Bacteriophage derived endolysin compounds are an effective treatment for MRSA

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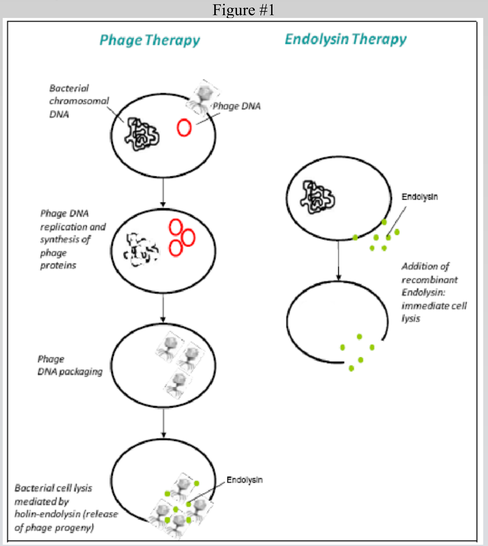
Abstract

Due to an increase in antibacterial resistance, there is a need for alternatives to antibiotics to treat the growing methicillin resistant *Staphylococcus aureus* (MRSA) epidemic. One alternative is phage therapy which uses bacteriophages that target and kill specific bacteria in infected hosts. Recently, an old technology is being re-visited to evaluate the use of bacteriophages to specifically target methicillin resistant *Staphylococcus aureus.* There are several bacteriophages that specifically target Staphylococcus aureus. Each bacteriophage has its own lytic enzymes that cause the bacterial cell to burst. When phage therapy was originally developed, a few issues were of concern regarding safety, stability, and treatment methods. These studies are indicating that bacteriophage derived endolysins are an effective treatment for MRSA. After successfully eliminating MRSA infections in several murine models, chimeric endolysin studies are moving to human clinical trials including the SAL200 formulation and CF-301. Benefits and advantages of these chimeric compounds are stable for storage and transportation, have increased activity at physiological pH, have increased plasma half-life, can be administered intravenously, and in some cases, demonstrate complete clearing of the infection in as little as 20 minutes. These studies are indicating that bacteriophage derived chimeric endolysins are an effective treatment for MRSA.

Figure #1



Raz, A. et al. (2017) Lysibodies are IgG Fc fusions with lysin binding domains targeting Staphylococcus aureus wall carbohydrates for effective phagocytosis. Proceedings of the National Academy of Sciences. 114(18):201619249 DOI: 10:1073/pnas.1619249114

The bacteriophage uses endolysin to lyse the cell wall of the bacteria.

Methods

In order to evaluate bacteriophage derived endolysins as an effective treatment for MRSA, several journal articles about studies testing the effectiveness of endolysins on MRSA infections in animal models and human clinical trials were compiled from studies in the past 15 years through searches on Pubmed. Endolysin treatments instead of whole phage treatments stood out as the most promising technology after reading journal articles about both treatment methods. Journal articles describing whole phage treatment were disregarded after reading about potential horizontal gene transfer and concerns that researchers had about shelf life and stability issues. Many literature reviews highlighted the fact that bacteriophage derived endolysin treatments are the most promising phage therapy technology. Due to the understanding of the modular nature of phage endolysins and the fact that researchers are utilizing this quality to create chimeric endolysins that are more effective than naturally occurring endolysins, chimeric endolysins became the main focus of this project. This literature review focused on two chimeric lysins in human clinical trials for treatment of MRSA.

Background

Methicillin Resistant *Staphylococcus aureus* (MRSA) has been a growing concern for hospitals in the past few decades, especially in cases of hospital-acquired MRSA. In some cases of MRSA, even last resort antibiotics, such as Vancomycin, are not sufficient enough to combat the infection. Alternative treatments outside of antibiotics are necessary for MRSA treatment. Bacteriophages, which are viruses that specifically attack specified bacteria, have been identified as effective at attacking and killing MRSA. Although not a new concept, advancements in phage therapy are making it more practical for human use. One of the key advancements in recent years has been the isolation of endolysin enzymes that cause the bacteria to lyse.

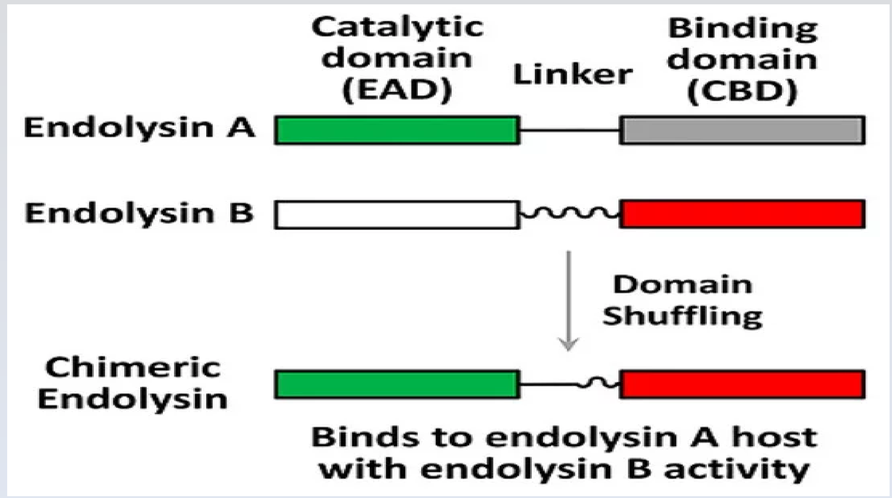
Bacteriophages bind to the bacteria at the cell wall binding domain (CDB) protein which is specific to that bacterial species. The phage injects its DNA into the bacteria, utilizes the host machinery to replicate and produce a specific endolysin that lyses the cell and releases the viral particles from the cell to continue the cycle. Although phage therapy may seem perfect at first glance, there are some limitations: phage therapy has the potential to change the bacterial DNA through horizontal gene transfer through the process of transduction. This could have unforeseen consequences to the pathogenesis of the commensal flora by altering the genetic makeup of the flora through horizontal gene transfer. High doses of bacteriophage into a host could induce the development of antibodies against the phage which could neutralize the phage before it is able to kill the bacterial infection. It is difficult to keep the bacteriophages stable for transport and storage because they have a narrow thermal stability window. Due to these drawbacks, researchers are focusing on isolating the apecific endolysins from these lytic phages and using them independently instead of whole bacteriophage treatments.

