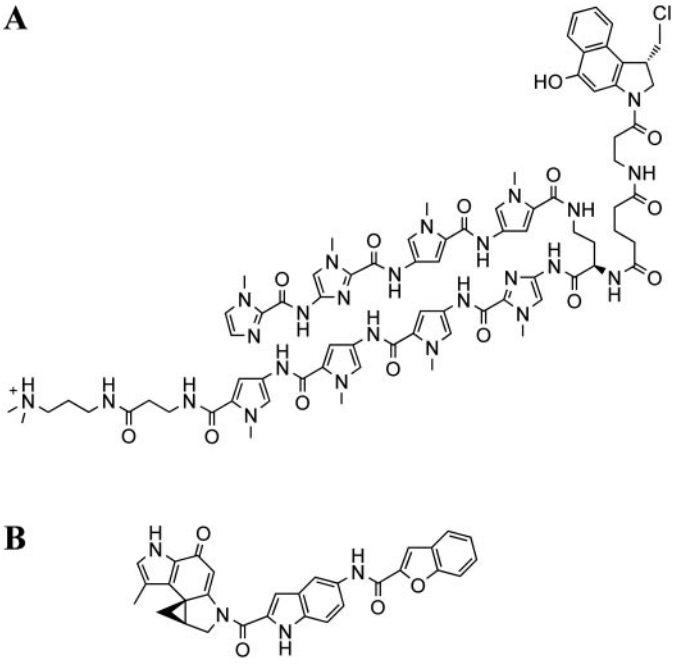

Fig. 1. Chemical structures of polyamide-CBI conjugate 1-CBI (A) and DNA alkylating agent adozelesin (B). 1-CBI is a hybrid molecule of a polyamide (polyamide 1) and the CPI analog adozelesin, and adozelesin is a synthetic analog of the antitumor antibiotic CC-1065. Both agents bind within the minor groove of DNA in a sequence-specific manner.

Intracellular SV40 DNA Replication Assay. BSC-1 cells were seeded at ~6×10^4 cells/well in a 12-well dish and grown for 48 h until 90 to 95% confluent. SV40 virions (10^6 pfu/ml) were pretreated with increasing concentrations of 1-CBI (0–100 nM) for 4 h at 37°C. Cells were then infected with pretreated SV40 virus in MEM containing 2% calf serum (MEM-2) for 2 h at 37°C. After virus-containing medium was removed, cells were incubated an additional 48 h at 37°C in fresh MEM-2. At 50 h after infection, SV40 DNA was extracted from the cells according to the QIAprep Spin Miniprep Kit procedure. Purified DNA was electrophoresed on a 1% agarose gel in 1× Tris-acetate electrophoresis buffer for 16 h, stained with Sybr green, and quantified using densitometry and ImageQuant program.

Intracellular SV40 Virus Production/Plaque Assay. BSC-1 cells were seeded at ~4×10^5 cells per 60-mm dish and grown for 72 h until 90 to 95% confluent. SV40 virions (10^6 pfu/ml) were pretreated with increasing concentrations of 1-CBI (0–100 nM) for 4 h at 37°C. After dilution of virus, cells were then infected with 200 μl of pretreated SV40 virus (100 pfu/ml) in MEM-2 for 2 h at 37°C. After virus-containing medium was removed, cells were overlaid with 5 ml of (1:1) MEM-2/1.8% bactoagar solution (day 0) and incubated an additional 9 days at 37°C. On postinfection day 4, 2.5 ml of MEM-2/bactoagar solution was added to each dish. On day 8, 2.5 ml of MEM-2/bactoagar solution containing 83 mg/ml Neutral red (Sigma-Aldrich, St. Louis, MO) was added to each dish to enhance visualization of the viral plaques. Plaques were counted on postinfection day 9, and viral pfu were calculated.

