Abstract

The presence of drug-resistant bacterial species and the infections caused by them constitute a serious threat to public health. According to data released in 2025 by the World Health Organization (WHO), antimicrobial resistance is directly associated with over a million deaths annually and indirectly with nearly 5 million deaths. New therapeutic strategies that enable overcoming this barrier are continuously being sought.

A promising alternative to currently available methods for combating bacterial infections is antimicrobial photodynamic therapy (aPDT). Its major advantage is the inability of bacteria to develop resistance to the processes leading to cell death or photoinactivation. However, the effectiveness of aPDT is significantly influenced by the amount of substance that accumulates inside the bacterial cell.

Combined photodynamic therapies are also gaining popularity; these involve the use of a photosensitizer (PS) in combination with additional chemical compounds, such as efflux pump inhibitors (EPIs), antibiotics, or cytostatics. This approach allows for obtaining an additive or synergistic effect compared to the effects of each therapy applied separately. It also contributes to a reduction in drug dosage and limitation of side effects. The combination of a PS with an EPI may have a real impact on one of the mechanisms inducing resistance and lead to an increase in the intracellular concentration of therapeutic substances.

Natural EPIs have attracted considerable interest. Over the last 30 years, nearly 75% of approved antibacterial products have been of natural origin. Numerous studies indicate that such substances may play the role of EPIs, contributing to enhancement of the applied therapy and to the resensitization of bacteria to substances to which they exhibited resistance.

In this dissertation, I aimed to develop alternative, combined aPDT therapeutic procedures based on compounds of natural origin, applicable both in aPDT therapy and in limiting the development of bacterial structures. Moreover, I demonstrated that digital holotomography (DHT) can be successfully applied as a quantitative measurement technique to monitor the effects induced by these substances and by the therapies in which they are used, at the level of individual bacterial cells. The doctoral dissertation consists of a cycle of three publications.

In the first, I presented a strategy for combining two appropriately selected PSs (Chlorin e6 and Pheophorbide a) in order to increase their intracellular accumulation and to obtain at least an additive therapeutic effect. I demonstrated that a combination of PSs with complementary physicochemical properties (anionic Chlorin e6 and cationic Pheophorbide a) leads to increased accumulation of PSs inside bacterial cells (*Escherichia coli*) and higher therapeutic efficiency.

Experimental verification using confocal microscopy and the quantitative phase imaging technique DHT confirmed that the effectiveness of the combined therapy (29.45%) exceeded the effectiveness of monotherapy (13.77% in the case of Chlorin e6 and 16.15% in the case of Pheophorbide a). The proposed approach constitutes an innovative direction in the development of photodynamic therapy and monitoring of its effects, fitting into global trends in the search for effective strategies to combat bacterial infections.

In the second publication, I focused on investigating the influence of natural EPIs (berberine, palmatine, piperine, curcumin, capsaicin, and coumarin) on the development of bacterial populations (*Escherichia coli, Proteus mirabilis, Bacillus cereus, Enterococcus faecalis*) and on the process of cellular structure formation. I demonstrated that inhibition of efflux pump activity leads to disruption of the mechanisms responsible for bacterial culture development and the formation of spatial structures by bacteria. The application of a bioreactor with reversed rotation enabled a quantitative description of growth curve parameters. A particularly pronounced effect was observed in the case of *E. faecalis* cells incubated with berberine, for which the mean value of the maximum growth rate was reduced by 53.8%. DHT enabled quantitative, three-dimensional analysis of changes in the refractive index (RI), dry mass of bacterial clusters, and the volume occupied by these structures. I experimentally demonstrated that the proposed therapeutic procedure led to a limitation of cell division dynamics and, in the case of *B. cereus*, to its inhibition, accompanied by cell elongation characteristic of stress-induced effects. In the case of *B. cereus*, I demonstrated that the implemented therapeutic procedure limited the increase in the volume of spatial structures formed by bacteria, indicating a slowdown or inhibition of the dynamics of their development. This indicates that the proposed procedure may counteract bacterial surface colonization.

In the third publication, I focused on the application of natural EPIs in combination with a PS, which constitutes an effective strategy for increasing the efficiency of aPDT. The studies were conducted on model bacteria: Gram-positive *Bacillus cereus* and Gram-negative *Escherichia coli*, focusing on the analysis of intracellular accumulation of the PS Chlorin e6. The use of low concentrations of selected EPIs (< 0.25 MIC) led to more efficient accumulation of the PS inside bacterial cells, which was experimentally confirmed using confocal microscopy and DHT. Increased PS accumulation translated directly into a clear increase in aPDT effectiveness, enabling potential reduction of the concentrations of active compounds used and limitation of undesirable therapeutic effects. In the case of *Bacillus cereus*, the combination of piperine with Chlorin e6 led to an increase in therapeutic effectiveness of 273.90%, whereas in the case of *Escherichia coli*, the application of berberine in combination with Chlorin e6 resulted in an increase in therapeutic effect of 227.72% compared to therapy based exclusively on the PS. The conducted studies clearly indicate that inclusion of EPIs in antibacterial treatment regimens enables the achievement of improved therapeutic effects.

The published results of the conducted studies confirm that the primary objective of the doctoral dissertation has been achieved. Alternative, combined antibacterial therapies based on EPIs of natural origin were identified, applicable both in aPDT therapy and in limiting the development of bacterial structures. Moreover, during the conducted research work, novel, label-free and non-destructive measurement procedures based on quantitative phase imaging – DHT were proposed, which may constitute an alternative to currently applied classical microbiological techniques. It is also worth emphasizing that widespread implementation of the proposed therapeutic procedures may contribute to limiting bacterial surface colonization, inhibiting their development, inducing photoinactivation, or reducing antimicrobial resistance not only in clinical practice but also in the food and cosmetic industries.

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