International Journal of Pharmacological Research www.ssjournals.com
ISSN: 2277-3312 Journal DOI:10.7439/ijpr

Enzybiotics- A Review

G.K.Sudhakar[1*], Venkatesh Kamath B[2] and Aravind Pai[3]

[1,3]*Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal*
[2]*Department of Pharmaceutical Biotechnology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal*

Corresponding author*: gk.sudhakar@manipal.edu

Abstract

When more number of bacteria are becoming resistant to antibiotics, the development of new disease-fighting agents has become essential. In this context, enzybiotics are alternative possibilities in fighting bacterial or fungal infections diseases. The applications of enzybiotics include different forms of prophylaxis and treatment of microbial infections. Many sources of enzybiotics include phage infected bacteria such as *Staphylococcus aureus, Streptococcus pneumonia* and *histeria monocytogenes*. Bacteriophages and antimicrobial peptides are main categories of enzybiotics. Lysozyme has antibacterial antiviral, anti-inflammatory, anticancer and immunomodulatory activities. Phage cocktail has great therapeutic potential for multi-drug resistant or super bug infection.

Key words: Enzybiotics, bacteriophages, lysins, bacteriocins

1. Introduction

Antibiotic abuse is partly responsible for continuing increase in the prevalence of multi-drug resistant bacteria[1]. When many bacteria become resistant to antibiotics, enzybiotics are used to fight against disease causing microorganism. Since antibiotic resistant drugs like methicillin, vancomycin threatens public safety, a new class of antibiotics known as enzybiotics are developed with new mechanism of action against drug resistant pathogens[2]. Enzybiotics help to fight bacterial or fungal diseases by using viruses or viral-derived lysins[3] and antimicrobial peptides[4].

The main characteristic of enzybiotics includes

1) New mechanism of antibacterial action
2) Capacity to kill antibiotic resistant bacteria
3) Less chances of developing bacterial resistance[5].

The term enzybiotics is a hybrid word from enzyme and antibiotic. It has been coined for phage encoded endolysins which attack and lyse bacteria when added exogenously[6]. It is referred to as bacteriophage enzyme with bacterial cell-wall degrading capacity. Hence it shows antibacterial and/or antifungal activity.

Enzybiotics consists of lytic enzymes that are naturally present in viruses, bacteria, and in body fluids such as tears, saliva and mucous. They include lysins, bacteriocins, autolysins and lysoenzymens, which mainly belong to the class of peptidoglycan hydrolases. Lysins are obtained from bacteriophages and bacteriocins while autolysins are obtained from phage infected bacteria.

1.1 Bacteriocins: Bacteriocins (narrow spectrum antibiotics) are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains[7]. They are made by non-pathogenic bacteria that normally colonize the human body. The loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body.

1.2 Lysins: Lysins or endolysins are double stranded DNA bacteriophage encoded enzymes that slice the covalent bonds in peptidoglycan[8]. These are basic enzymes having positive charge at pH lower than their isoelectric point. Peptidoglycan is a major structural component of bacterial cell wall, which encompasses of alternate residues of N-Acetylglucosamine and N-Acetylmuramic acid.

Endolysins are basically those enzymes which are used by bacteriophages to degrade the peptidoglycan of bacterial host from within the cell, resulting in cell lysis and release of progeny virions. They can obliterate these organisms when applied externally and serve as antimicrobial candidates by increasing bacterial drug resistance.

1.3 Lysozymes: Lysozymes, also known as muramidase or N-Acetylmuramide glycanhydrolase, damages the bacterial cell wall by catalyzing hydrolysis of 1,4 β-linkages between N-Acetyl D-glucosamine and N-Acetylmuramic acid residues in peptidoglycan. Lysozyme is abundant in number of secretions such as tears, saliva, human milk and mucous. It is also present in cytoplasmic granules of polymorphonuclear neutrophils. In human beings, lysozyme enzyme is encoded by LYZ gene[9]. Lysozyme is regarded to be a natural antibiotic[10]. It is a significant factor of innate immunity and a unique enzybiotic which exerts not only antibacterial activity but also antiviral, anti-inflammatory, anti-cancer and immunomodulatory activities[11].

