RESEARCH PAPER

Investigating AA10 Peptide Against Lux R Receptor Of Staphylococcus Aureus Using Peptide Docking And Zebrafish Toxicity Analysis

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ABSTRACT

Background: *Staphylococcus aureus* is the most common bacteria that causes a variety of clinical manifestations mainly skin infections among humans which includes impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome etc. The most common mode of transmission of *S. aureus* is through direct contact. The LuxR receptor plays a key role in the quorum-sensing system which increases the expression of many virulence factors. The function of Lux R as a quorum sensor is mediated by the binding of AHL (N-acyl-L-homoserine lactone) molecules to the N-terminal receptor of the proteins. **Aim:** The aim of the present study is to investigate the novel AA10 peptide against the LuxR receptor of staphylococcus aureus using peptide docking and zebrafish toxicity analysis. **Materials and method:** A novel peptide sequence with 10 amino acids containing Alanine, Cysteine, Glycine, cysteine, Glycine, Leucine, Leucine, Lysine, serine, alanine in sequence (ACGCGLLKSA) was designed using chem draw software. Helical wheel structure of the designed peptide AA10 was obtained from EMBOSS pep wheel software. The toxicity of the designed peptide was assessed using the Toxin pred software and its bioactivity was analyzed using Autodock software. A zebrafish embryo toxicity test was also conducted and the results were recorded. **Result:** The peptide designed contains 10 amino acids including Alanine, Cysteine, Glycine, cysteine, Glycine, Leucine, Leucine, Lysine, serine, alanine in sequence (ACGCGLLKSA). The designed peptide exhibits increased binding affinity with the LuxR receptor. The results of the zebrafish embryo toxicity test proved the non toxic nature of the designed peptide with an increased number of surviving zebrafish embryos. **Conclusion:** From the results of the present study, it can be concluded that the novel AA10 peptide exhibited good bioactivity and high binding affinity with the LuxR receptor in silico. It was also found to be non toxic in both in silico and in vivo analysis.

Keywords: AA10 peptide, LuxR receptor, quorum sensing, staphylococcus aureus, zebrafish

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INTRODUCTION

Staphylococcus aureus is the most common bacteria that causes a variety of clinical manifestations mainly skin infections among humans which includes impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome etc. (1) *Staphylococcus aureus* is a Gram-positive bacteria that are cocci-shaped and arranged in grape-like clusters.(2) It is a commensal of the normal human flora, located on the skin and mucous membranes. (3) The most common mode of transmission of *S. aureus* is through direct contact. (4)

The infections caused by S. aureus are attributed to the production of various exotoxins by the bacteria which includes pyrogenic toxin superantigens and exfoliative toxins, hemolysins (α, β, δ, and γ) and Panton-Valentine leukocidin. (5)(6) However in order to cause these human

diseases and to occupy multiple sites in the host body, staphylococci uses a special cell-to-cell communication known as quorum sensing that regulates the colonization of this bacteria and the expression of various virulence factors. (7)(8) Various bacterial processes such as sporulation, antibiotic resistance, biofilm formation and competence are controlled by the mechanism of quorum sensing. (9)(10)

The LuxR receptor plays a key role in the quorum-sensing system which increases the expression of many virulence factors. The function of Lux R as a quorum sensor is mediated by the binding of AHL (N-acyl-L-homoserine lactone) molecules to the N-terminal receptor of the proteins. (11)(12) Hence, Peptide-based antimicrobial treatment might show greater advantages than the existing treatment modalities as these peptides are known to bind to specific receptors and its action is confined to that particular target. It exhibits high affinity for particular receptors that are overexpressed than its normal levels. (13)(14)

The most suitable model for screening the potential use of novel drugs to treat various human diseases is the zebrafish. It has been proven by the similarity in the physiology and morphology of different organ systems by various phylogenetic analysis between the genomes of zebrafish and human. (15) (16) The zebrafish embryotoxicity test (ZFET), is widely used in the field of research as it provides a precise time span for the development of a vertebrate embryo and provides details of its early stages of life. It has been widely used to study metastasis, anticancer drug screening, and also to study drug toxicity for more than a decade. (17) Hence, the aim of the present study is to investigate the novel AA10 peptide against the LuxR receptor of staphylococcus aureus using peptide docking and zebrafish toxicity analysis.