Results

1-CBI Induces Thermolabile Lesions in Encapsidated SV40 DNA. The polyamide-CBI conjugate 1-CBI (Fig. 1) is a sequence-specific agent that binds and alkylates DNA. Previous studies have demonstrated several biological effects of 1-CBI including cell-growth inhibition, cell-cycle arrest, and (naked/minchromosomal) SV40 DNA damage (Wang et al., 2002). Given the abilities of 1-CBI to permeate cells and to damage both cellular/purified viral DNA, we sought to determine the efficacy of 1-CBI in damaging encapsidated SV40 DNA using an SV40 forms-conversion assay. With this assay, SV40 DNA lesions can be visualized on an agarose gel by conversion changes from form I DNA (supercoiled circular) to forms II (nicked circular) and III (linear) after treatment of virion.

We first determined the concentration-dependence of 1-CBI–induced DNA damage to encapsidated SV40 DNA. After incubating SV40 virions for 4 h with increasing concentrations of 1-CBI at 37°C, DNA lesions were converted to strand breaks by heating for 2 h at 70°C. Quantification of agarose gels (Fig. 2A) and resulting graphic analysis of DNA forms conversion (Fig. 2B) indicated that the amount of supercoiled form I DNA was reduced by nearly 50% (IC_{50} value) at approximately 500 nM (Fig. 2A, lane 6), whereas 1 μM 1-CBI converted more than 90% of form I to forms II and III (lane 7). At 2 μM 1-CBI, form III DNA increased significantly, with no detection of supercoiled form I DNA (Fig. 2A,

lane 8). Compared with nonheated DNA (forms profile not shown), nontreated control DNA remained undamaged after 2 h of heating (Fig. 2A, lanes 1 and 2). Treatment with the parent polyamide 1 (500 nM), which can bind reversibly to DNA as 1-CBI but not alkylate it, failed to induce SV40 DNA damage under the same experimental conditions (data not shown).

To compare the effects of 1-CBI treatment on naked versus encapsidated SV40 DNA and further confirm previous findings (Wang et al., 2002), purified viral DNA also was incubated for 4 h at 37°C with increasing concentrations of 1-CBI. Graphic analysis of DNA forms conversion indicated that the IC_{50} value of DNA strand damage by 1-CBI was approximately 50 nM (data not shown), comparable with the IC_{50} value (100 nM) obtained under related conditions (Wang et al., 2002).

In addition to concentration-dependent effects, the time-dependence of encapsidated SV40 DNA alkylation and damage by 1-CBI was determined under conditions identical with those described above. As shown in Fig. 2C, DNA damage is evident even after 15-min incubation at 37°C with 350 nM 1-CBI (lanes 3 and 4). This concentration of 1-CBI was chosen on the basis of the concentration-dependence studies so as to detect measurable DNA damage within the time period of interest. Approximate decreases of 50 and 80% form I SV40 DNA were apparent after 15-min and 16-h incubations, respectively (Fig. 2D). Taken together, these results demonstrate strongly the notable ability of 1-CBI to effectively penetrate virions and cause DNA damage in both a concentration- and time-dependent manner.

Under the experimental conditions used in this study, it is possible that 1-CBI could rebind to viral DNA during the postlysis incubation period and thus cause additional DNA damage. This phenomenon could explain the efficacy of 1-CBI–induced viral DNA damage reported here. It is difficult to remove the unbound agent from the reaction mixture; however, previous studies (McHugh et al., 1994; Wang et al., 2002) have demonstrated that some detergents, including SDS, are able to preclude select CPI agents from rebinding to DNA. Using reaction conditions similar to those used in this study, Wang et al. (2002) showed that 1% SDS effectively prohibited 1-CBI from binding minichromosomal SV40 DNA without causing removal of covalently prebound 1-CBI. Therefore, we are confident that the SV40 DNA damage observed in this study is not the result of postlysis 1-CBI–DNA interactions.

