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Induction of Topoisomerase II-Mediated DNA Cleavage by the Plant Naphthoquinones Plumbagin and Shikonin

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Plumbagin and shikonin, plant metabolites which have naphthoquinone structures, induced mammalian topoisomerase II-mediated DNA cleavage in vitro. Treatment of a reaction mixture containing these naphthoquinones and topoisomerase II at an elevated temperature (65°C) resulted in a great reduction in DNA cleavage, suggesting that the mechanism of the topoisomerase II-mediated DNA cleavage induced by these naphthoquinones is through formation of a cleavable complex, as seen with antitumor agents such as 4′-(9-acridinylamino)methanesulfon-*m*-anisidide and demethylepipodophyllotoxin ethylidene-β-glucoside. Lawson and lapacol, which are structurally related plant metabolites with naphthoquinone moieties, could not induce topoisomerase II-mediated DNA cleavage. Plumbagin and shikonin induced a similar DNA cleavage pattern with topoisomerase II which was different from the cleavage patterns induced with other known topoisomerase II-active drugs. A DNA-unwinding assay with T4 DNA ligase showed that shikonin, lawson, and lapacol did not intercalate into DNA, while plumbagin and 2-methyl-1,4-naphthoquinone intercalate into DNA, but to a lower degree than 4′-(9-acridinylamino)methanesulfon-*m*-anisidide does.

DNA topoisomerases are a class of enzymes that alter DNA conformation through a concerted breaking and rejoining of the DNA molecule, thereby controlling the topological state of DNA. They are reported to be involved in many important processes of DNA metabolism including replication, transcription, recombination, and chromosome segregation (38). In addition, topoisomerases have been potential targets for chemotherapy. Bacterial topoisomerase II (DNA gyrase) is well known as the primary target of quinolone antibacterial agents. Mammalian topoisomerase II has also been identified as the primary cellular target for a number of clinically important antitumor agents which include intercalating agents [e.g., 4′-(9-acridinylamino)methanesulfon-*m*-anisidide {*m*-AMSA}, adriamycin, and ellipticine] as well as nonintercalating agents (e.g., VP16 and VM26 [epipodophyllotoxin]) (6, 29, 34, 35). All of these drugs trap topoisomerase II in an intermediary conformation with DNA, termed the "cleavable complex," which can be detected as DNA double-strand breaks upon treatment of the complex with protein denaturants. Structure-activity studies of a large number of acridine derivatives, epipodophyllotoxin congeners, and antitumor quinolones have shown a strong correlation between antitumor activity and the ability to induce the cleavable complex (23, 31, 39). In addition, there is now good evidence that mammalian topoisomerase I is the cellular target of camptothecin, an alkaloid with antitumor activity which was isolated from the Chinese tree *Camptotheca acuminata*. Camptothecin derivatives have shown promising activities in clinical studies (12, 16).

Thus, important antitumor drugs, epipodophyllotoxins and camptothecin derivatives, have been developed from plant metabolites, and now their cellular target has been found to be topoisomerase. In order to identify new plant metabolites with antitumor activity, we screened plant extracts for their ability to induce a cleavable complex with purified mammalian topoisomerases. We found that the plant naphthoquinones plumbagin and shikonin are potent inducers of the cleavable complex formation with topoisomerase II in vitro.

In this report, we describe the nature of the cleavable complex induced by plant naphthoquinones and discuss the possible relations of these activities in vitro to their cytotoxicities and antimicrobial activities.

MATERIALS AND METHODS

Topoisomerase II isolation and nucleic acids. DNA topoisomerase II was isolated from calf thymus as described previously (13) and was partially purified with Bio-Rex 70 and hydroxylapatite (Bio-Gel HTP) and by P-11 phosphocellulose column chromatography. The topoisomerase II used in the present study was functionally pure, in that it contained neither topoisomerase I activity (topoisomerase I activity was determined by the relaxation assay by a previously described method [40]) nor endonucleolytic activity (inasmuch as there was no production of nicked or linear DNA in the relaxation assay). Topoisomerase II was kept at −20°C in a storage buffer containing 50% (vol/vol) glycerol, 0.5 mM dithiothreitol, 0.1 mM EDTA, and 30 mM potassium phosphate (pH 7.5). One unit of activity was the amount of topoisomerase II that relaxed half of the 0.4 μg of supercoiled pUL402 DNA, which contains the scaffold-associated regions from the *Drosophila* histone gene cluster (1) and sequences related to the topoisomerase II cleavage consensus (7, 11). Supercoiled pUL402 DNA was purified from *Escherichia coli* as described previously (24).

