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Cystocin, a Novel Antibiotic, Produced by *Streptomyces sp.* GCA0001: Biological Activities

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Cystocin belongs to the class of nucleoside antibiotics from *Streptomyces sp.* GCA0001. Cystocin showed good activity against Gram-positive bacteria, but showed less activity against the Gram-negative bacteria. Cystocin exhibited about two to four folds higher activity than puromycin. Especially, cystocin shows relatively strong activity against *Streptococcus* strains. Cystocin shows quite potent antitumor activity against all of the cells tested showing IC_{50} values of 0.10 to 0.14 μg/mL. This *in vitro* result indicates that the cytotoxocity of cystocin is two ten folds more active than puromycin's.

Key words: Cystocin, 3'-[*S*-Methyl-cysteinyl]-3'-amino-3'-deoxy-*N*,*N*-dimethyladenosine, Puromycin, Antibiotic activity, *Streptomyces*

INTRODUCTION

Cystocin, 3'-[*S*-methyl-cysteinyl]-3'-amino-3'-deoxy-*N*, *N*-dimethyladenosine, is a new antibiotic compound identified from *Streptomyces* sp. GCA0001. Cystocin belongs to the class of nucleoside antibiotics. Its structure was identified by NMR study (Sohng, *et al.*, 2002). Cystocin is similar to puromycin, where the terminal tyrosine is replaced by a methyl cysteine. Puromycin consists of a nucleoside segment (3'-amino-3'-deoxy-*N*,*N*-dimethyladenosin) to which an amino acid segment (4-methoxy phenylalanine) is covalently linked through 3'-amino group of the ribose ring (Fig. 1). The structure verification of cystocin was performed by comparing the resulted puromycin nucleosides from the hydrolysis of cystocin and puromycin (Sohng, *et al.*, 2002). Puromycin is wellknown antibiotic, and interferes with protein synthesis at the stage of chain growth by competing with the aminoacyl-*t*RNA, since it is structurally similar to the aminoacyl-adenosyl terminal of aminoacyl-tRNA (Poster, *et. al.*, 1952; Harris, *et al.*, 1971; Herris, *et al.*, 1977). As a result, puromycin is being successfully used as an antibacterial, antiprotozoal, and anticancer agent. This article describes the antibacterial and anticancer activity of cystocin.

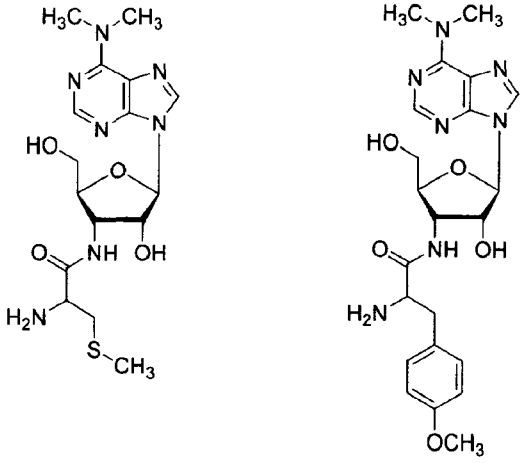

Fig. 1. The chemical structure of cystocin

MATERIALS AND METHODS

Materials

All the chemical used was analytical grade from Daejung Chemicals and Metals Company (Korea) and the medium components employed were purchased from Beckton Dickinson and Co. (USA).

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Microorganism

Streptomyces sp. GCA0001 was isolated from soil samples around Sunmoon University, Korea, and maintained on R_2YE agar (an accession number of KCTC0930BP) (Kieser, *et al.*, 2000).

Fermentation and isolation

Spores of *Streptomyces* sp. GCA0001 were inoculated into three 500 mL flasks containing 150 mL of seed medium (yeast extract 0.75 g, beef extract 0.45 g, tryptone 0.75 g, soluble starch 3.6 g, dextrose 0.75 g, calcium carbonate 0.6 g were added to 100 mL of water), and cultured on the rotary shaker with an agitation speed of 250 rpm for 3 days at 29 °C. To obtain enough material for analysis, fermentation was performed in a 14 L fermenter at 29 for 4 days with an aeration rate of 3.0 v/v/m and an agitation speed of 300 rpm, with 450 mL of the above solution as an inoculum. The fermentation medium consisted of: bacto-soyton 315 g, dextrin 450 g, calcium carbonate 63 g, cobalt chloride 0.216 g, and water to make a 9 L solution. The pH was adjusted to 7.4 prior to sterilization using 1.0 M HCl aqueous solution. The fermentation broth was centrifuged, and supernatant solution containing cystocin was extracted with chloroform repeatedly. This solution was dried to solid in vacuum, and $CHCl_3$:CH_3OH (9:1, v/v) solution of resulting residue was applied to silica gel column (silica 60, Merck) using the same $CHCl_3$-CH_3OH mixture as an eluent. To obtain the pure material, several applications to silica gel column were performed by changing the ratio of $CHCl_3$-CH_3OH mixture (Kharel, *et. al.*, 2002).

