Communications to the Editor

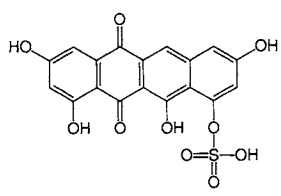
UCE1022, A NEW ANTITUMOR ANTIBIOTIC WITH TOPOISOMERASE I MEDIATED DNA CLEAVAGE ACTIVITY, FROM *Paecilomyces*

Sir:

Recent clinical studies of camptothecin derivatives such as CPT-11 and topotecan have demonstrated that these drugs have promising potentials as new antitumor agents[1]. The mechanism of action of these drugs is thought to depend on their ability to interact with the nuclear enzyme topoisomerase I[2,3]. Topoisomerase I catalyzes the passage of a DNA strand by concerted single-strand breaking and rejoining, thereby controlling the topological state of DNA[4]. Camptothecin and its derivatives, which are referred to topoisomerase I poisons, interrupt the breaking-rejoining cycle of topoisomerase I by stabilizing a covalent topoisomerase I-DNA complex termed "cleavable complex"[5]. Exposure of this cleavable complex to a denaturant leads to the formation of DNA single-strand breaks[5]. A number of studies have shown that the ability to stabilize cleavable complex is responsible for the antitumor activity of these drugs[6,7]. Hence, the identification of new drugs which induce cleavable complex with topoisomerase I is viewed as a promising approach to find clinically effective antitumor agents. In order to identify a 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[8], bulgarein[9] and UCE6[10] are potent inducer of topoisomerase I mediated DNA cleavage, and have now isolated from a culture broth of fungus UCE1022, a novel water-soluble compound with topoisomerase I mediated DNA cleavage activity.

The producing organism was isolated from soil collected in Koganei city, Tokyo, Japan and was assigned to the genus, *Paecilomyces* UOE1022 (FERM BP-4066). Fermentation was carried out at 25°C for 5 days under aeration and agitation in a 30-liter jar fermentor containing 18 liters of a culture medium consisting of soluble starch 5%, corn steep liquor 3%, 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. Along with the production of UCE1022, the structurally related antibiotic saintopin was also produced in a small quantity in this culture condition.

Fig. 1. Structure of UCE1022.



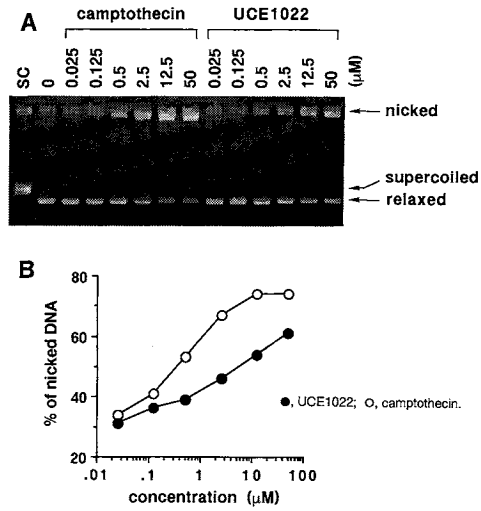
UCE1022 was isolated from the culture broth by the following steps. The fermentation broth (18 liters) was filtered, and the mycelial cake was extracted with MeOH (15 liters). The extract was diluted with deionized water (60 liters) and then applied to a column of Diaion HP-20 (1 liter) (Mitsubishi Chemical Industries Limited). The column was washed with deionized water - MeOH (7:3) and eluted with deionized water - MeOH (2:3). The active eluate was concentrated and further purified by silica gel chromatography (LiChroprep Si 60, 180 g) using EtOAc - MeOH - H_2O (3:2:1) as eluents. The active fractions were combined, concentrated and subjected to HPLC purification. HPLC was carried out on a packed column (YMC SH-343-5 S-5 120A AM ODS) and monitored at 254 nm. The mobile phase used was a mixture of MeOH and 10 mM ammonium acetate (1:1). Fractions containing UCE1022 were collected and concentrated to a small volume. To remove the salt, the concentrated solution was adsorbed on Diaion HP-20 column (0.2 liters) and eluted with

Table 1. Physico-chemical properties of UCE1022.