Drugs and biochemicals. Plumbagin (5-hydroxy-2-methyl-1,4-naphthalenedione) was isolated from *Dionaea muscipula*. Plants derived from seedlings of *D. muscipula* were subcultured every 4 weeks on half-strength Murashige-Skoog medium (27) containing 3% sucrose and 0.7% agarose at 25°C under a 16-h light, 8-h dark photoperiod. Plumbagin was extracted from plant materials with ethanol and was

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purified by two stages of silica gel chromatography by using *n*-hexane–ethyl acetate–methanol (50:5:1) and chloroform-methanol (100:1). The topoisomerase II-mediated DNA cleavage activity was monitored throughout the purification steps by the topoisomerase II-mediated DNA cleavage assay described below. The active fractions were concentrated and crystallized at 4°C and were identified by spectroscopic analysis. VP16 was obtained from the National Cancer Institute, and *m*-AMSA was a gift from the Warner-Lambert Co. Shikonin and lawson (naphthoquinones) and genistein (isoflavone) were purchased from Funakoshi Co. The other naphthoquinones, lapacol and 2-methyl-1,4-naphthoquinone (VK_3), were purchased from Aldrich Chemical Co., and quercetin (flavone) was from Kanto Chemical Co. For studies in vitro, stock solutions of the drugs were dissolved in methanol containing 20% dimethyl sulfoxide, stored at −20°C, and diluted in the same solvent before use. Proteinase K was obtained from Sigma Chemical Co., Bio-Rex 70 and hydroxylapatite (Bio-Gel HTP) were from Bio-Rad, and P-11 phosphocellulose was from Whatman. *Hin*dIII and T4 DNA ligase were purchased from Takara Shuzo Co., Ltd.

Agarose gel assay for topoisomerase II-mediated DNA cleavage. Reactions (20 μl) containing 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 1 mM ATP, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg of bovine serum albumin per ml, 0.4 μg of supercoiled pUL402 DNA, and calf thymus topoisomerase II with or without drug were incubated at 37°C. After 30 min, reactions were terminated by the addition of 2 μl of solution containing 5% sodium dodecyl sulfate (SDS) and 2.5 mg of proteinase K per ml. Following an additional incubation at 37°C for 30 min, the samples were electrophoresed through a 1.2% (wt/vol) agarose gel in 89 mM Tris-borate (pH 8.3)–2 mM EDTA buffer containing 0.1% SDS. A total of 100 U of topoisomerase II was used in the DNA cleavage assay. After electrophoresis, gels were stained with ethidium bromide and photographed over UV illumination. To determine the amount of linear DNA produced, the negatives were scanned with a Shimazu microdensitometer (CS-930). The area of the Gaussian peak of total DNA in each lane was measured, and the percentage of linear DNA was calculated.

Comparisons of the major cleavage sites were as follows. A total of 0.6 μg of pUL402 DNA linearized with *Hin*dIII (as substrate) and 150 U of topoisomerase II were used in the DNA cleavage assay. Other conditions of the topoisomerase II-mediated DNA cleavage assay were the same as those described above.

DNA-unwinding assay with T4 DNA ligase. The DNA-unwinding effects of the drugs were assayed by the method described by Camilloni et al. (4), with minor modifications. Plasmid pUL402 DNA was linearized with *Hin*dIII restriction endonuclease and recovered by phenol extraction and ethanol precipitation. Reaction mixtures (200 μl) containing 66 mM Tris-HCl (pH 7.6), 6 mM $MgCl_2$, 10 mM dithiothreitol, 0.7 mM ATP, 0.6 μg of linearized DNA, and drug were equilibrated at 15°C for 15 min; this was followed by incubation with an excess amount of T4 DNA ligase at 15°C for 60 min. The reactions were stopped by the addition of EDTA at a final concentration of 20 mM. Two-dimensional electrophoresis was performed as described previously (15), after treatment to remove the drug from the reaction mixture by extraction with phenol-ether and precipitation with ethanol.