Inhibition of SV40 DNA Replication by 1-CBI. Previous studies have demonstrated that direct 1-CBI treatment of infected BSC-1 cells can block intracellular SV40 DNA replication, but it does so at concentrations several orders of magnitude greater than that of its CPI parent (Wang et al., 2002). Regardless, the unexpected ability of 1-CBI to efficiently induce DNA lesions of encapsidated viral DNA prompted us to examine whether this effect could translate into inhibition of SV40 DNA replication when virus is pretreated with 1-CBI and is subsequently used to infect BSC-1 cells. After SV40 virions were treated for 4 h with increasing concentrations of 1-CBI at 37°C, BSC-1 cells were infected with pretreated virus for 2 h and then grown for an additional 48 h as described under *Materials and Methods*. After DNA purification and electrophoresis, gel quantification of DNA forms (Fig. 3A) indicated that 1-CBI caused a strong concentration-dependent inhibition of SV40 DNA replication, as indicated by the loss of DNA forms I to III. The concentration curve (Fig. 3B) shows that approximately 50% reduction of all three DNA forms occurred within the concentration range of 25 to 50 nM 1-CBI, with near complete loss of DNA forms at 100 nM 1-CBI.

Under related conditions, earlier studies have demonstrated the inability of the parent polyamide 1 (at 10 μM) to

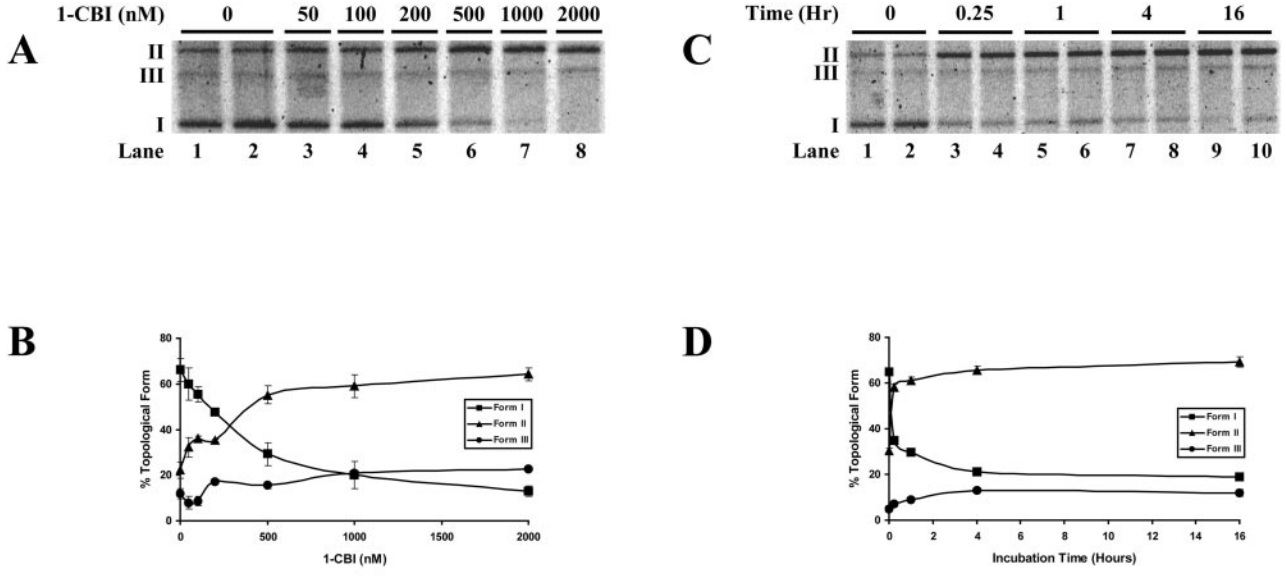

Fig. 2. 1-CBI–induced damage to encapsidated SV40 DNA. SV40 virions (~13 μg DNA) were treated with increasing concentrations of 1-CBI for 4 h at 37°C. Lysis solution (containing 1% SDS) was then added to all samples and incubated for 1 h at 37°C. DNA lesions were converted to strand breaks by heating at 70°C for 2 h. All samples were subjected to agarose gel electrophoresis followed by Sybr green staining (A) and DNA forms quantification (B). Positions of SV40 DNA forms I (supercoiled), II (nicked circular), and III (linear) are shown in A. B, the three forms of SV40 DNA are expressed as mean percentages ± S.E. C, time-dependent effect of 1-CBI–induced damage to encapsidated SV40 DNA. SV40 virions (~13 μg DNA) were treated with 0.35 μM 1-CBI for 0 to 16 h at 37°C. D, SV40 DNA was purified and subjected to agarose gel electrophoresis and DNA forms quantification as described above.

