Pharmaceutical Research, Vol. 18, No. 6, 2001 *Research Paper*

A Novel Topoisomerase II Poison GL331 Preferentially Induces DNA Cleavage at (C/G)T Sites and Can Cause Telomere DNA Damage

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Received January 17, 2001; accepted March 7, 2001.

Purpose. Topoisomerase II (Topo II) preferentially cuts DNA at alternating purine–pyrimidine repeats. Different Topo II poisons may affect Topo II to produce distinct drug-specific DNA cleavage patterns. GL331 is a new podophyllotoxin derivative exhibiting potent Topo II-poisoning activity. Therefore, the sequence selectivity of GL331-induced DNA cleavage was determined.

Methods. Human gastric adenocarcinoma SC-M1 cells were treated with GL331, and the resultant DNA fragments were isolated by SDS-K^+ precipitation. These DNA fragments were further cloned and sequenced to exhibit GL331-induced DNA cleavage sites. In addition, the telomere damage was detected by Southern blot analyses using a $(TTAGGG)_4$ probe. GL331's effect on telomerase was examined using the TRAP assay.

Results. The selective sequences of GL331-induced DNA cleavage were analyzed. The first nucleotide 3′-terminal to the cleavage sites was preferentially C or G and followed by the second nucleotide T. More than 50% of GL331-induced DNA cleavage fragments exhibited AT-rich sequences in the first 20 nucleotides. In addition, the telomeric damage was observed both from GL331-treated SC-M1 cells and *in vitro* incubation of genomic DNA with GL331 and purified human Topo II. Although GL331 treatment reduced cellular telomerase activity, *in vitro* reaction data suggested that GL331 was not a telomerase inhibitor.

Conclusions. GL331 preferentially induced Topo II-mediated DNA cleavage at (C/G)T sites. Because the telomeric repeat sequence contains GL331's GT preference site, the telomere was identified as one of the targets of GL331-induced DNA damage.

KEY WORDS: GL331; VP-16; topoisomerase II; telomere; DNA damage.

INTRODUCTION

Podophyllotoxin is a naturally occurring toxin from the plant *Podophyllum peltatum* (1). Derivatives of podophyllotoxin, represented by etoposide (VP-16), have been used as antineoplastic agents because they act as topoisomerase II (Topo II) poisons (2,3). Topo II is a nuclear enzyme responsible for modulation of the topological change of DNA via double-stranded DNA breaks coupled with strand passage and re-ligation. VP-16 does not inhibit Topo II to cut DNA but inhibits the rejoining step, sustaining the covalent binding of Topo II to DNA breaks (4,5). Topo II cleaves DNA at nonrandom sites, as observed by a variety of experimental systems (6,7). Topo II preferentially cuts DNA at alternating purine–pyrimidine repeats, but cleaves different DNA sequences when in the presence of Topo II poisons, yielding distinct drug-specific cleavage patterns (7–11). Recently, Topo II was shown to cleave the $(TTAGGG)_n$ telomeric repeats with the aid of VP-16 but not other tested agents (12). Telomere damage and the resulting G-rich fragments are able to inhibit the proliferation of cancer cells (13–15).

GL331 is a novel podophyllotoxin-derived Topo II-inhibiting compound (reviewed in Ref. 16), and the chemical formula is 4′-demethyl-4β-(4″-nitroanilino)-4-desoxypodophyllotoxin with a molecular weight of 520.48 (Fig. 1). GL331 shares many biochemical and physicochemical properties with its congener VP-16. However, GL331 has demonstrated more efficacious anticancer activity than VP-16 (16–19). It is interesting and important to study the mechanisms accounting for the superiority of GL331. In this study, the DNA cleavage sites induced by GL331 treatment were analyzed and found to particularly possess a predominance of (C/G)T at the 3′-terminal position. In addition, GL331 was shown to cause telomere damage both by *ex vivo* and *in vitro* analyses. Because the telomeric repeat sequence contains GL331's GT preference site, our data suggest that they are one of the targets of GL331-induced selective DNA damage.