Biological activities. (i) MIC determination. The in vitro antimicrobial activities of the naphthoquinones were determined on nutrient agar (0.1% glucose, 0.3% Bacto Tryptone, 0.3% meat extract, 1.6% agar) by a twofold serial dilution method by using final inocula of approximately 3.0×10^5 (*Staphylococcus aureus*), 6×10^4 (*Enterococcus faecium*), 8.0×10^4 (*Bacillus subtilis*), 1.0×10^4 (*Klebsiella pneumoniae*), 2.0×10^4 (*E. coli*), 1.2×10^4 (*Pseudomonas aeruginosa*), 2.0×10^4 (*Salmonella typhi*), 3.0×10^4 (*Proteus vulgaris*), 1.0×10^4 (*Shigella sonnei*), and 5.8×10^4 (*Candida albicans*) CFU per spot. The lowest concentration that inhibited growth of a bacterial strain after 18 h of incubation at 37°C was recorded as the MIC.

(ii) Cytotoxic activity. H-*ras*-transfected mouse BALB/c 3T3 (BALB/c 3T3 H-*ras*) cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). For determination of the cytotoxic activities of the drugs, BALB/c 3T3 H-*ras* cells (1.5×10^5 cells per well) were preincubated for 24 h at 37°C in 96-well plastic plates and were then treated with different dilutions of drug for 3 days. After washing the monolayer cells with phosphate-buffered saline, the concentrations of drugs required for 50% inhibition of cell growth were determined by a Giemsa stain method described previously (26).

RESULTS

Topoisomerase II-mediated DNA cleavage by naphthoquinones. In the course of screening plant extracts for compounds that stimulate topoisomerase II-mediated DNA cleavage activity, we found such an activity in the ethanol extract of *D. muscipula* grown on nutrient agar medium as described in Materials and Methods. *D. muscipula* Ellis. is a carnivorous herb with a restricted range in the bogs of coastal North and South Carolina (2). The purified active component has been shown to be identical to plumbagin, 5-hydroxy-2-methyl-1,4-naphthalenedione (Table 1), by nuclear magnetic resonance and mass spectrometric spectroscopic analyses. Plumbagin is found in the roots and aerial parts of members of the tribe *Plumbaginaceae* (36) and has also been reported to be a constituent of members of the tribe *Droseraceae* (28).

On the basis of the findings presented above, we screened plant metabolites with a naphthoquinone structure like that of plumbagin for their ability to induce topoisomerase II-mediated DNA cleavage by using purified calf thymus topoisomerase II and supercoiled pUL402 DNA as substrates. Figure 1 shows a photograph of agarose gels comparing topoisomerase II-mediated DNA cleavage activity in the presence of various amounts of naphthoquinones. In the case of plumbagin and shikonin, the linear-form DNA progressively appeared as the concentration of drug increased (from 0.5 μM in lanes c and h to 250 μM in lanes g and l of Fig. 1). For comparison, the antitumor drugs VP16 and *m*-AMSA, which have been shown to promote topoisomerase II-mediated DNA cleavage, were included (Fig. 1, lanes m to q and K to O, respectively).

To obtain more quantitative data, the amount of linearized DNA was measured by scanning the negatives with a densitometer. As shown in Fig. 2, plumbagin and shikonin showed comparable cleavage activities which were lower than those observed for *m*-AMSA and VP16. VK_3 showed weak topoisomerase II-mediated DNA cleavage activity that was one-half the activities of plumbagin and shikonin. In contrast, the other naphthoquinones lawson and lapacol had no effect on topoisomerase II activity up to the highest concentration tested (250 μM; Fig. 1, lanes r to v and A to E, respectively).

Reversibility of naphthoquinone-induced DNA cleavage.

TABLE 1. Comparison of biological activities of naphthoquinone compounds[a]

	plumbagin	shikonin	lawson	lapacol	VK_3
R_1	CH_3	HO, H	OH		CH_3
R_2	H	H	H	OH	H
R_3	OH	OH	H	H	H
R_4	H	OH	H	H	H
Relative topoisomerase II-mediated DNA cleavage activity (μg/ml)	5.64	6.34	>100	>100	18.58
Relative intercalation activity	+	-	-	-	+
Cytotoxicity IC_{50} (μg/ml)	0.26	0.063	18.5	1.4	0.11
Antimicrobial activity MIC (μg/ml)					
Organisms					
Staphylococcus aureus ATCC 6538P	1.6	2.1	>100	>100	8.3
Enterococcus faecium ATCC 10541	3.1	4.2	>100	>100	8.3
Bacillus subtilis No. 10707	0.2	0.3	12.5	2.9	0.1
Klebsiella pneumoniae ATCC 10031	6.3	>100	>100	>100	>100
Escherichia coli ATCC 26	12.5	>100	>100	>100	>100
Pseudomonas aeruginosa Bin H No.1	12.5	>100	>100	>100	>100
Salmonella typhi ATCC 9992	3.1	>100	>100	>100	>100
Proteus vulgaris ATCC 6897	0.4	>100	12.5	2.9	2.1
Shigella sonnei ATCC 9290	6.3	>100	>100	>100	>100
Candida albicans ATCC 10231	0.2	>100	>100	>100	1.0

