Artilysin®s as a novel enzyme-based approach to kill drug-resistant bacteria

In recent years world leading health care authorities have warned the global community about the threat of antimicrobial resistance. We risk being cast back into the dark ages when minor infections and injuries were often lethal. However, this threat is not unexpected, Sir Alexander Fleming, who won the Nobel prize for his discovery and isolation of penicillin in 1945, warned already in his acceptance lecture for bacterial resistance development. In spite of his warning words, the indiscriminate and inappropriate use of antibiotics has led to a global emergence and spread of resistance development, which has now reached alarming levels. Together with the unprecedented discovery void of new antibiotic classes, we may face a potential health crisis that will leave us unarmed against bacterial infections.

Therefore, new classes of antimicrobials have to be discovered and developed. Enzyme-based antimicrobials inspired by an even older discovery of Sir Alexander Fleming offer potential. Having a cold, curiosity inspired him to culture his nasal drippings and he observed their antibacterial effect. These enzymes, which are called muralytic enzymes, are widespread in nature and can be found in humans (e.g. tears, saliva, etc.) and animals (e.g. milk, eggs, etc.), but the most powerful variants are likely found in “virus-eating-bacteria” or bacteriophages.

“The enemy of my enemy is my friend” is an ancient statement, but is particularly true in the case of bacteriophages that kill an estimated 10-20% of the global bacterial population every day. Through millions of years of intimate coevolution between bacteriophage and bacterium, bacteriophages have evolved highly active muralytic enzymes or endolysins. Endolysins are produced within infected bacterial cells and at the end of the viral cycle, they digest the peptidoglycan layer which surrounds every bacterium, resulting in cell burst and the release of newly produced viral particles. The nice thing about endolysins is that purified endolysins are also active when you add them from the outside. This is particularly appealing for Gram-positive bacteria that have a peptidoglycan layer which is immediately accessible from the outside. Addition of purified endolysin to these bacteria results in a rapid cell death through peptidoglycan degradation, irrelative of the presence of existing resistance mechanisms. Endolysins can thus be used as antibiotics and their efficacy has meanwhile been shown for the treatment of mucosal and systemic Gram-positive infections in diverse animal models and food applications.

A major hurdle in the expansion of endolysins as antibiotics against Gram-negative pathogens was their outer membrane that shields the peptidoglycan layer and made them insensitive to lysis by exogenously applied endolysins. To tackle this barrier, endolysins were modified using protein-engineering to combine the self-promoted uptake mechanism of outer membrane permeabilizing peptides and peptidoglycan-degrading activity of endolysins to broaden the use of endolysins. These research efforts resulted in a novel class of engineered endolysins, coined Artilysin®s. They are able to eradicate multi-drug resistant Gram-negative bacteria. A well characterized Artilysin is

Art-175, which kills the notorious multi-drug resistant bacterium *Pseudomonas aeruginosa* upon contact, indifferent of the presence of any existing antibiotic resistance mechanism. Strains resistant against Art-175 could not be selected. Case studies with dogs suffering otitis support potential applicability of Art-175 against topical, drug-resistant infections.

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