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Synergistic antibacterial effect of tetrandrine and cell-wall permeable agents against *Staphylococcus aureus*

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황색포도상구균에 대한 Tetrandrine과 세포벽투과성약제의 상승적 항균효과

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원광대학교 : 권동렬, 강옥화, 김성배, 금준호, 문수현

Objectives

Tetrandrine (TET) is a bis-benzylisoquinoline alkaloid that is the main active component in the root of *Stephania tetrandra* S. Moore of the Menispermaceae family, which has been used traditionally for the treatment of arthritis, silicosis and hypertension in Asia including Korea and China. We hypothesized that direct effects of TET on bacterial cell wall might be responsible for the synergistic activity from the combination of TET and many agents against *S. aureus*.

Materials and Methods

The following materials were purchased from commercial sources: Tetrandrine (TET); Ampicillin (AMP); Oxacillin (OXA); N,N´-Dicyclohexylcarbodiimide (DCCD); Sodium azide (NaN3); Triton X-100; Ethylenediaminetetraacetic acid (EDTA); lipopolysaccharide (LPS) ; peptidoglycan (PGN) from Sigma-Aldrich; Tris-(hydroxymethyl) aminomethane (TRIS) were from Amresco (San Francisco, CA).

Bacterial strains and growth conditions : 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); KWCAM 113095 to KWCAM 123091.

Minimum inhibitory concentrations (MICs) value determination assay : The MICs assay was performed according to the method described by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009), Saiful *et al* (2008) and Mohtar *et al* (2009).

Antibacterial assay with membrane permeability assay : To elucidate whether antibacterial activity of TET was associated with the altered membrane permeability, antibacterial susceptibility of TET was examined in the presence of detergents. To increase the permeability of the membrane, the concentration of TET ¼ MIC, as a fractional inhibitory concentration (FIC) determined in a combination assay with other therapeutic agents, was added to bacterial cells. NaN_3 and DCCD were used as a inhibitor of ATPase.

Combination assay between TET and peptidoglycan : This combination assayed included TET plus PGN. This was confirmed by adding PGN in MHB. Serial dilutions of the PGN were mixed in 1/2 MIC of TET. For the controls, we added the LPS to the cultures.

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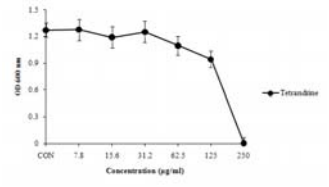
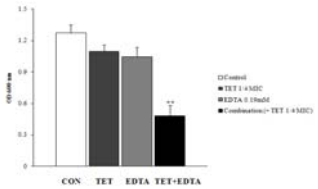
Results

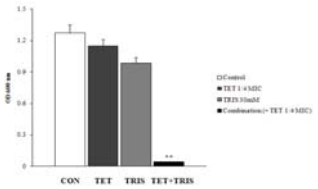
To investigate the effects of enhanced membrane permeability on the activity of TET, the anti-bacterial activity of TET under increased membrane permeability was examined using EDTA, TRIS and TX. The *S. aureus* showed an increasing susceptibility toward TET by 3 permeability agent. TET may directly bind to the *S. aureus* cell wall and interfere with its integrity. PGN blocked the antibacterial activity of TET.

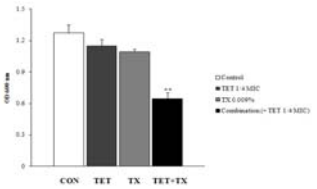
Table 1. Antibacterial activity of TET, AMP and OXA against *S. aureus* (μg/ml).

Bacteria Strains	Tetrandrine	Ampicillin	Oxacillin
ATCC 25923	250	0.9	1.9
ATCC 33591	250	62.5	> 250
KWCAM 113095	250	125	125
KWCAM 113102	125	> 250	> 250
KWCAM 123090	250	31.2	> 250
KWCAM 123091	125	31.2	> 250





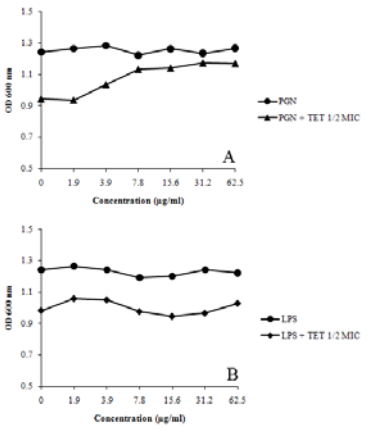




Direct binding of TET with peptidoglycan (PGN) or lipopolysaccharide (LPS) of the cell wall.

PGN from *S. aureus* was added to MH broth containing TET only (A) The same amount of LPS was used as a control (B). ●, TET (125 μg/ml) only; ▲, PGN (0 to 62.5 μg/ml) + TET (125 μg/ml); ◆, LPS (0 to 62.5 μg/ml) + TET (125 μg/ml).

DISCUSSION



There was a clear synergistic effect against strains of *S. aureus* from three cases. It is believed that the metal chelator sets metal ions apart from the membrane, resulting in PGN (or LPS) and protein dissociation which lead to cell lysis. These results indicate damage of the cell wall by TET. The blocking of the antibacterial activity of TET and the synergistic effect of TET and PGN against *S. aureus* demonstrated the direct binding of TET with PGN. TET may synergize the activity of β-lactams mainly because both TET and β-lactams directly or indirectly attack the same site, PGN on the cell wall. The TET-induced damage of the bacterial cell wall and the possible interference with its biosynthesis through direct binding with PGN may be the major reasons for the synergism against *S. aureus*.