[a] Topoisomerase II-mediated DNA cleavage activities were compared by determining the concentration (in micrograms per milliliter) of drug that induced 10% linear DNA from total DNA. As a positive control, the relative topoisomerase II-mediated DNA cleavage activity of VP16 was 1.06 μg/ml (data not shown). Intercalating activity is signified as the presence of a drug concentration-dependent DNA band shift in the DNA-unwinding assay. The intercalation activities of naphthoquinones (250 μM) were shown equal to that obtained with *m*-AMSA at the following concentrations: 2.5 to 12.5 μM (+), 12.5 to 50 μM (++), and ≥50 μM (+++); −, no effect. For cytotoxicity, the values indicated the concentration (in micrograms per milliliter) of drug required for 50% inhibition of cell growth (IC_{50}) of BALB/c 3T3 H-*ras* over 72 h of exposure. Antimicrobial activity was shown by the MIC determined by an agar dilution method.

The formation of a cleavable complex by antitumor drugs has been shown to be reversed by several treatments of the reaction, such as dilution, increased salt concentration, or elevated temperature (6, 17, 29, 34, 35). To test whether naphthoquinone-induced DNA cleavage with topoisomerase II is reversible, reaction mixtures were heated to 65°C before termination with SDS and proteinase K, and an aliquot of the mixture was withdrawn at various time intervals during the heat treatment. The reversibility of the topoisomerase II-mediated DNA cleavage induced by plumbagin, shikonin, and VP16 was analyzed by agarose gel electrophoresis; and the amount of linear DNA was quantified by densitometric analysis as described above. As shown in Fig. 3, heat treatment (65°C) rapidly reversed the cleavable complex induced by plumbagin and shikonin in a manner similar to that observed with VP16. Within 20 min, the DNA cleavage activities of plumbagin and shikonin were suppressed to drug-free background levels (about 5% of linear DNA was

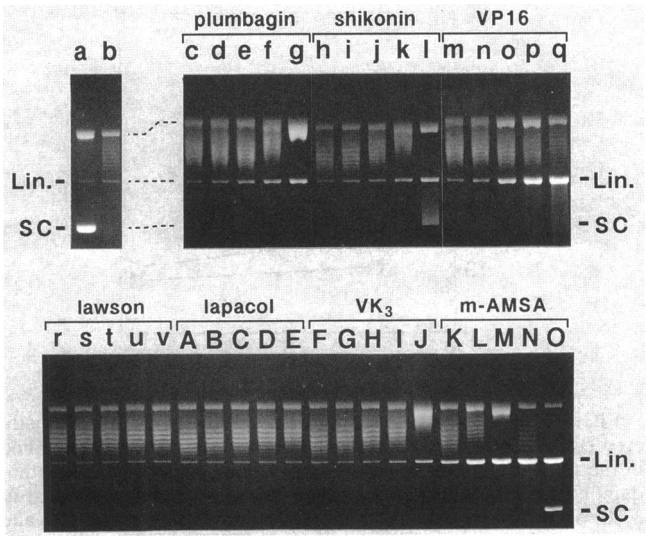

FIG. 1. Topoisomerase II-mediated DNA cleavage by naphthoquinones and antitumor drugs. Cleavage of supercoiled DNA was analyzed by the agarose gel assay described in the text. Lane a, substrate DNA alone; lane b, substrate DNA plus topoisomerase II (no drug); lanes c to g, plumbagin; lanes h to l, shikonin; lanes m to q, VP16; lanes r to v, lawson; lanes A to E, lapacol; lanes F to J, VK_3; lanes K to O, *m*-AMSA. Drug concentrations were 0.5 μM (lanes c, h, m, r, A, F, and K), 2.5 μM (lanes d, i, n, s, B, G, and L), 12.5 μM (lanes e, j, o, t, C, H, and M), 50 μM (lanes f, k, p, u, D, I, and N), and 250 μM (lanes g, l, q, v, E, J, and O). Lin., linear DNA; SC, supercoiled DNA.

produced in the absence of drug). These results suggest that the mechanism of DNA cleavage induced by naphthoquinones in vitro is through the formation of a reversible, cleavable complex, which has been reported for other known topoisomerase inhibitors.