EXPERIMENTAL

Materials and Methods

Cell Culture

Human gastric adenocarcinoma cell line SC-M1 was grown in MEM supplemented with 10% fetal bovine serum, 2 mM of L-glutamine, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (18).

Cloning and Sequencing of GL331-Induced DNA Fragments

SC-M1 cells were treated 1 h with 10 μM of GL331, and the resultant protein-linked DNA breaks were isolated by the SDS-K^+ precipitation method as described (20). The SDS/K^+-precipitated DNA fragments were further treated with proteinase K digestion and phenol/chloroform extraction, and added with glycogen before further ethanol precipitation. These purified DNA fragments were subjected to blunt-ending by T4 DNA polymerase and then ligated with the *Sma* I-digested pBluescript II SK(+) plasmid. The ligation mixture was further introduced into *E. coli* DH5α. After selection against ampicillin, *E. coli* transformants were picked up and proved to have insert DNA by 10% polyacrylamide gel electrophoresis of the polymerase chain reaction (PCR) products using T3 and T7 primers. These inserts were finally determined by direct sequencing on both strands utilizing T3 and

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ABBREVIATIONS: Topo II, topoisomerase II; DIG, digoxigenin; TRAP, telomeric repeat amplification protocol.

0724-8741/01/0600-0846$19.50/0 © 2001 Plenum Publishing Corporation

GL331 VP-16

Fig. 1. Molecular structures of GL331 and VP-16.

T7 primers and an ABI PRISM 377 XL DNA sequencer (Perkin Elmer, Foster City, CA).

Telomeric Restriction Fragments Assay

Ten micrgrams of genomic DNA was digested with *Hinf* I and resolved by 1% agarose gel electrophoresis. The DNA was denatured for 30 min with 0.5 M NaOH plus 1.5 M NaCl and neutralized with 0.5 M Tris-Cl (pH 8) plus 1.5 M NaCl for another 30 min prior to blotting onto the Hybond-N^+ membrane (Amersham Pharmacia, Piscataway, NJ). The membrane was hybridized 2 h at 42°C with digoxigenin (DIG)-labeled $(TTAGGG)_4$ oligonucleotides in Rapid Hybridization Buffer (Amersham Pharmacia). Then, the membrane was washed once with 5X SSC (1X SSC = 0.15 M NaCl plus 0.015 M Na_3-citrate) plus 0.1% SDS at room temperature for 20 min and twice with 0.5X SSC plus 0.1% SDS at 42°C for 15 min. The membrane was further rinsed with the solution containing 0.1 M maleic acid, 0.15 M NaCl, and 0.3% Tween-20 (pH 7.5) and incubated for 30 min with blocking solution (Boehringer Mannheim, Indianapolis, IN). The membrane was finally incubated for 30 min with the alkaline phosphatase-conjugated anti-DIG antibody in blocking solution (1: 10000 dilution), and after two washes, the telomeric fragments on the membrane were detected by adding CDP-Star™ substrate to produce chemilluminescence (Amersham Pharmacia).

Telomeric Repeat Amplification Protocol (TRAP)

The telomerase activity was assayed based on the protocol of the TRAPeze telomerase detection kit (Intergen Co., Purchase, NY). Cell lysates were prepared in 200 μl lysis buffer consisting of 0.5% CHAPS, 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 0.1 mM benzamidine, and 5 mM β-mercaptoethanol. Then, each TRAP reaction was performed at 30°C for 30 min in a 50 μl of reaction mixture containing 20 ng of cell lysate, 20 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween-20, 1 mM EGTA, 250 μM dNTPs, Primer Mix (TS, RP, K1, and TSK1 templates supplied in the TRAPeze kit), and 2 units of Taq polymerase (Boehringer Mannheim), and followed by 33 cycles of PCR reactions at 94°C for 0.5 min and 59°C for 0.5 min. One half of each reaction mixture was resolved by 12.5 % polyacrylamide gel electrophoresis, and after staining with SYBR green (Molecular Probes Inc., Eugene, OR), the image of the telomeric repeat pattern was printed by Alpha-InnoTech IS500 Digital Imaging System (Avery Dennison, CA).

