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Antibacterial Activity of Usnic acid against *Staphylococcus aureus*

[1]National Institute of Horticultural & Herbal Science, RDA
[2]College of Pharmacy and Wonkwang-Oriental Medicines Research Institute, Wonkang University
Sin-Hee Han[1], Young-Seob Lee, Su-Hwan Lee[1], Young-Guk Kim[1], Chung-Berm Park[1]
Su-Hyun Mun[2], Sung-Bae Kim[2], Joon-Ho Keum[2], Ok-Hwa Kang[2], Dong-Yeul Kwon[2]

황색포도상구균 (*Staphylococcus aureus*) 에 대한 Usinc acid 의 항균활성

농촌진흥청 : 한신희*, 이영섭, 이수환, 김영국, 박충범
원광대학교 : 문수현, 김성배, 금준호, 강옥화, 권동렬

Objectives

Methicillin-resistant *Staphylococcus aureus* (MRSA) along with other resistant bacteria have become a big social and clinical problem. Thus, Discovery and development of bioactive compounds from natural products agents as alternatives to the very few antibiotics, need to be done urgently required. The aim of this work is to evaluate the relationship between membrane permeability and ABC transporter-inhibiting effect in order to gain more insight into the mechanism of *S. aureus*.

Materials and Methods

Bacterial strains : Isolates used in this study included two American Type Culture Collection (ATCC) strains; ATCC 25923 and ATCC 33591. The clinical isolates of bacterial strains were obtained from Wonkwang University Hospital (Iksan, Korea).

Determination of antibacterial activity by the disc diffusion method : Sterile paper discs were loaded with 20 µL of UA (varying concentrations: 10 and 100 µg/disc) dissolved in DMSO. The bacterial suspensions were diluted to match the 0.5 McFarland standard scale, and were further diluted to obtain the final inoculum. The MHA was poured into Petri dishes and inoculated with 100 µL of the suspension containing 1.5×10^8 cfu/mL of bacteria. The inhibition zone diameter around each of the discs was measured and recorded at the end of the incubation period.

Minimum inhibitory concentrations (MICs) value determination assay : The MICs value determinations against the *S. aureus* were undertaken for the UA in double-broth microdilution method involving 96-well microtitre plates. Serial twofold dilutions of the UA, dissolved in DMSO dissolved in MH Broth were prepared in test

주저자 연락처 (Corresponding author) : Sin-Hee Han, E-mail : herbman@korea.kr Tel : 043-871-5545

tubes prior to the addition overnight microbial suspension (10^8 CFU/ml) followed by incubation at 37°C for 24 h.

Membrane permeability or ATPase-inhibitors assay : To elucidate whether antibacterial activity of UA was associated with the altered membrane permeability, antibacterial susceptibility of UA was examined in the presence of detergents or ATPase-inhibiting agents. To increase the permeability of the membrane, the concentration of UA ¼ MIC, as a fractional inhibitory concentration (FIC) determined in a combination assay with other therapeutic agents, was added to bacterial cells in the presence of 0.19 mM EDTA. NaN_3 were used as a inhibitor of ATPase. The antibacterial susceptibility of UA, in the presence of 0.00125% NaN_3 was also carried out at the same condition.

Results

Table 1. The disc diffusion test of UA against *S.aureus* strain.

Strain		Inhibition zone(mm)	
		10μg	100μg
MSSA	ATCC 25923	13	17
MRSA	ATCC 33591	12	16
	DPS 1	17	20
	DPS 2	13	15
	DPS 3	15	17
	|	|	|
	DPS 15	14	15

Table 2. The MICs of UA and MIN against *S.aureus* strain.

Strain		MIC (μg/mL)	
		UA	Minocycline
MSSA	ATCC 25923	15.62	0.9
MRSA	ATCC 33591	15.62	31.25
	DPS 1	7.8	15.62
	DPS 2	31.25	15.62
	DPS 3	15.62	31.25
	|	|	|
	DPS 15	31.25	15.62

FIG. Membrane permeability or ATPase-inhibitors assy