Identification of major DNA cleavage sites induced by topoisomerase II in the presence of naphthoquinones. Mapping studies of the topoisomerase II-mediated DNA cleavage sites have revealed that different DNA cleavage patterns are observed when drugs from the different chemical classes are compared, while drugs from the same chemical class show

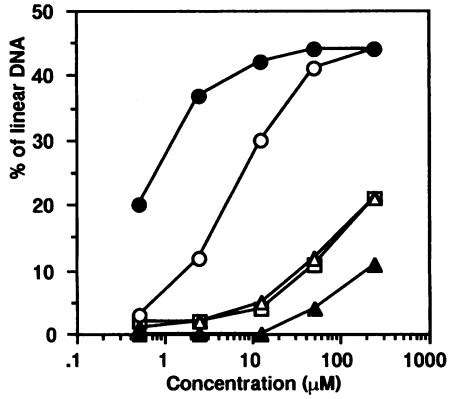

FIG. 2. Quantitative comparison of topoisomerase II-mediated DNA cleavage by naphthoquinones and antitumor drugs. The percentage of DNA linearized by topoisomerase II in the presence of drug was determined by scanning photographic negatives with a densitometer. ●, *m*-AMSA; ○, VP16; □, plumbagin; △, shikonin; ▲, VK_3.

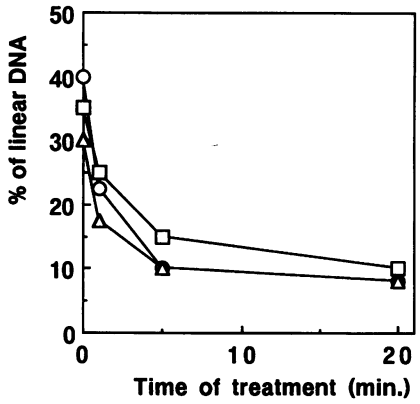

FIG. 3. Heat reversal of drug-induced topoisomerase II-mediated DNA cleavage. DNA cleavage assays were done as described in the text. A reaction mixture (100 μl) containing either naphthoquinone or VP16 (250 μM for each compound) was incubated at 37°C for 30 min. The reaction mixture was then heated to 65°C, and an aliquot (20 μl) was withdrawn at various times during the heat treatment. SDS and proteinase K treatment were done as described in the text. The percentage of DNA linearized by topoisomerase II in the presence of drugs was determined by scanning the photographic negatives with a densitometer. ○, VP16; □, plumbagin; △, shikonin.

similar cleavage patterns (5, 35). The major cleavage sites induced by plumbagin and shikonin were compared with those induced by the plant flavonoids genistein and quercetin, which induce topoisomerase II-mediated DNA cleavage (25, 41). The flavonoids genistein and quercetin are also natural products of plant origin, similar to the naphthoquinones plumbagin and shikonin, while flavonoids are a chemical class different from the naphthoquinone chemical class.

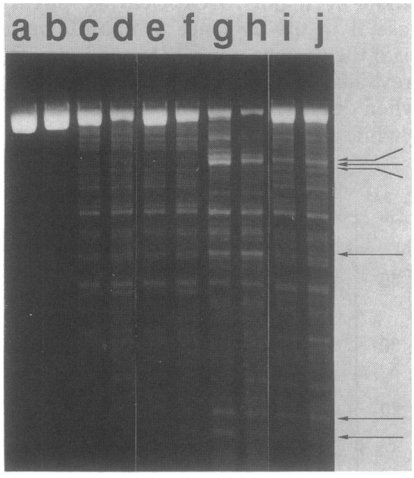

FIG. 4. Comparison of DNA cleavage patterns induced by naphthoquinones and flavonoids. Plasmid pUL402 DNA linearized with *Hin*dIII was used in the assay. The topoisomerase II-mediated DNA cleavage assay was done as described in the text. Lane a, linear DNA alone; lane b, linear DNA plus topoisomerase II (no drug); lanes c to j, drug plus topoisomerase II (lanes c and d, plumbagin; lanes e and f, shikonin; lanes g and h, genistein; lanes i and j, quercetin). Drug concentrations were 50 μM (lanes c, e, g, and i) and 250 μM (lanes d, f, h, and j). The arrows indicate the major fragments produced by genistein and/or quercetin but not detected in the lanes of naphthoquinones in the assay.