RESULTS

GL331 Induces Topo II-Mediated DNA Cleavages at Selective Sequences

Different Topo II poisons may differentially affect Topo II to produce distinct drug-specific DNA cleavage patterns. To determine the sequences cleavable by GL331-affected Topo II, the DNA fragments precipitated by SDS-K^+ from GL331 or VP-16-treated SC-M1 cells were ligated with pBluescript II SK(+) vector DNA and introduced into *E. coli* DH5α. After ampicillin selection, 85 and 52 colonies were picked up from the GL331 and VP-16 groups, respectively. Among them, 25 of 85 and 9 of 52 colonies were proven to contain inserts as determined by 10% polyacrylamide gel electrophoresis of the PCR products utilizing T3 and T7 primers. Furthermore, the sequences of these inserts were determined. As listed in Table I, we show the first 20 nucleotides of both 5′-ends of each GL331 or VP-16-induced DNA fragment. They represented the adjacent nucleotides 3′ to the Topo II cleavage sites. When we looked at these sequences, A/T contents of ≥ 60% (12/20) were found in 26 of GL331's 50 sequences (52%) and 4 of VP-16's 18 sequences (22%), suggesting that the selective sites of GL331-affected Topo II were preferentially adjacent to AT-rich sequences. The nucleotide frequencies are listed in Table II. In the GL331 group, the first nucleotide occurred significantly as C or G with frequencies at 48 and 38%, respectively ($P = 0.0011$ by chi-square analysis if compared with the random nucleotide frequency 25%), and the second nucleotide was predominantly T, at a frequency of 52% ($P = 0.0488$). No significant selectivity was found to occur at other positions. The hierarchy of frequencies of the first two nucleotides has the order: GT = CT (24%) > CC (14%) > CA (10%) > GG (8%) > GA (6%) > AG = AT = TG (4%) > AC (2%) > GC = CG = AA = TA = TC = TT (0%). On the other hand, C was identified as the first nucleotide in 56% of VP-16's 18 sequences, but it was not statistically significant, probably because the sample number was not enough ($P = 0.1369$).

GL331 Induces Telomere Damage but Is Not a Telomerase Inhibitor

Telomeric repeats TTAGGGTTAGGG . . . could be cleaved by Topo II with the aid of VP-16 (12). Considering that GL331 is a VP-16 congener and the telomeric repeat sequence contains GL331's GT preference site, we explored whether GL331 could also affect Topo II to cleave the human telomere DNA. Genomic DNA was extracted from GL331 or VP-16-treated SC-M1 cells, and subjected to Southern blot analysis using $(TTAGGG)_4$ as the probe. When cells were treated with 10 μM of GL331 for 48 h, their telomere damage accumulated to a sufficient level so that the telomere shortening pattern was detectable; after 60 h or longer, many telomeres were too damaged to hybridize with the probe and thus the telomeric band intensity decreased (Fig. 2A). In the parallel experiment, 10 μM of VP-16 caused less telomere damage. Only the smearing band pattern representing the telomere shortening was observed after 60 h of VP-16 treatment (Fig. 2A). To confirm the telomere damage really resulted from the Topo II-mediated cleavage, genomic DNA was incubated with Topo II in the presence of 1 μM GL331 or 10

Table I. List of the First 20 Nucleotides of Both 5′-Ends of Each GL331 and VP-16-Induced DNA Fragment[a]