inhibit cell-free SV40 DNA replication (Wang et al., 2002). In addition, our results indicate that polyamide 1 (at 0.5 μM) is unable to induce DNA lesions in encapsidated SV40 DNA. Therefore, we examined whether this lack of activity would also occur in intact virions. As expected, pretreatment with the nonalkylating parent polyamide 1 (500 nM and 2 μM) did not result in any appreciable SV40 DNA replication inhibition (data not shown).

Inhibition of SV40 Virus Production by 1-CBI. As a consequence of its capacity to inhibit viral SV40 DNA replication via encapsidated viral DNA damage, 1-CBI was tested for similar activity in terms of virus replication. As described under *Materials and Methods*, SV40 virions were treated for 4 h with increasing concentrations of 1-CBI at 37°C, and then BSC-1 cells were infected with pretreated virus for 2 h and grown for an additional 9 days. Effects on virus production were assessed by plaque-formation assay, a technique used to estimate the number of infectious particles in a sample. In our studies, this method was applied to estimate the number of viral plaques produced after infection, replication, and lysis of infected cells. A summary of the results from two independent studies is illustrated in Table 1. Consistent with viral DNA damage and DNA replication data, pretreatment of virions with 1-CBI resulted in a concentration-dependent inhibition of SV40 virus replication, as demonstrated by decreased plaque formation in comparison to control plaque formation. At 1 nM 1-CBI, plaque formation decreased by nearly 50% in comparison to control numbers, whereas more than 90% plaque formation was inhibited at 100 nM 1-CBI.

In terms of SV40 DNA replication inhibition, as well as activity in mammalian cells, adozelesin is 100 to 1000 times more active than 1-CBI under conditions of direct treatment of virally infected cells (Cobuzzi et al., 1996; Wang et al., 2002). As a result of this large disparity in cellular activity, we wished to determine whether this pattern of activity would hold true for adozelesin and 1-CBI under conditions in which the virus is initially pretreated with each alkylator.

A comparative summary of DNA damage effects induced by 1-CBI and adozelesin is shown in Table 2. At first, as a standardized comparison between adozelesin and 1-CBI, naked SV40 DNA was incubated with adozelesin under conditions identical with those of 1-CBI. Similar to 1-CBI, treatment with adozelesin also resulted in a concentration-dependent forms conversion, with an approximate IC_{50} value of 50 nM. We also evaluated the concentration-dependent DNA-damaging effects of adozelesin on encapsidated SV40 DNA. Agarose gel quantification showed that at 500 nM and 2 μM adozelesin, the concentration of supercoiled form I DNA was reduced by almost 50% and 90%, respectively (data not shown). In comparison, at a concentration of nearly 500 nM, 1-CBI was also able to reduce the amount of supercoiled DNA (form I) by approximately 50%. Thus, under similar conditions in a virion context, 1-CBI is surprisingly able to damage naked and encapsidated SV40 DNA as efficiently as adozelesin.

To relate DNA damage with DNA replication inhibition, we also investigated the inhibition of intracellular SV40 DNA replication by adozelesin. Under treatment conditions identical with those of 1-CBI, SV40 virions were pretreated with adozelesin and then were used subsequently to infect BSC-1 cells. In comparison to 1-CBI (IC_{50} range of 25–50 nM), adozelesin caused nearly 50% SV40 DNA replication inhibition within the concentration range of 2 to 10 nM (Table 2). Therefore, as determined from these IC_{50} values, 1-CBI is nearly as effective at inhibiting SV40 DNA replication even though adozelesin is a much stronger inhibitor of SV40 replication when treating virally infected cells (Cobuzzi et al., 1996; Wang et al., 2002). The fact that DNA replication and virus production are more sensitive to 1-CBI than naked/encapsidated DNA damage demonstrates perhaps that its DNA-damaging potency is enhanced under conditions of continuous viral replication. In contrast, such a biological phenomenon is not evident with adozelesin.