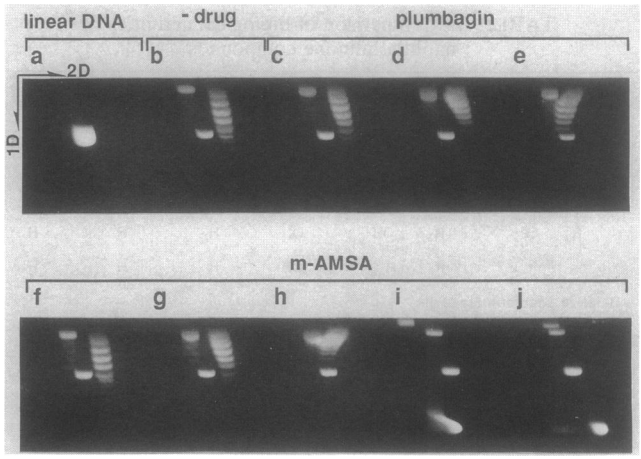

FIG. 5. Unwinding of DNA by plumbagin and *m*-AMSA. Unwinding measurements were made as described in the text. Lane a, linear DNA alone; lane b, linear DNA plus T4 ligase (no drug); lanes c to e, plumbagin plus T4 ligase; lanes f to j, *m*-AMSA plus T4 ligase. Drug concentrations were 0.5 μM (lane f), 2.5 μM (lane g), 12.5 μM (lanes c and h), 50 μM (lanes d and i), and 250 μM (lanes e and j).

As shown in Fig. 4, the patterns of DNA cleavage produced by plumbagin and shikonin were similar (lanes c and d and lanes e and f, respectively) and were distinctly different from those produced by the flavonoids genistein and quercetin (lanes g and h and lanes i and j, respectively). The major DNA fragments observed with the flavonoids genistein and quercetin were not detected in the lanes containing plumbagin and shikonin in the assay. In addition, the pattern of DNA cleavage produced by VP16 as shown in a previous report (40) is different from those observed with plant naphthoquinones and flavonoids. These results indicate that the topoisomerase II-mediated DNA cleavage sites induced by naphthoquinones are different from those induced by other plant metabolites, the flavonoids.

Intercalation activity of naphthoquinone. Most of the antitumor drugs which have topoisomerase II-mediated DNA cleavage activities are intercalating agents, such as *m*-AMSA, adriamycin, and ellipticine, and we therefore examined whether naphthoquinone can intercalate into plasmid DNA by using linearized pUL402 DNA and T4 DNA ligase (4). *m*-AMSA was included as a control in the assay. When T4 DNA ligase rejoins the free ends of linear DNA in the presence of an intercalating drug, the DNA molecule supercoils negatively, shifting the DNA band to the negative form (Fig. 5, lanes f to j). As shown in Fig. 5, plumbagin produced a concentration-dependent DNA band shift, indicating a decrease in the linking number (Fig. 5, lanes c to e), although their potencies were an order of magnitude lower than that of *m*-AMSA in this regard (Fig. 5, lanes f to j). VK_3 showed a weak intercalation activity (one-fifth that of plumbagin). In contrast, shikonin, lawson, and lapacol did not show intercalation activities even at 250 μM (data not shown). Thus, plumbagin is a weak intercalator like *m*-AMSA, whereas shikonin does not intercalate into DNA.

Biological activities. To determine whether the topoisomerase II-mediated DNA cleavage activity of the naphthoquinones correlated with other biological activities, cytotoxicity was measured by using BALB/c 3T3 H-*ras*, and antimicrobial activities were determined (Table 1). Plumbagin (relative topoisomerase II-mediated DNA cleavage activity, 5.64 μg/ml; relative intercalation activity, +), shikonin (relative