MATERIALS AND METHOD

The present study was conducted in the department of cariology, Saveetha dental college, Chennai, TamilNadu, India. A novel peptide sequence with 10 amino acids containing Alanine, Cysteine, Glycine, cysteine, Glycine, Leucine, Leucine, Lysine, serine, alanine in sequence (ACGCGLLKSA) was designed using chem draw software. It is a downloadable application for drawing chemical structures for various purposes. It was used to save time and reduce costs by identifying the amino acids required for the peptide synthesis that are likely to have the desired properties before synthesizing them in vitro.

Helical wheel structure of the designed peptide AA10 was obtained from EMBOSS pep wheel software which represented the polar (basic/ uncharged) and the non polar components. It is used to draw a helical wheel diagram for the desired protein sequence. This displays the sequence in a helical representation as if looking down the axis of the helix. It is useful to study the physical and chemical properties of residues around a helix.

The toxicity of the designed peptide was assessed using the Toxin pred software and its bioactivity was analyzed using Autodock software. The steps in molecular docking include ligand and target preparation, preparation of docking and scoring parameters. The third step involves the use of a graphical interface to run the docking program (AutoDock). Finally, the obtained results are analyzed and evaluated.

AA10 peptide was synthesized using CEM microwave peptide synthesizer. In-vivo toxicity analysis of AA10 peptide was done on zebrafish larva where the 100 newly fertilized zebrafish eggs were exposed to the test chemical at two different concentrations (25g/ml and 50g/ml), standard antibiotic (amoxicillin 50g/ml) for a period of 96 hrs. The samples were monitored for every 24 hrs, At the end of the exposure period, the number of zebrafish that had survived was assessed. The results are represented in the form of figures and graphs.

RESULTS

The peptide designed contains 10 amino acids including Alanine, Cysteine, Glycine, cysteine, Glycine, Leucine, Leucine, Lysine, serine, alanine in sequence (ACGCGLLKSA). The helical structure of the designed peptide is depicted in Figure 1. The designed peptide exhibits increased binding affinity with the LuxR receptor when assessed by molecular docking with the value of -121kcal/mol (Figure 2). The physico chemical properties such as molecular weight (922.13g/mol), iso electric point (pH 8.12), net charge at pH 7 (0.9) were assessed. It also exhibits poor water solubility (Figure 3). The designed peptide was found to be non toxic and the bioactivity was found to be 0.88 (Figure 4). The results of the zebrafish embryo toxicity test proved the non toxic nature of the designed peptide with an increased number of surviving zebrafish embryos (Figure 5).