Clone	T3-primer ends	T7-primer ends
GL331		
G1:	C T T G T G G C C A G C T T C G T T C T	G G G A A A A C A A C T A G A A C G A A
G5:	C T G A T T T G T G G C A A T G C C T C	C T C A T C A C T T T C C A G C C C A G
G7:	G T G T C C C A G G T A T G A A G A G T	C T G C T T G T T G T G T T C A G A G G
G12:	G T A G G C T T A T C A C T C C C A G C	C C C A A A T C T A T A C A G A C T C A
G14:	G T C T T G A A C C C C T G A C C T C A	C T C T A G T G G T T T T G C A G T T G
G15:	G T A A A G A A A T G T A A T G C T A A	C T C A A A T C T A T T C A G A C T C G
G16:	C T A G G A G A A G T C G G G A G G C T	G G C A G A G G T G A G T T G G A T G G
G18:	G A T T G G C G A G C G T G C T A T T C	C T G T A T T T T G G G A A A T T G A T
G21:	C A A A G A C T T T A T G A C T A A A A	C T T T A C T T T A A T C A G A T C C A
G23:	G T T G T G T T T T T A T A T T T G T G	C C C T T T T A T A A G A T C A C T A C
G31:	C T A G T A T A A T A T T T A T G T A A	C C T A A G T C T G G C C T A T G A G T
G35:	C T C C T C A A T T C A T A T A T T T C	G T G A A G T C A G T C A T A A A A C T
G37:	G T C C T T T G T T T A T G C T T T C A	C C T G T C T C A A A C A A A C A A A T
G41:	C T C A G G A G G C T G A G G C A G G A	C A C T G C A A C C T C T G C C T C C T
G45:	G G A A A A A T C A A T A T T G T T A A	G T C T A A T A A T G T G A T G C C T G
G46:	A T C C A T T T T A G A G G A G G A A G	C A T C C C T T C C C A G A C T C C A G
G50:	C C T C T A C A A A A T A T A A C A A T	G G G T T A T T T G T A T T T T G C T G
G53:	A T C C G T T T T G T G T T T T C A G T	T G A T T T C A G A C G A A G A A A A T
G55:	G A T C A C C C A G A T A C A T A A A G	G T A T T T G T A T A G T T C C C A A A
G57:	C A T C T C A A A A A C C A A A A A C C	C C C A A A G T G C T G G G A T T A T A
G60:	A C G G C T T T T G C T C C A C C T C T	A G T A A G G A A A C A C T G G G G G T
G64:	G T C T T T T G G C T A A A T A A A T A	T G T C C T T G C C T C C T T T C C A G
G66:	G T T C G A G A C C A G C C T G G T C A	G T C T C A C T T T G T T G A C C A G G
G75:	C A C T G T G G T T C T A C T T T G C A	C C C C T C A C A G A A A T G C T T G G
G82:	G A C A T A G T T T G G G C C A C T G C	A G T G G T G A T A A C T G A A T C A T
VP-16		
V4:	C C T C T A C A A A A T A T A A C A A T	G G G T T A T T T G T A T T T T G C T G
V7:	C G C T T G A A C C C A A G A G A T T G	C T A C T G C A C T T A G G G A A C T G
V15:	G T A G T T G T T A A A G G C C C A G T	A G C A A A G C T T T T T C C C T G T G
G19:	C G C A G T T T G G G A A C C A T G A C	C T C T T C C C A G T C A C C A C C C T
V23:	G G G A C A T G G C A T T C C T G A T G	C A C C A G A A T C C T A G C A T C A G
V32:	C T T T C C A C T C A A T T C C A C T C	C A G T G G A A T G G A A T G G A A T G
V35:	G A G C A G A A A C A A G C A G A A G A	G T C T C T T C T G C T A T T G T T T A
V49:	A G T T C T T G T T T A G G T G A C T C	C A T G G C A G T C A C C T A A A C A A
V51:	T C C A G C C T G G G C A G C A G	C T G C T G C C C A G G C T G G A

[a] Cloning of GL331 and VP-16-induced DNA fragments were described in Materials and Methods. Totally, 25 and 9 clones were proven to contain GL331 and VP-16-induced DNA fragments, respectively. These fragments were finally determined by direct sequencing on both strands utilizing T3 and T7 primers and an ABI PRISM 377 XL DNA sequencer (Perkin Elmer).

