Abstract

Streptococcus agalactiae – the most common representative of Group B *Streptococcus* (GBS) despite being a common commensal bacterium in adults, is a leading source of invasive infections in newborns, but recently also in pregnant women, the elderly and immune-compromised patients. GBS infections may appear as a wide spectrum of diseases like: bacteremia, sepsis, pneumonia, urinary tract infections, acute and chronic osteomyelitis and others. First-line antimicrobials against GBS are β-lactam antibiotics. With the rising decrease of microbial susceptibility to penicillin and resistance to other antibiotics, there is a huge need for alternative therapies. Antimicrobial Photodynamic Inactivation (aPDI) is one of the proposed therapies which provide an alternative solution for antibiotic resistance in bacteria. It requires the simultaneous presence of photosensitizer (PS), light of a specific wavelength and environmental oxygen. As a results, there are generated Reactive Oxygen Species (ROS), which cause damage in cellular membranes, proteins and nucleic acids, and finally, lead to bacterial cell death.

The aim of the study was the evaluation of aPDI bactericidal activity against *S. agalactiae* serotypes and representatives of physiological flora of the human vagina, evaluation of photo- and cytotoxicity of aPDI to human keratinocytes, creation of the extended logistic model of aPDI for various levels of irradiance, evaluation of the possibility of aPDI resistance/tolerance development, determination of the mutagenic potential of aPDI and finally evaluation of aPDI potential in the decolonization of *S. agalactiae* from the mice vagina.

Rose Bengal (RB) mediated aPDI was chosen as the most effective aPDI variant in decreasing of *S. agalactiae* viability *in vitro*. It caused a lower viability decrease in representatives of physiological flora of the human vagina than for *S. agalactiae*. Computer modelling studies resulted in the creation of an extended logistic aPDI model which effectively describes the dynamics of *S. agalactiae* mortality in the whole tested irradiation power range. RB-mediated aPDI was proven effective also against *S. agalactiae* biofilm grown in stationary and continuous flow models, as well as in multispecies biofilm culture. Used conditions of aPDI did not have significant photo- or cytotoxic effect against human keratinocytes and did not show substantial mutagenic effect in prokaryotic or eukaryotic cells. However, I observed that with the treatment of consecutive cycles of sub-lethal doses of aPDI, the aPDI tolerance development in *S. agalactiae* occurs. Tolerant bacteria have significantly altered phenotype and expression levels of oxidative stress-related genes but can still be eradicated with higher PS concentration and light dose. In the mouse model of *S. agalactiae* colonization a decrease in *S. agalactiae* viability was observed. Moreover, changes in the viability of different bacterial groups were investigated. Histopathology studies did not show any cytotoxic effect of aPDI in vaginal tissues. In conclusion, RB-mediated aPDI is effective in decreasing of *S. agalactiae* viability both *in vitro* and *in vivo* and is safe to use. However, its application in clinical practice requires further investigation.