Appearance	Reddish purple powder
Molecular formula	$C_{18}H_{10}O_{10}S$
HRFAB-MS (NBA):	
Obsd	416.9919 $(M-H)^-$
Calcd for $C_{18}H_9O_{10}S$	416.9917
UV λ_{max}^{MeOH} nm (ε)	241 (22,000), 274 (27,500), 306 (16,100), 339 (9,800), 483 (12,300)
IR ν_{max}^{KBr} cm^{-1}	3360, 3210, 1625, 1600, 1400, 1330, 1265, 1235, 1045
Rf value[a]	0.25
Solubility:	
Soluble	H_2O, MeOH, EtOH
Slightly soluble	EtOAc
Insoluble	$CHCl_3$, *n*-hexane

[a] Silica gel TLC (Merck 5715), *n*-hexane - EtOAc - MeOH - AcOH (6:4:1:1, v/v).

Fig. 2. Topoisomerase I mediated DNA cleavage activity of UCE1022 and camptothecin.



In panel A, topoisomerase I was purified from calf thymus as described previously[5]. Stock solutions (25 mM) of camptothecin and UCE1022 were dissolved in dimethyl sulfoxide and H_2O respectively, stored at −20°C and diluted in methanol 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 pUL402 DNA. In panel B, the percentage of nicked DNA induced by drugs in the presence of topoisomerase I was determined by scanning the photograph negatives with a densitometer.

deionized water-MeOH (2:3) after washing with deionized water. The elute was lyophilized to yield 13.2 mg of UCE1022 as a reddish purple powder.

The physico-chemical properties of UCE1022 are summarized in Table 1. UCE1022 was readily soluble in H_2O, MeOH and EtOH but slightly soluble in EtOAc, insoluble in $CHCl_3$ and *n*-hexane. The UV spectrum and the IR spectrum of UCE1022 suggested similar structure to those of saintopin and UCE6[8,10]. The molecular formula of UCE1022 was determined as $C_{18}H_{10}O_{10}S$ by HRFAB-MS. The structure of UCE1022 (Fig. 1) was assigned to be (1,3,8,10,11-pentahydroxynaphtacene-5,12-dione 10-*O*-sulfate) by ^1H and ^{13}C NMR spectroscopic studies. The uniqueness of the structure of UCE1022 is that it contains a sulfate ester at the C-10 position, which is different from saintopin[8] and UCE6[10] as well as other topoisomerases poisons. The structure determination and chemical properties of UCE1022 will be reported in a separate paper[11].

The topoisomerase I mediated DNA cleavage activity of UCE1022 was compared with the well known topoisomerase I poison, camptothecin, using purified calf thymus topoisomerase I and plasmid pUL402 DNA *in vitro* (Fig. 2A). As the concentration of UCE1022 was increased, supercoiled DNA was converted to nicked DNA to a somewhat lesser extent than camptothecin. In the absence of topoisomerase I, UCE1022 did not induce any changes on the supercoiled structure of pUL402 DNA (data not shown). To obtain the quantitative data, the amount of nicked DNA was measured by scanning the negatives with densitometer (Fig. 2B). At a drug concentration of 2.5 μM, UCE1022 produced nicked DNA at a yield of 47% of substrate DNA, which was lower than that (67%) observed for camptothecin. The DNA cleavage activity of camptothecin reached a maximum and saturated at 12.5 μM, in contrast, the activity of UCE1022 increased dose dependently at concentrations above 12.5 μM. On the other hand, UCE1022 did not show any inhibitory activity against topoisomerase II, in the assay using purified calf thymus topoisomerase II and plasmid pUL402 DNA *in vitro*. In addition, UCE1022 did not inhibit DNA ligase, another enzyme that acts on DNA (*i.e.*, DNA ligation

activity of T4 DNA ligase on linearized plasmid pBR 322 DNA) (data not shown). These results indicate that UCE1022 selectively inhibits the breakage-rejoining reaction of topoisomerase I by stabilizing a cleavable complex.

UCE1022 shows cytotoxic activity against a human tumor cell line, HeLa S3 (IC_{50} 6.1 μM) *in vitro*. Detailed studies on the mechanism of action and antitumor activity of UCE1022 are in progress.

Acknowledgments

The authors are grateful to Ms. MITSUKO JITSUKAWA and Ms. MACHI KUSUNOKI for technical assistance.

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(Received March 25, 1994)

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