μM VP-16 and then subjected to a Southern blot using the telomere probe. The result shows that 1 μM of GL331 induced a 50% decrease of the detectable telomeric level in comparison with only 29% reduction induced by 10 μM of VP-16 (Fig. 2B). Because 1 μM of GL331 and 10 μM of VP-16 induced comparable levels of *in vitro* plasmid DNA cleavage (Ref. 20 and data not shown), GL331 seemed more effective than VP-16 in causing the Topo II-mediated telomere breakage. In addition, the TRAP method was used to determine the telomerase activity in the cell lysates from SC-M1 cells treated with GL331 or VP-16 for time periods. Telomerase activity was observed to be decreased in the cells treated 48 h with GL331 or VP-16, and seemed to be completely inhibited after the 72-h treating course (Fig. 3A). To determine whether GL331 and VP-16 would act as inhibitors of telomerase, GL331 or VP-16 was added during the *in vitro* reactions of the telomerase activity assay. As shown in Fig. 3B, GL331 and VP-16 did not affect the TRAP assay, indicating that GL331 and VP-16 were not inhibitors of telomerase and Taq DNA polymerase. Reduction of SC-M1 cellular telomerase activity after GL331 or VP-16 treatment could be attributed to cells undergoing the death pathway. The data suggest that GL331 and VP-16 specifically inhibited the Topo II activity.

DISCUSSION

Podophyllotoxin is a naturally occurring plant toxin with demonstrated potent microtubule-interfering activity that causes severe toxicity and may thus limit its use in cancer treatment (1). GL331 and VP-16, both derived from podophyllotoxin, exhibit less inhibitory activity against microtubule assembly but act as potent poisons of Topo II (2,3,20). They can inhibit Topo II to rejoin the DNA breaks, and

Table II. List of Nucleotide Frequencies of the DNA Sequences in Table I

	GL331																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	0.10	0.16	0.16	0.30	0.30	0.30	0.20	0.30	0.30	0.28	0.28	0.24	0.30	0.32	0.32	0.34	0.20	0.34	0.34	0.30
C	0.48	0.16	0.40	0.24	0.10	0.22	0.14	0.20	0.14	0.16	0.20	0.24	0.22	0.10	0.24	0.20	0.36	0.18	0.26	0.14
G	0.38	0.16	0.16	0.16	0.22	0.20	0.22	0.18	0.12	0.28	0.18	0.24	0.14	0.26	0.20	0.18	0.20	0.14	0.22	0.28
T	0.04	0.52	0.28	0.30	0.38	0.28	0.44	0.32	0.44	0.28	0.34	0.28	0.34	0.32	0.24	0.28	0.24	0.34	0.18	0.28
	C/G[a]	T[b]	N[c]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	VP-16																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	0.11	0.22	0.11	0.22	0.17	0.22	0.33	0.33	0.17	0.17	0.33	0.5	0.44	0.00	0.22	0.39	0.44	0.31	0.25	0.19
C	0.56	0.11	0.39	0.28	0.22	0.22	0.28	0.28	0.33	0.33	0.17	0.17	0.11	0.28	0.44	0.17	0.17	0.44	0.06	0.19
G	0.28	0.33	0.28	0.11	0.22	0.33	0.11	0.17	0.33	0.33	0.22	0.06	0.22	0.33	0.17	0.33	0.17	0.13	0.13	0.44
T	0.06	0.33	0.22	0.39	0.39	0.22	0.28	0.22	0.17	0.17	0.28	0.28	0.22	0.39	0.17	0.11	0.22	0.13	0.56	0.19
	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

