



**Karolinska
Institutet**

Institutionen för Mikrobiologi, Tumör och Cellbiologi (MTC)

***Staphylococcus aureus* α -toxin – natural
fragments and effects on intestinal epithelial cells**

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
Institutet offentligen försvaras i
MTC:s föreläsningssal, Theorellsväg 1, Solna

Torsdagen den 16 Juni, 2011, kl 09.30

av

Young-Keun Kwak

M. Eng.

Huvudhandledare:

Prof. Roland Möllby
Karolinska Institutet
Institutionen för Mikrobiologi,
Tumör och Cellbiologi (MTC)

Bihandledare:

Med. Dr. Beatrix Vécsey-Semjén
Kungliga Tekniska Högskolan
Skolan för teknikvetenskaplig
kommunikation och lärande

Fakultetsopponent:

Docent Cecilia Rydén
Uppsala Universitet
Institutionen för Infektionssjukdomar och
medicinsk biokemi och mikrobiologi

Betygsnämnd:

Docent Olof Hultgren
Örebro Universitet
Mikrobiologi/Laboratoriemedicinska
Länskliniken

Docent Mats Andersson
Karolinska Institutet
Universitetsförvaltningen (UF)

Docent Karin Artursson
Sveriges Lantbruksuniversitet
Statens Veterinärmedicinska Anstalt

ABSTRACT

Staphylococcus aureus is a human pathogen, commonly found in healthy humans as a part of normal flora. α -toxin is thought to be largely responsible for the pathogenesis of the bacterium. This cytotoxin has been shown to irreversibly damage the membrane of a great variety of cells including erythrocytes, endothelial cells, and mouse adrenocortical cells. α -toxin is secreted as a water soluble monomer that binds to mammalian cell membranes, oligomerizes and forms a heptameric transmembrane pore. Pore formation leads to osmotic shock and cell death.

One of the aims of the present work was to understand the biological relevance of four naturally occurring α -toxin fragments isolated from *S. aureus* culture medium. All four fragments bound to and formed transmembrane channels in egg-phosphatidyl glycerol vesicles. Oligomer formation on the lipid membrane was a prerequisite of channel formation. Interestingly, alleviated hemolytic activity was partially recovered by acidification of the medium. We have demonstrated that some toxin fragments can be proteolytically generated from intact staphylococcal α -toxin by *S. aureus* extracellular co-expressed proteases. All isolated fragments induced intoxication of mouse adrenocortical Y1 cells *in vitro*. Only one fragment, missing the first eight N-terminal amino acids, induced irreversible intoxication of Y1 cells in the same manner as the intact toxin.

After we established that α -toxin fragments indeed were biologically active, we demonstrated that α -toxin treatment induced loss of junctional proteins (ZO-1, ZO-3, E-cadherin, and occludin) in human intestinal Caco-2 cells. Surprisingly, when α -toxin was applied from the basolateral side of the model epithelium the trans-epithelial resistance (TER) decreased significantly in a dose- and time-dependent manner; while no significant changes in TER were induced by application from the apical side. To investigate the influence of α -toxin on calcium homeostasis of Caco-2 cells, we measured $[Ca^{2+}]_i$ in calcium (1 mM) containing and calcium free buffer. $[Ca^{2+}]_i$ was significantly increased about 10 min after the addition of α -toxin, whereas no significant signal increase was observed in calcium free buffer. Our result shows a positive correlation between an increase in intracellular $[Ca^{2+}]$ and epithelial barrier function and suggests that α -toxin treatment might be involved in maintaining TER against external stimuli. Our finding gives a possible explanation of how bacteria disseminates in the blood-stream of sepsis patients: in *S. aureus*-associated sepsis, the membrane damaging α -toxin may circulate in the blood and affect the intestinal barrier, resulting in translocation of enteric bacteria. This might lead to the spread of endotoxin (LPS) in the blood and further aggravate sepsis syndrome.

In order to prove this hypothesis we performed animal experiments in order to determine whether bacterial translocation across the intestinal epithelium can be induced by intravenous application of α -toxin. The present work indicates a strong correlation between intestinal hyperpermeability and bacterial translocation and suggests that α -toxin may thus aggravate the septic condition. Demonstrating α -toxin involvement in serious inflammatory disease syndrome, caused by a vicious circle of *E. coli* endotoxemia and bacterial translocation, will be a challenge for the future.