topoisomerase II-mediated DNA cleavage activity, 6.34 μg/ml; relative intercalation activity, −), and VK_3 (relative topoisomerase II-mediated DNA cleavage activity, 18.58 μg/ml; relative intercalation activity, +) were cytotoxic with 50% inhibitory concentrations of 0.26, 0.063, and 0.11 μg/ml, respectively. In contrast, the cytotoxicities of lapacol and lawson (relative topoisomerase II-mediated DNA cleavage activity, both >100 μg/ml; relative intercalation activities, both −) were lower (50% inhibitory concentrations, 1.4 and 18.5 μg/ml, respectively) than that of plumbagin. Plumbagin exhibited a broad spectrum of antimicrobial activity against both gram-positive and -negative bacteria and *C. albicans*. Shikonin and VK_3 showed antimicrobial activities against only gram-positive bacteria. Lapacol and lawson, which did not show topoisomerase II-mediated DNA cleavage activity or intercalation activity, exhibited marginal activity only against *B. subtilis* and *P. vulgaris*. The topoisomerase II-active drugs plumbagin, shikonin, and VK_3 showed significantly greater cytotoxic and antimicrobial activities compared with those of the non-topoisomerase II-active drugs lapacol and lawson. However, the relative topoisomerase II-mediated DNA cleavage activity did not correlate quantitatively with cytotoxicity among the topoisomerase II-active drugs.

DISCUSSION

Topoisomerases are viewed as the primary cellular targets of many drugs that have been used extensively in cancer chemotherapy. Among the antitumor drugs which induce a DNA-cleavable complex with topoisomerases, epipodophyllotoxins and camptothecin derivatives have been developed from plant metabolites (6, 16). In the present study, we showed that the plant naphthoquinones plumbagin and shikonin also induce topoisomerase II-mediated DNA cleavage in vitro.

Plumbagin is one of the well-known plant naphthoquinones which is reported as a constituent of several genera within the plant kingdom and has been studied for its biological activity (10, 14, 19). Krishnaswamy and Purushothaman (21) reported that plumbagin isolated from *Plumbago zeylanica* causes a regression of tumor growth in methylcholanthrene-induced fibrosarcomas in Wistar rats. It was also active against P388 lymphocytic leukemia in vivo at a dose of 4 mg/kg of body weight but was not active against an L-1210 lymphoid leukemia (21). Shikonin is a well-known pigment from the root of *Lithospermum erythrorhizon* and has been used as a material to prepare an ointment which is used for the treatment of wounds and burns (18). It has been suggested that the antimicrobial activity of shikonin is one of the mechanisms responsible for the effects (18). In addition, Sankawa et al. (32) reported that shikonin has antitumor activity against a murine tumor model with intraperitoneally inoculated sarcoma 180 ascites cells at a dose of 5 to 10 mg/kg/day.

However, the critical biochemical targets of plumbagin and shikonin have yet to be identified. Here we presented data showing that the plant naphthoquinones plumbagin and shikonin induce topoisomerase II-mediated DNA cleavage. These topoisomerase II-active drugs showed significantly greater cytotoxicity in comparison with those of the non-topoisomerase II-active plant naphthoquinones lapacol and lawson. Although the relative topoisomerase II-mediated DNA cleavage activity did not correlate quantitatively with cytotoxicity among the topoisomerase II-active drugs, it is considered that permeability and metabolic inactivation in cells may be different among these compounds with different substituents (e.g., R_1, CH_3 and 4-oxo-2-hexeneyl; R_3 and R_4, OH). From these results, we suggest that topoisomerase II is an important cellular target of plumbagin and shikonin, leading to their antitumor and cytotoxic activities.

It has been reported that the mechanism of action of drugs targeting mammalian topoisomerase II is very similar to those of nalidixic acid and the related quinolone antibacterial agents which induce a DNA-cleavable complex with DNA gyrase, a bacterial topoisomerase II; both types of drugs trap the topoisomerase II-DNA-cleavable complex in very similar manners (3, 9, 30). In agreement with these common properties, it has been previously shown that VP16, which is an antitumor drug which enhances mammalian topoisomerase II-mediated DNA cleavage activity, is also a poison of bacterial DNA gyrase (22). Furthermore, several new derivatives of quinolone antibiotics have recently been shown to induce a DNA-cleavable complex with mammalian topoisomerase II and possess antitumor activities in vivo (39). Taking these findings together, it is likely that the antibacterial activities of plumbagin and shikonin might be mediated through the action of bacterial topoisomerases. This hypothesis should be determined in vitro by using purified DNA gyrase and the new, recently identified bacterial topoisomerases (8, 20, 33, 37).

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