[a] The nucleotide frequency was obtained by calculating the ratio of the nucleotide's occurrence. For example, the first nucleotide occurs as A in 5 of GL331's 50 sequences; therefore, the frequence of A as the first nucleotide is 5/50 = 0.1 in GL331-induced DNA fragments.
[b] $P < 0.05$ by chi-square analysis if compared with the random nucleotide frequency (0.25).
[c] N represents A, C, G, or T.

therefore, inhibit cancer cell proliferation (20,21). The Topo II-mediated DNA cleavage occurs at nonrandom sites, and some drugs could affect the sequence preference of Topo II resulting in drug-specific cleavage patterns. In this study, the GL331-specific DNA cleavage pattern was determined. The preference for (C/G)T as the two nucleotides immediately 3′ of the cleavage site was observed with GL331-induced DNA cleavage in SC-M1 cells (Fig. 4A). These selective sites employed by GL331-affected Topo II have never been reported from other Topo II poisons. VP-16 possesses a preference for C at −1 position (the first nucleotide 5′ of the cleavage site) and a purine at −2 (11). In addition, the intercalating agent *m*-AMSA prefers +1 A (A as the first nucleotide 3′ of the cleavage site) and +4 T as Topo II sites (6,11); adriamycin tends to recognize the −1 A and +5 T positions (22), and ellipticine induces −1 T and +5 A preference at cleavage sites (9).

Recently, VP-16 was shown to change Topo II to cleave the telomeric repeat sequence 5′-TTAGG*GTT-AGG*G . . . (cleavage sites designated as *, Ref. 12). Telomeres are located at the ends of eukaryotic chromosomes and function to protect chromosomes from degradation, fusion, and recombination (reviewed in Ref. 23). Telomere shortening can be observed after each cell dividing cycle, and when telomeres were induced by drugs to reach a critical low length, the cells experienced crises and died (12–15). From our experimental data, we found that GL331 was more potent than VP-16 in inducing the telomere damage *in vitro* and *ex vivo*. Based on the suggested (C/G)T sites for GL331, we think that GL331 induced more efficient telomere breakage at the abovementioned cleavage sites than VP-16 (Fig. 4B). The G-rich DNA ends after telomere damage have been reported to efficiently induce p53 accumulation and inhibit the proliferation of cancer cells (13–15). Interestingly, GL331 has also demonstrated higher anticancer efficacy in several experimental systems if compared with VP-16 (16–19). Therefore, the GT site selectivity could be one of the mechanisms that render GL331 a more efficient inhibitor of cancer cells.

Introduction of DNA damage is one of the main mechanisms employed by anticancer agents to inhibit the proliferation of cancer cells. VP-16 and GL331 can inhibit Topo II and sustain the covalent binding of Topo II to DNA breaks (4,5,20). The interaction among VP-16, Topo II, and DNA may occur through an intercalation-like mechanism (24,25). The planar ABCD ring structure of VP-16 inserts between the base pairs, whereas the pendant E ring and the glycosidic moiety stretch out along the DNA minor groove and may bind DNA and/or Topo II. Distinct DNA damage patterns induced by VP-16 and GL331 were probably attributed to the difference of two compounds' chemical structures. The structural difference between GL331 and VP-16 is the 4β-*O*-glycosidic moiety of VP-16 was replaced by a nitroanilino group. It was previously reported that the 4β-N-aromatic substituents were more potent Topo II poisons than alkyl substituents (26,27). In addition, the 4β-N-aromatic substituents caused more protein-linked DNA breaks *ex vivo* and were more efficacious than VP-16 to inhibit Topo II *in vitro* (28). It appears that the 4β-N-aromatic moiety may render GL331 differently interacting with DNA and Topo II, causing a distinct DNA cleavage pattern.

