P-85

Antibacterial Activity of Tetrnadrine and Synergy Effect with Ethidium bromide against Multiple-Resistant *Staphylococcusaureus*

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

다재내성 포도상구균에 대한 Tetrandrine의 항균활성과 Ethidium bromide 와의 상승효과

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

Objectives

Multiple-resistant *Staphylococcus aureus* (MRSA) along with other resistant bacteria have become a big social and clinical problem. Recently, efflux mechanism has been generally known as the main contributor of resistance to antibiotics. This study therefore aimed to evaluate tetrandrine (TET) as potential antibiotic agents or potential 'multidrug efflux pump inhibitors' (EPIs) against MRSA.

Materials and Methods

The following materials were purchased from commercial sources: tetrandrine (TET); ampicillin (AMP); oxacillin (OXA) were from Sigma-Aldrich.

Bacterial strains : Isolates used in this study included two American Type Culture Collection (ATCC) strains; four Culture Collection of Antimicrobial Resistant Microbes at Seoul Women's University in Korea (CCARM) strains; KWMrI 1039 to KWMrI 1053. 15 clinical isolates were obtained from 15 different unique patients at Wonkwang University Hospital (Iksan, Korea).

Determination of Minimum Inhibitory Concentrations (MICs) and Combination Effect : The MICs assay was performed according to the method described by Saiful *et al* and Mohtar *et al*. The MICs value determinations against the 21 *S. aureus* isolates were undertaken for the standard efflux substrate (ethidium bromide; EtBr) and TET in double-broth microdilution method. Combinations of TET with antibiotics were investigated as previously described. Susceptibility tests were carried out in 96-well microtiter plates by a two-fold standard broth microdilution, with the levels of two antibacterial agents in MH Broth.

Time-Kill Assay : It was performed according to the method described by YuJie Fu *et al* and Choi *et al*. For this assays, final concentration of suspension of the strains was adjusted to approximately 10^6 cfu/ml of an overnight culture was used. The time and extent of killing curve was determined by plotting viable colony counts (cfu/ml) against time in MH agar.

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

Results

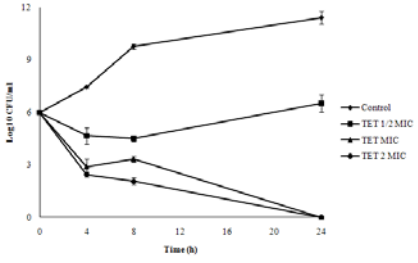
The results of the MICs and Efflux assay are as shown in Table 1 and 2. TET showed a similar level of MICs in each strains. For this assay, all the tested pathogenic organisms showed activity by reducing TET and EtBr MICs value by 2 to 4 fold.

Strains	TET	AMP	OXA
ATCC 25923	250	0.9	1.9
ATCC 33591	250	62.5	> 250
CCARM 3090	250	31.2	> 250
CCARM 3091	125	31.2	> 250
CCARM 3095	250	125	125
CCARM 3102	125	> 250	> 250
KWMrI 1039	250	31.25	> 250
KWMrI 1040	250	31.25	> 250
⋮	⋮	⋮	⋮
KWMrI 1041	250	31.25	> 250
KWMrI 1050	250	125	> 250
KWMri 1051	125	125	> 250
KWMrI 1052	250	62.5	> 250
KWMri 1053	125	125	> 250

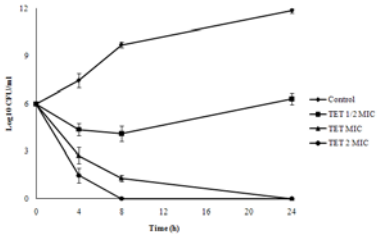
Strain	Alone	Combination			
	EtBr	Tetrandrine		EtBr	
ATCC 25923	31.2	62.5	(4)	7.8	(4)
ATCC 33951	15.6	125	(2)	7.8	(2)
CCARM 3090	7.8	62.5	(4)	3.9	(2)
CCARM 3091	15.6	31.2	(4)	7.8	(2)
CCARM 3095	31.2	125	(2)	7.8	(4)
CCARM 3102	15.6	62.5	(2)	7.8	(2)
KWMrI 1039	7.8	62.5	(4)	3.9	(2)
KWMrI 1040	31.2	62.5	(4)	7.8	(4)
KWMrI 1041	7.8	62.5	(4)	3.9	(2)
⋮	⋮	⋮		⋮	
KWMrI 1050	7.8	62.5	(4)	3.9	(2)
KWMrI 1051	31.2	62.5	(2)	15.6	(2)
KWMrI 1052	15.6	62.5	(4)	7.8	(2)
KWMrI 1053	31.2	31.2	(4)	15.6	(2)

DISCUSSION

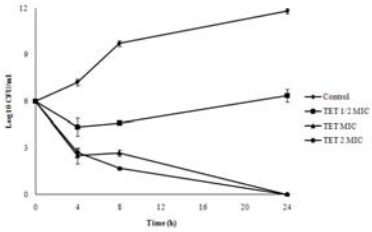
The presence of TET resulted in a four-fold reduction in EtBr MICs for all strains possessing efflux-related resistance. EtBr is a planar molecule, and TET potentiated its action against the *S. aureus* strain, suggesting that an additional MDR can be involved in this case as well. So combination of a TET with antimicrobial agent that are known pump substrates could reduce the morbidity and mortality shown in many countries due to the seriousness of *S. aureus* infections. There are several mechanisms that can resist antibiotic and toxic molecules in bacteria, and drug efflux transporters as MDR pumps are capable of such antibiotic resistance, because they selectively pump toxic molecules out to the extracellular condition by keeping toxic molecules to a minimum in an intracellular condition. The results of the present study appear to be promising, and may enhance the use of natural products as drugs.



Time-kill curves for KWMrI1039 of *S. aureus* using tetrandrine(TET)



Time-kill curves for ATCC 25923 of *S. aureus* using



Time-kill curves for ATCC 33591 of *S. aureus* using tetrandrine(TET).