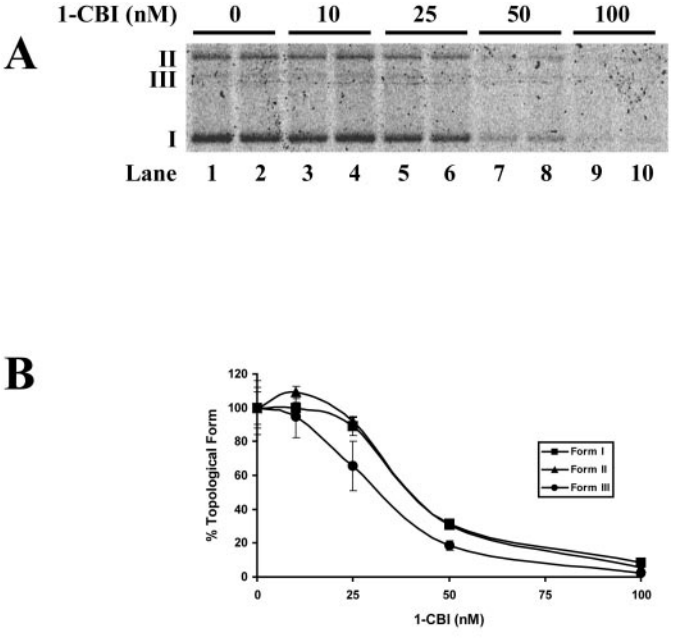

Fig. 3. 1-CBI–induced inhibition of in vivo SV40 DNA replication. BSC-1 cells were infected with 1-CBI–pretreated SV40 virions (10^6 pfu/ml) for 2 h at 37°C. After removal of virus, BSC-1 cells were allowed to grow for 48 h under normal growth conditions. SV40 DNA was then purified and subjected to agarose gel electrophoresis followed by Sybr green staining (A) and DNA forms quantification (B). Positions of SV40 DNA forms I (supercoiled), II (nicked circular), and III (linear) are indicated in A, with SV40 DNA forms expressed as mean percentages ± S.E. (B).

Discussion

The polyamide conjugate 1-CBI has been shown to exhibit inhibitory biological effects in SV40-treated mammalian cells, albeit with a greatly reduced activity compared with its CPI parent adozelesin. However, given its sequence-selectiv-

TABLE 1

1-CBI–induced inhibition of in vivo SV40 virus production: viral plaque assay

BSC-1 cells were infected with 1-CBI–pretreated SV40 virions as described under *Materials and Methods*. Cells were stained with Neutral red, and viral plaques were counted on postinfection day 9. Total plaque numbers are expressed as mean percentages of total control plaque numbers (100% = 1.9×10^7 pfu/ml). Plaque-forming units were calculated as follows: (total number of plaques) × (SV40 virion dilution factor)/(infection volume).

[1-CBI]	Viral Plaques
	%
Control	100
1 nM	50
10 nM	47
100 nM	8

ity for SV40 DNA (Wang et al., 2002) and its limited membrane permeability in mammalian cells, an important question to consider is whether 1-CBI demonstrates comparable and/or enhanced activity in SV40 virions. Therefore, because of 1-CBI's limited activity in cells and its lack of testing in a biological target other than cells (Wang et al., 2002), the objective of this study was to evaluate 1-CBI for its ability to penetrate SV40 virions and damage encapsidated DNA. In particular, we sought to determine how the presence of virion capsid proteins and chromatin structure would influence 1-CBI in terms of uptake efficiency and DNA-damage ability in comparison to adozelesin.