In addition to acting as a Topo II poison, GL331 could induce a plethora of cellular mechanisms that are quantitatively or qualitatively different from those induced by VP-16. During the apoptotic induction, the activities of protein tyrosine kinases are decreased in cancer cells treated with GL331 or VP-16, whereas the activities of protein tyrosine phosphatases are in contrast induced only in GL331-treated cancer cells (17). The accumulating results that indicate the mechanism differences between GL331 and VP-16 treatments will help us to understand the clinical significance of GL331 in cancer therapy. In conclusion, we report herein that GL331 preferentially induced DNA damage at (C/G)T sites, and that the telomeric repeat sequence was identified as one of the targets.

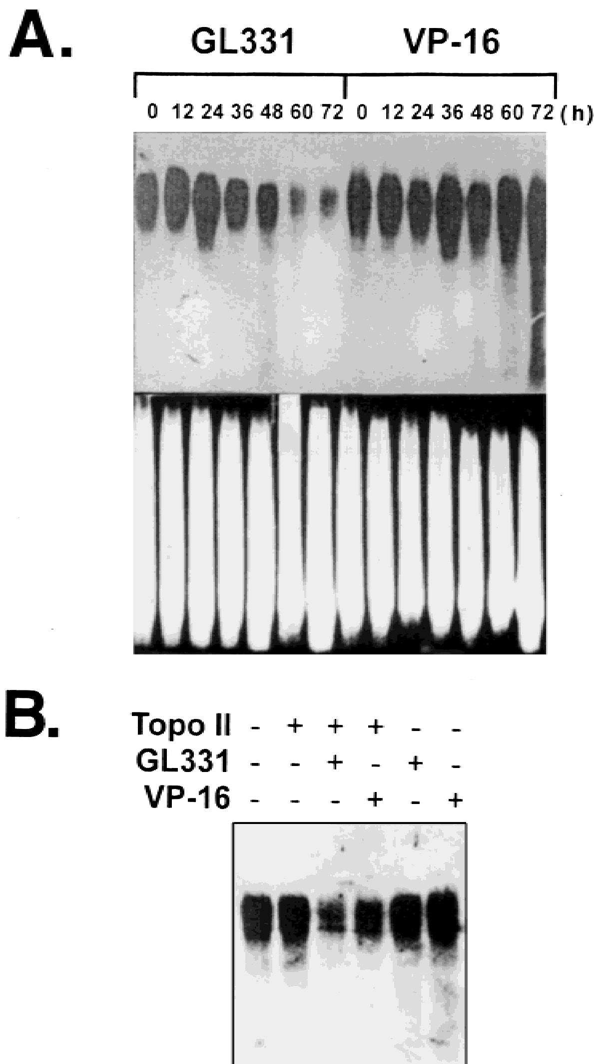

Fig. 2. Telomere damage induced by GL331. (A) Investigation of telomere shortening from the DNA of SC-M1 cells treated with 10 μM of GL331 or VP-16 for different time points. Cellular DNA was isolated and digested with *Hinf* I, and was subjected to Southern blot analysis utilizing digoxigenin (DIG)-labeled $(TTAGGG)_4$ as the probe. The telomeric fragments were detected by a CDP-Star™ chemilluminescence system (Amersham Pharmacia). Lower panel shows the ethidium bromide staining result to demonstrate the equal levels of sample loading. It is noted that reduction of telomeric band intensity was induced by GL331 at 60 h or longer, probably because many telomeres were too damaged to hybridize with the $(TTAGGG)_4$ probe. The representative data from at least three independent experiments are shown. (B) Investigation of telomere damage from the DNA *in vitro* incubated with 10 units of purified human Topo II (TopoGEN, Inc., Columbus, OH) plus 1 μM of GL331 or 10 μM of VP-16. After Topo II and drug incubation at 37°C for 2 h, the DNA was digested with *Hinf* I prior to Southern blot hybridization with the DIG-labeled $(TTAGGG)_4$ probe. The image was printed and quantitated by an Alpha-InnoTech IS500 digital imaging system. In comparison with the control incubation without Topo II and drug, the telomeric band intensities were reduced down to 50 and 71% of control by the presence of Topo II plus GL331 and VP-16, respectively. The ethidium bromide result to monitor the equal loading is not shown. The data are representative of three independent experiments.