Endolysins are modular enzymes made up of multiple domains each with a different function. Endolysins are cell wall hydrolases that are produced during the infectious cycle of the bacteriophage. The endolysin cleaves the integral peptidoglycan bonds of the susceptible gram positive bacteria which causes rapid cell lysis. The concept of phage endolysin therapy is to isolate that endolysin and to use it to cause "lysis from without". Lysis from without is lysis of the bacteria through the addition of an outside agent. Some domains are cell wall binding domains (CBDs), which bind to specific epitopes in their target bacteria. Other domains are enzymatically active domains (EADs), which break down the peptidoglycan layers in the bacterial cell wall. These domains can be separated from each other and retain their function. This is the basis for chimeric endolysins, Chimeric lysins are combinations of effective CBDs and EADs, such that they are optimized for storage, plasma half-life, and activity at physiological pH. Domains can also be swapped between endolysins from different phages or endolysins can be bound together to form dimers or oligomers. The different domains are linked together with flexible interdomain linkers (Gerstmans, 2017). By creating dimers or oligomers, the plasma half-life can be significantly increased due to reduction in renal clearance due to the increased molecular weight of the compound. SAL200 and CF-301 are chimeric lysins which are currently in human clinical trials.

By using the enzymes that bacteriophages use to lyse the bacterial cell wall (endolysins) alone, it is possible to stimulate lysis-from-without (Fischetti, 2018). This is when the lytic enzymes produced by the bacteriophage bind to the cell wall of the target bacteria and stimulate lysis from the cell surface instead of from inside the bacteria The advantages of endolysins include: thermal stability, long shelf life, lack of inducible resistance, less risk of horizontal gene transfer, and a longer plasma half-life (Zhang, 2016).

Researchers have found several different endolysins derived from many different MRSA specific bacteriophages, which have bactericidal activity against *Staphylococcus aureus*. Chimeric endolysins appear to be the most effective alternative treatment for MRSA, and currently, there are a couple of chimeric endolysin treatments that are in human clinical trials. The most promising are SAL200 (Jun, 2017) and CF-301 (Schuch, 2014).

Figure #2



Kim, B. et al. (2019) Phage-Derived Antibacterials: Harnessing the Simplicity, Plasticity, and Diversity of Phages. Viruses. 11(3), 268. DOI: 10.3390/v11030268

Chimeric endolysins are a combination of multiple domains from different bacteriophages in order to improve the endolysin.

Discussion

Researchers have attempted to isolate and use CBDs from phages alone and have found that they can attenuate bacteria and allow the immune system to clear the infection (Yang, 2018). Phage endolysins can be combined with lysostaphin or antibiotics for a synergistic killing of MRSA. Combining endolysins with previously ineffective antibiotics can work synergistically to make the antibiotic effective again (Daniel, 2010). This is because endolysins are great at breaking down biofilms. Once the outer layer of the biofilm is removed it allows the antibiotic to have direct access to the bacteria making the antibiotic synergistically effective. Some researchers have developed chimeric phage endolysins by combining endolysins and CBDs from different phages.

SAL200 and CF-301 are chimeric endolysins which are currently in human clinical trials. Both chimeric endolysin treatments are injectable and can be used to combat systemic MRSA. SAL200 is in phase 1 clinical trials wherein it was shown to be safe in 57 healthy Korean men (Jun, 2017). CF-301 is currently in phase 2 clinical trials. After finding no adverse events in 20 healthy volunteers in phase 1 trials, phase 2 trials are ongoing with 115 adult patients with complicated *Staphyloccocus aureus* bacteremia including endocarditis from MRSA (Fischetti, 2018). CF-301 has demonstrated the ability to eliminate systemic MRSA infections within hours of treatment (Schuch, 2017). These treatments were both found to have strong bactericidal activity against MRSA, long plasma half-life, and don't induce resistance. Bacteriophage derived endolysin compounds are an effective treatment for MRSA and will likely be approved for human use in the near future.

Results

Comparison of Chimeric Endolysins

	Plasma half-life	Rapid elimination of Infections	Synergistic benefits	Lack adverse effects	Clinical Trials	Source
SAL200	2 min to 9hours	X	With lysostaphin	X	Phase 1	(Jun, 2017)
CF-301	Up to 11.3 hours	X	With daptomycin	X	Phase 2	(Schuch, 2017)

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