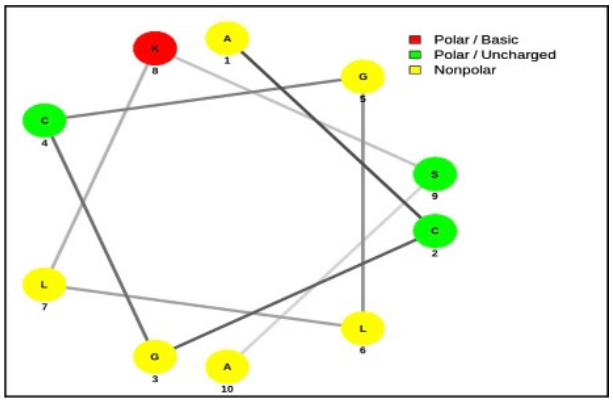

Figure 1: Helical structure of AA10 peptide

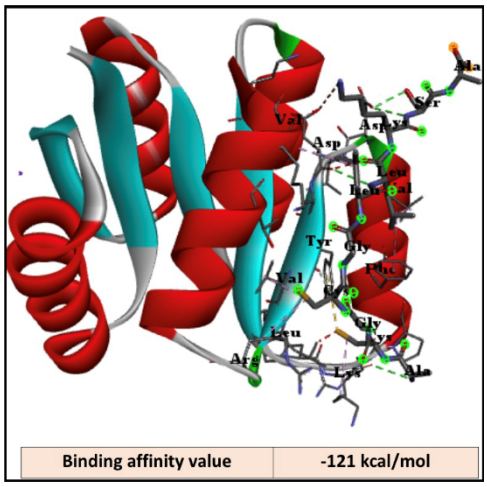

Figure 2: Molecular docking

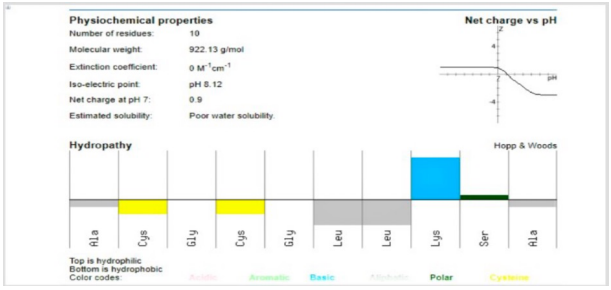

Figure 3: physicochemical properties of AA10 peptide

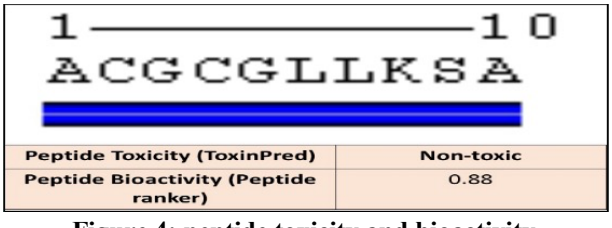

Figure 4: peptide toxicity and bioactivity

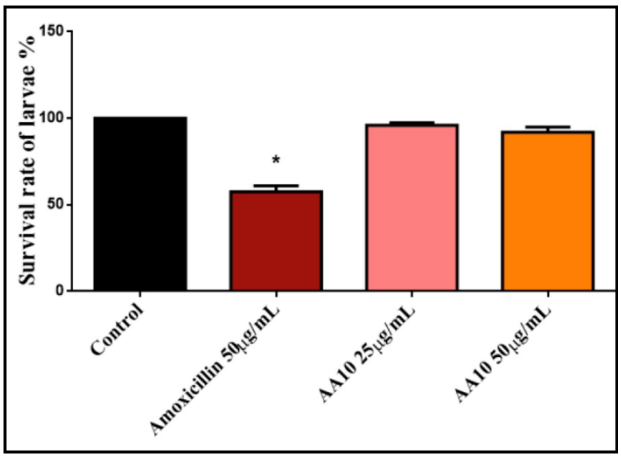

Figure 5: zebrafish embryo toxicity test

DISCUSSION

The AA10 peptide designed in the present study can be used against the quorum sensing mechanism mediated by the LuxR receptor of staphylococcus aureus due to its high affinity to the receptor and non toxic nature proven by both in silico and in vivo approaches. The discovery of novel peptides have gained popularity in the recent decades (18)where the results of Marlyn et al., reported a novel series of pyrido [3, 2, 1- de] phenanthridin -6 synthesized through in silico approaches and in-vivo assessment. (19) Similarly, The results of Tecla et al., reported a new peptide KKVTMTCSAS which was therapeutic in *Galleria mellonella* candidal infection, showing non toxic effects on mammalian cells. (20)

Previous studies have proved that the accessory gene regulator (*agr*) system of *Staphylococcus aureus* is involved in the MRSA-mediated injury during skin infection. (21,22) Targeting these MRSA quorum sensing proves to be a promising alternative to antibiotic therapies. (23) The results of (24) were in accordance with the present study where the properties of the peptide LL-37 was assessed using in *silico* tools and it was found that the antimicrobial effects of dimeric peptide and Catoid on *Staphylococcus* strains were very significant.

The results of Xiayou Lu et al., reveals that the novel RK22 peptide developed from *Hirudinaria manillensis* killed *S. aureus* by inhibiting biofilm formation. It also showed good stability of plasma, very less cytotoxicity and hemolytic activity. The administration of RK22 peptide has significantly inhibited *S. aureus* infection and also its resistant strains. (25) Similarly, in another study conducted by Shen Yang et al., a novel peptide GPVR 10 was developed and was found to have significant minimum inhibitory concentration against *S. aureus*. (26)

The present study employed a comprehensive virtual screening approach using structure-based peptide screening to predict the efficacy of binding with their target receptors. The limitations of the present study was the small sample size. However, a similar study can be conducted on a larger scale in future with invivo analysis on animals with similar resemblance to humans. genome.

CONCLUSION

From the results of the present study, it can be concluded that the novel AA10 peptide exhibited good bioactivity and high binding affinity with the LuxR receptor in silico. It was also found to be non toxic in both in silico and in vivo analysis.

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