1.4 Autolysin: An autolysin is an enzyme that hydrolyses the components of biological cell or tissue. Autolysins exists in all bacteria containing peptidoglycan. The peptidoglycan matrix is very rigid, thus these enzymes break down the matrix in to small sections so that growth and division of cell can occur. They act by hydrolyzing β (1, 4) bond between N-Acetylglucosamine and N-Acetylmuramic acid. Gram positive bacteria regulate autolysins with teichoic acid molecules attached to tetra peptide of peptidoglycan matrix[12]. The major autolysins of *Staphyloccus epidermidus* and *Staphylococcus aureus* play an important role in cell separation. Their mutants are also reduced reduced in virulence. Presence of lyt A gene in all isolates of *Streptococcus pneumonia* shows that irrespective of site of isolation, kind of infection caused, autolysin is an obligate necessity for this organism[13].

2. Other potential enzybiotics

Among other antimicrobial peptide gene families, the defensins and cathelicidins can also be used as enzybiotics. These endogenous antibiotic peptides play a crucial role in the innate immunity system. They are first line of defense in protecting the internal and external body surfaces of the host. Another lysin, isolated from phage P68 of *Staphylococcus aureus*, shows antimicrobial activity when used along with the antibiotic Gentamicin[14].

2.1 Fusion proteins: The two fusion proteins consist of streptococcal λ SA2 endolysin endopeptidase domain fused to *Staphylococcal* cell wall binding domains from either lysostaphin or *Staphylococcal* phage K endolysins Lys K. Both chimeras were synergistic with lysostaphin against *Staphylococcus aureus.* The potential of fusion peptidoglycan hydrolases as enzybiotics for the treatment of *Staphyloccus aureus* induced mastitis has been reported[15].

2.2 Virion-associated peptidoglycan hydrolases (VAPGH): VAPGH are phage-encoded lytic enzymes that locally degrade the peptidoglycan of bacterial cell wall during infection. They act by generating a small hole through which the phage tail tube crosses the cell envelope to eject the phage genetic material at the beginning to the infection cycle. The unique properties of VAPGH's include high specificity, remarkable thermostability and modular organization. The potential of VAPGH is versatile as enzybiotics. Various enzybiotics (source, types and their enzymatic specificity) along with antimicrobial range is given in table 1.

Table 1: Various representative enzybiotics

Enzybiotic name	Enzybiotic class	Source	Enzymatic specificity	Antibacterial range
Ply C	Lysin	Phage C1	Amidase	*S. pyrogens* groups C and E
P al	Lysin	Phage DP	Amidase	*S. pneumoniae*
λ SA 2-E	Endolysin	Staphylococcal phage	Endopeptidase	*S. aureus*
P ly G	Lysin	Phage gamma	Amidase	*B. anthracis*
Lyt A	Autolysin	S.pneumoniae	Amidase	*S. pneumoniae*
Lysostaphin	Bacteriocin	S. simulans	Endopeptidase	*S. aureus, Staphylococci*
Hen egg white lysozyme	Lysozyme	Hen's egg white	Muramidase	Gram positive bacteria

3. Bacteriophages therapy

Bacteriophages therapy has proven to be medically superior to antibiotic therapy with many advantages such as

1. Phages are more efficient against multi-drug resistant pathogenic bacteria
2. Phage specificity ensures lack of interference with normal microbial flora
3. Quick response to the appearance of phage resistant bacterial mutants
4. Cost of phage treatments lower than antibiotic treatment
5. Rare side effects

Phage lytic enzymes are used to eradicate Gram positive pathogens resistant to antibiotics. P 16-17 consists of inferred N-terminal D-alanyl-glycyl endopeptidase domain of P 16 and C-terminal cell wall binding domain of minor coat protein P 17 and shows antimicrobial activity towards *Staphyloccus aureus.* P 16-17 increases antimicrobial efficacy of antibiotic Gentamicin. Thus, this synergistic effect was found useful to reduce the effective dose of aminoglycoside type antibiotics[16].

3.1 Phage cocktail and recombinant phages: Phage cocktail has great therapeutic potential for multidrug resistant bacterial infection when compared with monophage in reducing bacterial mutation and rescue frequency. Recombinant phages efficiently kill target bacteria. Genetically modified enzybiotics like Bacteriocins have great potential in generation of antimicrobials and fight against many challenges in disease control[17]. Lysins are effective antibacterial in the fight against infectious disease where Multi-Drug Resistant is prevalent. Since bacteriophages are the most abundant biological entities on earth, they are rich natural source of these enzymes.

