Photodynamic inactivation of multidrug-resistant *Staphylococcus aureus* and reduction of its virulence using novel gallium(III)-coordinated porphyrins

MSc Klaudia Szymczak

Staphylococcus aureus is a Gram-positive pathogen of the ESKAPE group, responsible for about 80% of all skin infections. Its virulence consists of factors such as toxin production, biofilm formation and the ability to internalize into host cells. The intracellular survival of *S. aureus* in host cells is associated with recurrent infections, which contribute to therapeutic failures. Due to low penetration, antibiotics are not highly effective against intracellular *S. aureus*. Alternative antimicrobial therapies are currently being sought that can effectively reduce the intracellular bacterial reservoir. One therapeutic option may be antimicrobial photodynamic inactivation (aPDI), based on a chemical compound, known as a photosensitizer, which is excited when exposed to light of a given wavelength in an aerobic environment. As a result of the three components action, reactive oxygen species are generated, which contribute to bacterial death by damaging cellular biomolecules such as proteins, DNA and lipids. Gallium(III)-coordinated porphyrins (Ga^{3+}MPs) are dual-functional compounds, i.e. they exhibit the photodynamic properties of a photosensitizer in the light-dependent pathway, while in the light-independent pathway they block iron-dependent metabolism by mimicking the structure of the natural ligand – heme. Ga^{3+}MPs are heme analogues and can thus be recognized by specific receptors for heme from the iron surface determinant (Isd) system. Once accumulated in the bacterial interior, gallium ions are released from the porphyrin ring, interfering with iron ion-dependent metabolic pathways.

This dissertation focuses on investigating the effectiveness of aPDIs against *S. aureus* based on the use of novel photosensitizing compounds - gallium (III)-coordinated porphyrins. The aim of the study is to assess whether excitation of Ga^{3+}MPs with green light leads to a significant reduction in the activity of virulence factors such as toxins, biofilm and intracellular survival of *S. aureus*. The effectiveness of aPDI was tested on the following models: planktonic cultures; *in vitro* and *ex vivo* biofilm (porcine skin model), and an infection model of human keratinocytes. On the infection model, three strategies for implementing the photodynamic method were proposed to study: I) the ability to eliminate bacteria released from the host, II) the effect on adherence and internalization III) the effectiveness of aPDI in reducing intracellular *S. aureus* load.

The results presented in this dissertation indicate that aPDI has high antimicrobial efficacy against *S. aureus* in suspension cultures, as well as on biofilm models. It was shown that aPDI effectively inactivates the biological functionality of important virulence factors, such as staphylococcal enterotoxin C and toxic shock toxin TSST-1. The photodynamic inactivation can be an effective method for eliminating bacteria released from the host. Pretreatment with aPDI contributes to a significant reduction in bacterial adherence to the host, but without a significant effect on internalization. The results also showed that aPDI effectively decreases GFP signal derived from intracellular *S. aureus*. This is the first study that presents the efficacy of novel Ga^{3+}MPs combined with green light in reducing the intracellular *S. aureus*. This significantly extends the therapeutic potential of the Ga^{3+}MPs-mediated photodynamic method against recurrent staphylococcal infections.