Synopsis of the Ph. D. Thesis

Antimicrobial and Anti-apoptotic Role of Nanoconjugated Vancomycin against Drug Resistant *Staphylococcus aureus*

By

Subhankari Prasad Chakraborty

M. Sc. in Human Physiology

Department of Human Physiology with Community Health

Vidyasagar University

Midnapore, Paschim Medinipur

West Bengal, India

Pin-721 102

2012

Staphylococcus aureus is most frequently isolated pathogen causing pneumonia, bloodstream infections, skin and soft tissue infections and surgical wound infections with prevalence rate ranging from 4.6% - 54.4%. *S. aureus* have evolved resistance to both synthetic and traditional antibiotics. More than 90% *S. aureus* strains are resistant to penicillin. Methicillin, a semi synthetic penicillin was used to treat Penicillin Resistant *Staphylococcus aureus* but resistance finally emerge in 1962. Vancomycin, a glycopeptide antibiotic continues to be an important antimicrobial agent to treat MRSA but resistance finally emerges in 2002 in a patient in the USA. The mounting problem of antibiotic resistance of *S. aureus* has prompted renewed efforts toward the discovery of novel antimicrobial agents.

The present work is aimed to identify Vancomycin Sensitive *Staphylococcus aureus* (VSSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) from post operative pus sample. VSSA and VRSA infection induced redox signaling in lymphocytes and as well as inflammatory response and apoptosis in T-lymphocytes were also studied. Concurrently, we have approached nanoconjugated vancomycin as an alternative remedies against it. CMC-EDBE-FA was synthesized via reaction of the carboxyl group of carboxymethyl chitosan with the primary amine group of FA-EDBE in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Vancomycin was loaded onto it, the complex is called nanoconjugated vancomycin, and the successful binding was confirmed by DLS and TEM study. Different antimicrobial test and biochemical estimation revealed that, CMC-EDBE-FA nanoparticles had no antimicrobial activity, *in vitro* and *in vivo* cytotoxicity. Nanoconjugated vancomycin exerts *in vitro* antimicrobial activity against VSSA and VRSA strains as evidenced by decreased MIC and MBC value, tolerance level, biofilm formation, cell viability, cell wall thickness and as well as increased membrane Na^+, K^+-ATPase activity, intracellular potassium released and leakage of cellular constituents.

To examine the VSSA and VRSA-induced oxidative stress in lymphocytes, we have measured viable bacteria count in blood, some serum inflammatory markers such as C-reactive protein, myeloperoxidase activity, nitrite release, level of TNF-α and IL-10, and superoxide generation, nitrite generation, lipid peroxidation, protein oxidation, glutathione system and different cellular antioxidants status after 3, 5, 10, 15 days of in vivo VSSA and VRSA challenge (5×10^6 CFU/mL). Nitrite generation, lipid peroxidation,

protein oxidation, glutathione system and antioxidant status were measured in T-lymphocytes of VSSA and VRSA infected mice. To evaluate the alteration of inflammatory response in VSSA and VRSA infected T-lymphocytes nitric oxide (NO) generation, iNOS2 expression level in mRNA level, Th1 cytokines (TNF-α, IL-12) and Th2 cytokines (IL-10 and TGF-β) release in serum as well as expressions in mRNA level were studied. Cell viability and DNA fragmentation was assessed as indicator of apoptosis. To examine the involvement of NO generation in apoptosis, the expression of TNF-α, caspase-8, pro-caspase-3 in T-lymphocytes were carried out. The expression of stress protein (p38MAPK) and cell survival proteins (pAkt) were also carried out in VSSA and VRSA infected T-lymphocytes.

The present study clearly established that, in vivo VSSA and VRSA infection can induce maximum alteration of serum inflammatory markers and oxidative damage in lymphocytes. We have found evidenced by enhanced CRP level, MPO activity, release of TNF-α, NO and diminished release of IL-10, and as well as enhanced NO generation, lipid peroxidation, protein oxidation, and diminished cellular antioxidant defense system. Although, NO generation and iNOS expression in mRNA level were increased significantly ($P<0.05$) in VSSA and VRSA infected T-lymphocytes. Beside that, up regulated pro-inflammatory (Th1) cytokines (TNF-α ,and IL-12) with concomitant down regulated anti-inflammatory (Th2) cytokines (TGF-β, and IL-10) were observed in VSSA and VRSA infected T-lymphocytes, as a result the balance of Th1/Th2 cytokine was shifted towards the Th1 response. Our results indicated that, VSSA and VRSA infection decreased the cell viability and increased the DNA fragmentation. Over expressed TNF-α, caspase-8, no changes of caspase-9 expression and decreased level of pro-caspase 3 indicated apoptosis of VSSA and VRSA infected T-lymphocytes. Hence, the apoptosis pathway is through the extrinsic pathway (mitochondria independent). Moreover, VSSA and VRSA infection activated the p38MAPK, and down regulated the pAkt level. We have also studied to reduce toxicity by treatment of vancomycin and nanoconjugated vancomycin at a dose of 100 mg/kg bw/day and 500 mg/kg bw/day in VSSA and VRSA infected mice, respectively. Our results also clearly indicated that, VSSA and VRSA infection-induced excess radical generation, lipid and protein damage, were significantly *($P<0.05$)* reduced by nanoconjugated vancomycin treatment. Antioxidant status was also effectively restored after nanoconjugated vancomycin treatment. Moreover, in VSSA and VRSA infected mice NO generation was effectively modulated and Th1/Th2 cytokines

regulation were shifted towards Th2 response due to treatment of nanoconjugated vancomycin. Nanoconjugated vancomycin treatment also increased the cell viability and rationalized the DNA fragmentation in VSSA and VRSA infected T-lymphocytes. Moreover, nanoconjugated vancomycin treatment down regulated the p38MAPK, TNF-α expression, caspase-8 expression, as a result suppressed the pro-caspase 3 activation, and concurrently, up-regulated the pAkt expression during VSSA and VRSA infection. Vancomycin treatment exhibited protective role against only in Vancomycin Sensitive *Staphylococcus aureus* (VSSA) infection but not against Vancomycin Resistant *Staphylococcus aureus* (VRSA) infection.

Hence, these findings suggested that, VSSA and VRSA infection can induce the oxidative stress, inflammation, and ultimately provoked to apoptosis through TNF-α dependent pathway in T-lymphocytes. It may be suggested that the use of nanoconjugated vancomycin acts as a modulator and reduce the immune cell damage, which may be used as potential therapeutic agent against staphylococcal infection.