Bioinformatic and proteomic studies provide new opportunities for domain swapping construction of chimeras and production of specifically engineered designer lysins with many applications[18]. Furthermore, a combination of bacteriophages and antibiotic synergistic action can offer better therapeutic value on the basis of studies of both *in vitro* and *in vivo* on the behaviour of phage-bacterium system[19].

4. Conclusion

Phage therapy is more specific, accurate and thus can complement and replace current antibiotics by facilitating virus way out from the host. Enzybiotics (lysozymes, lysins, Bacteriocins, defensins, cathelicidins) have a novel mode of antimicrobial action than those of traditional antibiotics. Due to rapid progress in the fields of Biotechnology, enzyme technology and molecular biology along with the knowledge of evolution of natural enzymes, it is hoped that phages which are present abundantly in the biosphere could be trapped and utilized in finding of more potent enzybiotics.

References

1. English BK, Gaur AH. The use and abuse of antibiotics and the development of antibiotic resistance. *Adv Exp Med Biol.* 2010; 659:73-82.
2. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med*. 2004; 10(12 Suppl):S122-9.
3. Fischetti VA. Bacteriophage lytic enzymes: novel anti-infectives. *Trends Microbiol*. 2005; 13(10):491-6.
4. Gordon YJ, Romanowski EG. A Review of Antimicrobial Peptides and Their Therapeutic Potential as Anti-Infective Drugs. *Curr Eye Res.* 2005; 30(7): 505–515.
5. Kusuma C, Jadanova A et al. Lysostaphin-Resistant Variants of *Staphylococcus aureus* Demonstrate Reduced Fitness *In Vitro* and *In Vivo*. *Antimicrob Agents Chemother.* 2007; 51(2): 475–482.
6. Wu H, Lu H et al. EnzyBase: a novel database for enzybiotic studies. *BMC Microbiology* 2012, 12:54.
7. Farkas-Himsley H. Bacteriocins--are they broad-spectrum antibiotics? *J Antimicrob Chemother.* 1980 ;6(4):424-6.
8. Borysowski J, Weber-Dabrowska B, Górski A. Bacteriophage endolysins as a novel class of antibacterial agents. *Exp Biol Med* (Maywood). 2006;231(4):366-77.
9. Peters CW, Kruse U, Pollwein R, Grzeschik KH, Sippel AE. The human lysozyme gene. Sequence organization and chromosomal localization. *Eur J Biochem.* 1989;182(3):507-16.
10. Glynn AA. Lysozyme: antigen, enzyme and antibacterial agent. *Sci Basis Med Annu Rev.* 1968:31-52.
11. Sava G. Pharmacological aspects and therapeutic applications of lysozymes. *EXS*. 1996; 75:433-49.
12. Thomas JS, Steve AB, Simon JF Autolysins of Bacillus subtilis: multiple enzymes with multiple functions. *Microbiology*. 2000; 146(2): 249-262
13. Sourav S, Patricia A, et al. Detection of pneumolysin and autolysin genes among antibiotic resistant Streptococcus pneumoniae in invasive infections. *Indian J Med Microbiol*. 2010;28(1):34-9.
14. Manoharadas S, Witte A, Bläsi U. Antimicrobial activity of a chimeric enzybiotic towards *Staphylococcus aureus*. *J Biotechnol*. 2009; 139(1):118-23.
15. Schmelcher M, Powell AM et al. Chimeric Phage Lysins Act Synergistically with Lysostaphin To Kill Mastitis-Causing *Staphylococcus aureus* in Murine Mammary Glands. *Appl Environ Microbiol.* 2012; 78(7): 2297–2305.
16. Hermoso JA, García JL, García P. Taking aim on bacterial pathogens: from phage therapy to enzybiotics. *Curr Opin Microbiol*. 2007;10(5):461-72.
17. Gillor O, Nigro LM, Riley MA. Genetically engineered bacteriocins and their potential as the next generation of antimicrobials. *Curr Pharm Des*. 2005; 11(8):1067-75.
18. Fenton M, Ross P, McAuliffe O, O'Mahony J, Coffey A. Recombinant bacteriophage lysins as antibacterials. *Bioeng Bugs*. 2010;1(1):9-16.
19. Skurnik M, Strauch E. Phage therapy: facts and fiction. *Int J Med Microbiol*. 2006; 296(1):5-14.