Analysis of DNA damage using encapsidated SV40 DNA indicated that 1-CBI could alkylate DNA and induce DNA breaks in a concentration-dependent manner (IC_{50} = 500 nM) as efficiently as adozelesin (see Table 2). However, under the same experimental conditions, DNA strand damage was virtually undetectable with the nonalkylating parent polyamide 1, even at elevated concentrations (10 μM). Examination of the forms conversion data obtained for both naked and encapsidated DNA (Fig. 2B and Table 2) indicated that the higher order structure of virally packaged, chromatin-bound SV40 DNA does not significantly affect the extent of 1-CBI–induced DNA damage. Therefore, these results are consistent with earlier findings that showed 1-CBI could damage both naked and isolated minichromosomal SV40 DNA with equal efficiency (Wang et al., 2002). In addition, an increase in linear form III DNA (Fig. 2A) was evident only at elevated 1-CBI concentrations (2 μM), indicating a lack of uniquely vulnerable DNA sequences (i.e., "hot spots") in the encapsidated SV40 genome to which 1-CBI may bind. Thus, these DNA-damage profiles are similar to those demonstrated for isolated minichromosomal SV40 DNA and support the monoalkylating ability of 1-CBI reported previously (Wang et al., 2002).

The accessibility of encapsidated SV40 DNA to alkylation by 1-CBI, compared with naked DNA, would seem likely to be time-dependent. For naked viral DNA, it has been demonstrated that CPI agents such as CC-1065 quickly (~15 min) induce maximum DNA damage (McHugh et al., 1994), whereas 1-CBI requires much longer (~16 h) time periods (Wang et al., 2002). For encapsidated viral DNA, our results demonstrated that at a 1-CBI concentration (0.35 μM) that induces detectable DNA forms conversion, a significant loss of form I DNA was evident even after 15-min incubation at 37°C, with maximum DNA lesion damage at 16-h incubation (Fig. 2, C and D). This concentration-dependent rate of DNA forms conversion is nearly identical with that reported for 1-CBI–induced damage to naked SV40 DNA (Wang et al., 2002). Therefore, the ability of 1-CBI to access and alkylate viral DNA is not hindered by the presence of either viral coat proteins (capsid) or chromatin structure components. Furthermore, given the virtual inactivity of the parent polyamide 1 at 10 μM, our findings suggest that the addition of the alkylating moiety to the polyamide 1 confers on 1-CBI potent and unique SV40 DNA-damaging capabilities in a virally encapsidated genome.

As a result of induced lesions, the effectiveness of a DNA-damaging agent is usually associated with its ability to inhibit DNA replication. Likewise, we examined 1-CBI–induced inhibition of DNA replication in virally infected BSC-1 cells using 1-CBI–treated virions. Examination of the forms data (Fig. 3, A and B) indicated that approximately 50% reduction of SV40 DNA replication occurred at a concentration range of 25 to 50 nM 1-CBI. In contrast to virion pretreatment, under conditions of direct 1-CBI treatment of virally infected BSC-1 cells, we confirmed that elevated concentrations of 1-CBI (>2 μM) are required for DNA replication inhibition (data not shown), consistent with previous reports (Wang et al., 2002). Therefore, the efficiency of DNA replication inhibition by 1-CBI is enhanced nearly 2 orders of magnitude after pretreatment of SV40 virions with 1-CBI before BSC-1 cell infection (Table 2). These results demonstrate that despite the ability of 1-CBI to quickly enter SV40 virions and consequently damage encapsidated SV40 DNA (Fig. 2C), 1-CBI is unable to effectively inhibit intracellular viral DNA replication when administered to virally treated cells, even during extended incubation periods (48 h). Unlike in SV40 virions, this ineffectiveness may be caused by the limited uptake of this conjugate molecule into mammalian cells (Belitsky et al., 2002; Best et al., 2003).

