Streszczenie w języku angielskim

"Humoral immune response of *Galleria mellonella* to bacterial infection with *Pseudomonas aeruginosa*"

The increasing drug resistance of microorganisms is one of the main threats to public health. The search for new drugs requires a thorough understanding of the pathogenesis mechanisms of bacteria resistant to antibiotics. One such pathogen is Gram-negative *Pseudomonas aeruginosa*, which produces numerous virulence factors, among others proteolytic enzymes. Larvae of the greater wax moth (*Galleria mellonella*) are being increasingly used as model organism in studies of pathogenesis and bacterial virulence factors. The innate immune system of insects and mammals is characterized by a high degree of structural and functional homology. It is worth mentioning that a positive correlation between virulence of *P. aeruginosa* in mammalian and invertebrate models was found. In the fight against microorganisms, insect's organism triggers mechanisms of humoral response in which antimicrobial peptides and the phenoloxidase system play an important role.

Antimicrobial peptides, called immune peptides, are synthesized in response to infection, mainly in the fat tissue and in the hemocytes. Activation of the phenoloxidase system occurs as a result of the conversion of zymogen prophenoloxidase (proPO) to phenoloxidase (PO), carried out by prophenoloxidase activating enzyme (PPAE). The final product of the phenoloxidase-catalysed reaction is melanin, which helps the host organism to fight pathogens. The investigations were aimed at analyzing selected aspects of *G. mellonella*'s innate immune response after infection with two strains of *P. aeruginosa* (entomopathogenic and clinical isolates), as well as after injection of bacterial virulence factor – alkaline protease. The bacterial strains used in the studies produce a different set of extracellular proteinases. The alkaline protease was purified from the culture fluid of the clinical strain of *P. aeruginosa* by ion-exchange chromatography and confirmed in the obtained chromatographic fraction with LC-MS-MS/MS and with immunoblotting.

Using the radial diffusion method and bioautography method, it has been shown that infecting insects with *P. aeruginosa* bacteria increases both the antimicrobial and antifungal activity in the hemolymph of *G. mellonella* larvae. The increase in antimicrobial activity in hemolymph was associated with the induction of the synthesis of low molecular weight immune peptides. The studies also analyzed the protein-peptide profile in haemolymph extracts of

infected larvae using different electrophoretic techniques (Tricine SDS-PAGE and IEF SDS-PAGE) and RP-HPLC chromatography. It was demonstrated for the first time that the infection of insects with the *P. aeruginosa* induced the synthesis of six immune peptides, the number of which increased significantly with the development of bacteraemia. After just 8 hours from infection of the larvae, the level of three peptides increased, i.e. Gm defensin, anionic peptide 1 and proline-rich peptide 2, and after 15 hours the amount of three constitutive peptides increased, namely cekropin D-like peptide, moricin-like peptide B and proline-rich peptide 1. The level of constitutively present peptides in the hemolymph, such as anionic peptide 2, apolipoforicin, and Gm gallerimycin did not change after infecting insects with the pathogen. In addition, 15 hours after the injection of the pathogen, the amount of superoxide dismutase, which plays an important role in the homeostasis of antioxidative processes in insect, has clearly increased in the extracts studied.

After the immunization of larvae with a small dose of the alkaline protease of *P. aeruginosa*, a similar set of peptides was observed in the extracts of hemolymph, compared to peptides identified after infection with bacteria. Moreover, as a result of the tests carried out using the spectrophotometric and electrophoretic methods, activation of the phenoloxidase system was observed in the hemolymph of G. *mellonella* larvae immunized with alkaline protease in the first hours after injection. However, after about 15 hours there was a dose-dependent, almost complete inhibition of phenoloxidase activity. *In vitro* studies have shown that the alkaline protease does not affect activated phenoloxidase, but clearly inhibits the proPO activation process. Based on the obtained results, it was found that the alkaline protease may play a different role during infection depending on the severity of the infection. On the one hand, it is able to initiate the occurrence of immunological reactions, while on the other hand, it is also able to overcome the humoral immune response of *G. mellonella* larvae.

The greater wax moth *G. mellonella* proved to be a useful model organism for studying the reactions constituting the immune response of insects and for testing the pathogenicity and virulence factors of *P. aeruginosa*. Understanding the interactions that occur in the host-opportunistic pathogen system may contribute to the discovery of a potential therapeutic target to fight the infection caused by *P. aeruginosa*, i.e. inhibiting the activity of extracellular proteolytic enzymes.

Keywords: phenolic oxidase system, antimicrobial peptides, *Galleria mellonella*, alkaline protease, *Pseudomonas aeruginosa*