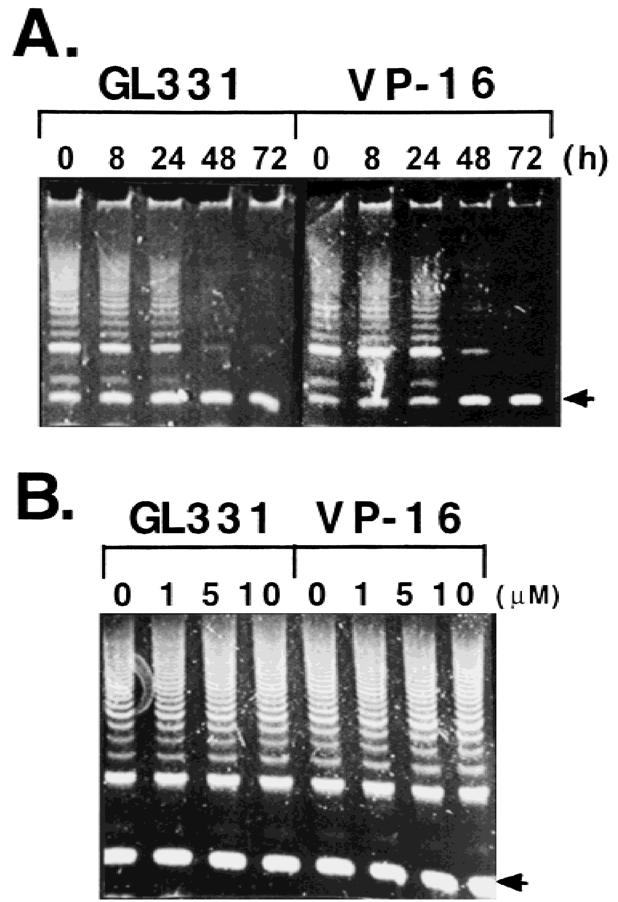

Fig. 3. Effect of GL331 on the telomerase activity. (A) Assay of telomerase activity from SC-M1 cells treated with 10 μM of GL331 or VP-16 for different time points. Lysis of drug-treated cells and TRAP reactions were performed according to the method of the TRAPeze telomerase detection kit (Intergen). The TSK1 template supplied by the kit was added in each TRAP reaction to obtain a 36-bp product as the internal control (i.e., the bands indicated by the arrowhead). The representative data from at least three independent experiments are shown. (B) Assay of telomerase activity when GL331 or VP-16 is present in the reactions. Various doses of GL331 or VP-16 were added in the cell lysate from untreated SC-M1 cells before the TRAP reaction. The arrowhead indicates the 36-bp bands of internal control. The representative data from two independent experiments are shown.

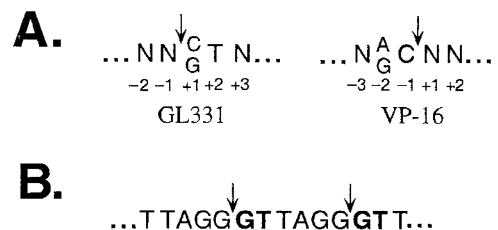

Fig. 4. (A) The preferential DNA cleavage sites for GL331 and VP-16-affected Topo II. The arrows indicate the cleavage positions, and the N represents A, T, C, or G. (B) The proposed telomere DNA cleavage sites induced by GL331 and VP-16. Based on the data shown in Fig. 2 and the suggested preferential (C/G)T sites for GL331, we think that GL331 is more potent than VP-16 to induce telomere breakage at these sites.

ACKNOWLEDGMENTS

GL331 was kindly provided by Genelabs, Inc.

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