Aleksandra Taraszkiewicz

Title: **Mechanistic aspects of *Candida albicans* photodynamic inactivation induced by imidazoacridinone derivatives as photosensitizing compounds.**

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Summary

Candida albicans is a human opportunistic pathogen. It colonizes mucosal surfaces of the respiratory, gastrointestinal, genitourinary tracts and skin surfaces, causing infection in 50-70% of healthy individuals. However, in the case of long-term antibiotic treatment, the use of catheters and artificial valves, skin damage due to burns, and immunocompromised patients, the risk of infection increases. Infections caused by *C. albicans* can be divided into superficial (e.g.. vaginal and oral thrush, onychomycosis) and deep (internal organ mycosis, e.g, fungal endocarditis or bladder infection). As a result of invasion of the fungus into the blood, a potentially life-threatening systemic fungal infection can be developed. Among patients in intensive care units, *Candida* spp., is the fourth most common infection-causing-pathogen. In case of microorganisms resistant to treatment, the lack of effective antifungal therapy has become a problem. Therefore, the development of alternative treatment methods is important. One of them can be photodynamic inactivation (PDI), which is based on the interaction of three factors: light, a photoactive compound and oxygen. The photoactive compound absorbs the light, resulting in the transfer of electrons from the ground state to the excited state. There are two types of photodynamic reactions. In type I photodynamic reaction, the excited electron from the photoactive compound is transferred to the substrates: biomolecules (e.g., leading to the formation of lipid radicals), water (e.g., to form hydroxyl radical $HO^{\cdot}$) and oxygen (to form superoxide anion $O_2^{\cdot-}$). Photogenerated radicals can directly affect cell structures, deoxyribonucleic acid or undergo dismutation process. Type II photodynamic reaction involves energy transfer from the photosensitizer molecules in the excited state to the molecular oxygen in its ground state. In this reaction, highly reactive oxygen in its excited state, known as singlet oxygen ($^1O_2$) is generated. Imidazoacridinone derivatives (IA) have been synthesized by prof. J. Konopa's research group from the Technical University of Gdansk and have been

developed as potential anti-cancer drugs. The only report of the light dependent biocidal activity of IA, was concerning the tumor cell lines. Imidazoacridinone derivatives accumulated in the lysosomes, which after irradiation were damaged. Imidazoacridinones photoactivation proved to be very promising as a potential method to control the growth of microorganisms that are human pathogens.

The aim of this study was to analyze the compounds from the imidazoacridinone group in photodynamic inactivation of *Candida albicans*: determination of their photosensitizing properties, the type of photodynamic reaction and the impact of light parameters on the efficiency of PDI. Furthermore, IAs accumulation in *C. albicans* cells was measured, type of cell death resulting from the PDI was determined and also the possibility of inducing resistance to the PDI was studied. In the performed experiments three reference strains and clinical strains of *C. albicans* were used. Results showed that the effectiveness of the PDI varies depending upon the IA derivative and the *C. albicans* strain (the difference was noticeable for both: the reference and clinical strains). It has been shown that both types of photodynamic reaction: I (radical photogeneration) and II (singlet oxygen generation) can be responsible for the biocidal effect of the photosensitizing compounds. Additionally, it has been shown that the PDI efficiency is dependent on the power of the delivered light. In case of IA-based PDI, the best results were obtained with the use of the medium power of light (7 mW/cm^2). It has also been shown that *C. albicans* undergoes apoptosis after the treatment with PDI. Sequential treatment of *C. albicans* cells with sub-lethal doses of light and a photosensitizing compound showed changes in response to the photodynamic reaction. However, no resistance to the PDI treatment was observed. On the basis of the performed experiments, it was found, that the accumulation of IA is indispensable, but it is not the only essential factor for the effective PDI. A clear correlation between the effectiveness of the PDI and IAs accumulation, as well as the singlet oxygen and free radicals generation yield has not been observed.