UCE6, A NEW ANTITUMOR ANTIBIOTIC WITH TOPOISOMERASE I MEDIATED DNA CLEAVAGE ACTIVITY, FROM ACTINOMYCETES

Sir:

DNA topoisomerases are nuclear enzymes that catalyze the concerted breaking and rejoining of DNA strands, thereby controlling the topological states of DNA[1]. There is now good evidence showing that topoisomerase I is the principal intracellular target for clinically important antitumor drugs, camptothecin and its derivatives[2~4]. These drugs, referred as to "topoisomerase I poisons" interfere with the breakage-rejoining reaction of topoisomerase I by stabilizing a tight topoisomerase I-DNA complex termed "cleavable complex" which prevents the final rejoining step of the reaction. Exposure of this cleavable complex to a denaturant leads to the formation of DNA single strand breaks[5]. Several lines of evidence indicate that the ability to induce topoisomerase I mediated DNA cleavage is responsible for the antitumor activity of these drugs[2~5]. In order to identify a specific new topoisomerase I poison, we have screened cultures of actinomycetes and fungi for their ability to stabilize cleavable complex *in vitro*. We found that saintopin[6] and bulgarein[7] are potent inducers of topoisomerase I mediated DNA cleavege, and have now isolated UCE6, a novel compound with topoisomerase I mediated DNA cleavage activity, from a culture broth of actinomycetes.

The producing organism was isolated from a soil collected at Dohshi river in Yamanashi Prefecture, Japan. Fermentation was carried out at 28°C for 7 days under aeration and agitation in 30-liter jar fermentor containing 18 liters of a culture medium consisting of soluble starch 5%, soybean meal 1.5%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.05%, pH 7.0. The fermentation broth (18 liters) was filtered, and the mycelial cake was extracted with MeOH. The extract was diluted with an equal volume of deionized water and then applied to a column of Diaion HP-20 (Mitsubishi Chemical Industries Limited). The column was washed with deionized water-MeOH (1:9) and eluted with MeOH. The active eluate was concentrated and further purified by silica gel chromatography (LiChroprep Si60) with *n*-hexane-EtOAc-MeOH (2:2:1). The active fractions were combined, concentrated and precipitated by the addition of cold deionized water at 4°C to yield 38 mg of UCE6.

The physico-chemical properties of UCE6 are summarized in Table 1. UCE6 was obtained as a reddish orange powder, readily soluble in DMSO but insoluble in H_2O and $CHCl_3$. The molecular formula of UCE6 was determined as $C_{24}H_{20}O_8$ by HR-FABMS. The UV spectrum and the IR spectrum suggest the presence of a polyaromatic quinone structure. The structure of UCE6 (Fig. 1) was determined by ^1H and ^{13}C NMR spectroscopic studies. The structure determination and chemical properties of UCE6 will be reported in a separate paper[8].

The topoisomerase I mediated DNA cleavage activity of UCE6 was studied *in vitro* using purified calf thymus topoisomerase I and plasmid pUL402 DNA. Fig. 2 shows a photograph of agarose gel electrophoresis comparing the topoisomerase I mediated DNA cleavage activity of UCE6 with well

Table 1. Physico-chemical properties of UCE6.

Appearance	Reddish orange
Specific rotation	$[\alpha]_D^{22} = +350°$ (MeOH, *c* 0.0021)
Molecular formula	$C_{24}H_{20}O_8$
HR-FABMS (NBA):	
Obsd	437.1242 $(M+H)^+$
Calcd for $C_{24}H_{21}O_8$	437.1236
UV λ_{max}^{MeOH} nm (ε)	217 (23,500), 240 (25,300), 283 (26,100), 294 (25,300), 307 (27,600), 334 (14,800), 477 (15,000)
IR ν_{max}^{KBr} cm^{-1}	3361, 1695, 1655, 1603, 1441, 1377, 1323, 1277, 1217
Rf value[a]	0.61
Solubility:	
Soluble	DMSO, DMF, pyridine
Slightly soluble	MeOH
Insoluble	$CHCl_3$, EtOAc, *n*-hexane, H_2O

[a] Silica gel TLC (Merck 5715), *n*-hexane-EtOAc-MeOH (5:5:1).

Fig. 1. Structure of UCE6.

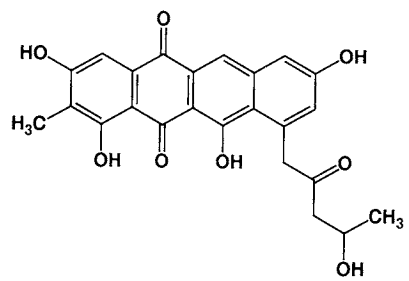
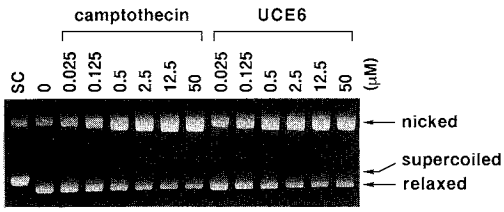


Fig. 2. Topoisomerase I mediated DNA cleavage activity of UCE6 and camptothecin.



Topoisomerase I was purified from calf thymus as described previously[5]. Stock solutions of drugs were dissolved in dimethyl sulfoxide at 50 mM, stored at −20°C and diluted in methanol containing 40% dimethyl sulfoxide before use. 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/ml of bovine serum albumin, 0.4 μg of pUL402 DNA, and DNA topoisomerase I with or without drug were incubated at 37°C. After 30 minutes, reactions were terminated by the addition of 2 μl of solution containing 5% SDS and 2.5 mg/ml of proteinase K. Following additional incubation at 37°C for 30 minutes, the sample were electrophoresed through a 1.2% agarose gel in 89 mM Tris-borate (pH 8.3), 2 mM EDTA, 0.1% SDS containing 0.5 μg/ml ethidium bromide. Lane SC: substrate supercoiled pUL402 DNA.

known topoisomerase I poison, camptothecin. As the concentration of UCE6 was increased, supercoiled DNA was converted to nicked DNA. The topoisomerase I mediated DNA cleavage activity of UCE6 was dose dependent and comparable to that of camptothecin. On the other hand, UCE6 did not interfere with the breakage-rejoining reaction of topoisomerase II using purified calf thymus topoisomerase II and plasmid pUL402 DNA *in vitro*, and also did not inhibit another enzyme that acts on DNA such as DNA ligase (*i.e.*, DNA ligation activity of T4 DNA ligase on linearized plasmid pBR 322 DNA) (data not shown). These results indicate that UCE6 selectively inhibit the breakage-rejoining reaction of topoisomerase I by stabilizing a cleavable complex. In addition, in the absence of topoisomerase I, UCE6 did not induce any changes on the supercoiled structure of pUL 402 DNA (data not shown).

UCE6 shows cytotoxic activity against a human tumor cell line, HeLa S3 (IC_{50} 0.018 μM) *in vitro*, which is comparable to that of camptothecin (IC_{50} 0.016 μM). UCE6 did not exhibit antimicrobial activity against either Gram-positive or Gram-negative bacteria (data not shown). Detailed studies on the mechanism of action and antitumor activity of UCE6 are in progress.

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