Antibacterial activities

MICs were determined by an agar dilution method. Cystocin and standard antibiotics (puromycin and ciprofloxacin) were solubilized in DMSO and diluted to autoclaved water. Final concentrations of the antibiotics were tested in the range 100-0.002 μg/mL. The MIC tests were carried out by Korea Research Institute of Chemical Technology (Daejeon, Korea).

SRB assay

The antitumor activities were examined by sulforhodamine B assay (SRB). The cell lines employed were A549 (human non-small cell lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF-498 (human CNS) and HCT-15 (human colon). Cystocin and puromycin were tested in the range 0.0001 μM-100 μM by 10 times dilution along with doxorubicin (stock solution: 2 mM in DMSO) as standard antitumor compounds. The SRB assay was carried out by Korea Research Institute of Chemical Technology (Daejeon, Korea).

RESULTS AND DISCUSSION

Antibacterial activities *in vitro*

The MICs of cystocin, puromycin and ciprofloxacin against 20 strains of Gram-positive and Gram-negative bacteria are shown in Table I. Cystocin showed good activity against Gram-positive bacteria, but showed less activity against the Gram-negative bacteria including *Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca* and *Enterobactor cloacae*. Cystocin exhibited about two to four folds higher activity than puromycin. Especially, cystocin shows relatively strong activity against *Streptococcus* strains (MIC: 3-6 μg/mL). But the antibacterial activities of cystocin and puromycin were not as powerful as that of ciprofloxacin.

Antitumor activities *in vitro*

The cytotoxic activity of cystocin was examined with five cell lines by SRB assay. Puromycin and doxorubicin were tested comparatively as reference compounds. Cystocin shows quite potent antitumor activity against all of the cells tested showing IC_{50} values of 0.10 to 0.14 μg/mL (Table II). This *in vitro* result indicates that the cytotoxocity of cystocin is two-ten folds more active than puromycin's and also comparable with that of doxorubicin.

Table I. Antibacterial activities *in vitro* of cystocin, puromycin and ciprofloxacin

Organism	MIC (μg/mL)		
	Cystocine	Puromycin	Ciprofloxacin
Streptococcus pyogenes 308A	3.125	6.250	3.125
Streptococcus pyogenes 77A	3.125	6.250	0.391
Streptococcus faecium MD8b	6.250	6.250	0.391
Staphylococcus aureus SG511	6.250	6.250	0.195
Staphylococcus aureus 285	6.250	12.50	0.781
Staphylococcus aureus 503	6.250	6.250	0.391
Escherichia coli 0786.250	12.50	25.00	0.004
Escherichia coli DC0	50.00	100.0	0.195
Escherichia coli DC2	1.563	1.563	0.098
Escherichia coli TEM	50.00	>100	0.013
Escherichia coli 1507 E	50.00	>100	0.013
Pseudomonas aeruginosa 9027	>100	>100	0.195
Pseudomonas aeruginosa 1592E	>100	>100	0.195
Pseudomonas aeruginosa 1771	>100	>100	0.195
Pseudomonas aeruginosa 1771M	25.00	100.0	0.049
Salmonella typhimurium	25.00	50.00	0.007
Klebsiella oxytoca 1082E	3.125	3.125	<0.002
Klebsiella aerogenes 1522E	100.0	100.0	0.013
Enterobacter cloacae P99	50.00	>100	0.013
Enterobacter cloacae 1321E	25.00	25.00	0.004

Table II. Antitumor activities *in vitro* of cystocin, puromycin and doxorubicin

Compounds	IC_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF-498	HCT-15
Cystocin	0.14	0.10	0.11	0.13	0.14
Puromycin	0.31	0.38	0.16	0.28	1.08
Doxorubicin	0.01	0.038	0.107	0.098	0.091

Puromycin is an aminoacyl nucleoside, which is structurally similar to the 3'-terminal of aminoacyl-*t*-RNA (Suhandolink, 1970; Suhandolink, 1979). It is broad spectrum antibiotic with antitumor activity that results from inhibition of protein synthesis. It is well recognized that puromycin takes the place of aminoacyl-*t*-RNA site on the ribosome and thereby interrupting the growth of the polypeptide chain by forming peptidyl puromycin (Herris, *et al.*, 1977). It has already been proved that the aromatic aminoacyl residue linked through 3'-amino group of puromycin ribose ring is not necessary for the biological activity (Fong, *et al.*, 1978). Cystocin is different only in amino acid segment (*S*-methyl-cysteinyl) in comparison with puromycin and, the antibacterial and antitumor activities of cystocin were found to be similar or two-ten folds more than puromycin's. This gives the possibility on the further modification on the amino acid segment for better anti-bacterial and anti-tumor activities.

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