It is surprising that, compared with adozelesin (IC_{50} range, 2–10 nM), our results indicated that 1-CBI (IC_{50} range, 25–50 nM) was nearly as effective at inhibiting intracellular SV40 DNA replication when treating BSC-1 cells with 1-CBI–treated virions (Table 2). Because 1-CBI has been shown to be much less effective at blocking intracellular SV40 DNA replication than the parent adozelesin by almost 3 orders of magnitude under conditions of direct treatment of infected cells (Cobuzzi et al., 1996; Wang et al., 2002), we would have predicted 50% inhibition of SV40 DNA replication to occur at a 1-CBI concentration nearly 1000-fold greater than the calculated concentration of 25 to 50 nM. In addition, even though adozelesin is a much more potent DNA replication inhibitor than 1-CBI in mammalian cells, no enhanced activity (i.e., increase in DNA replication sensitivity compared with DNA damage) was evident with this agent as was observed with 1-CBI under conditions of SV40 virion pretreatment (Table 2). Because adozelesin equally inhibits SV40 DNA replication (IC_{50} range, 2–10 nM) under conditions of direct treatment of infected cells (Cobuzzi et al., 1996) or treatment of uninfected cells with pretreated virus (Table 2), these results may reflect a difference in drug uptake compared with 1-CBI. Furthermore, the increased sensitivity of viral DNA replication compared with DNA damage suggests that 1-CBI's DNA-damaging potency may be enhanced under conditions of continuous viral replication.

TABLE 2
Summary of SV40 virion/DNA studies: effects of 1-CBI and adozelesin
Experiments were conducted as described under *Materials and Methods*.

	Approximate IC_{50} Values	
	1-CBI	Adozelesin
	nM	
Growth inhibition[a]		
BSC-1 cells	300	0.1
DNA damage		
Naked SV40 DNA	50	50
Encapsidated SV40 DNA	500	500
DNA replication inhibition		
Encapsidated SV40 DNA	25–50	2–10
Viral production inhibition		
SV40 virion	1–10	N.D.

N.D., not determined.
[a] B. J. Philips, unpublished results.

Overall, these findings for 1-CBI are consistent with a direct relationship between DNA damage and DNA replication inhibition and strongly suggest that SV40 virions themselves may be acting as an efficient transport conduit or conveyance mechanism for 1-CBI to penetrate mammalian cell membranes, further enhancing its antiviral potency. Therefore, it is not surprising that 1-CBI also effectively blocked SV40 virus production in a concentration-dependent manner under conditions of virion pretreatment with 1-CBI ($IC_{50} \sim 5$ nM) (Table 1).

To our knowledge, this study is the first to demonstrate, as a proof-of-principle, direct inhibitory effects at multiple biological levels for a hybrid polyamide-CBI agent or any other type of DNA-damaging agent in a virion context. Because 1-CBI has been shown to target cellular DNA in a sequence-specific manner (Wang et al., 2002), enhanced replication inhibition of polyamide-CBI conjugates may be possible by targeting unique viral sequences such as promoter regions. For example, Takahashi et al. (2003) described recently the synthesis of a polyamide-CBI conjugate derived from duocarmycin A that selectively targets telomeres and exhibits widespread antitumor activity.

Because 1-CBI is much less cytotoxic to mammalian cells than adozelesin (~3 orders of magnitude) under related conditions (Table 2), it is conceivable that a similar class of unique polyamide-CBI conjugates could be generated for use as potent antiviral agents. Such agents would be advantageous in comparison to classic CPI drugs in that they would possess strong antiviral replication activity yet, unlike the later, demonstrate significantly reduced cytotoxicity toward uninfected cells. Such a scenario could be envisioned in which newly released viruses, after host cell lysis, would be vulnerable to binding by such antiviral agents before infecting other cells, thus preventing subsequent cellular infection. In contrast, noninfected cells would remain virtually unaffected by these antiviral agents, given their reduced cytotoxicity coupled with their unique viral DNA sequence preference. At this point, it is unknown how factors such as chemical structure and stability, binding affinity, and sequence specificity may affect viral loading of 1-CBI. Future studies will attempt to define the nature of these features that may underlie the efficacy of this conjugate molecule as a potential viral inhibitor.

Acknowledgments

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