Abstract

In the recent past, the increasing number of reports on peptides exhibiting prospective antibiotics and cargo delivery abilities has highlighted the tremendous potential of peptide therapeutics in the field of pharmaceutical research and development. The repertoire of the peptides includes naturally existing and synthetically developed peptides. However, the ratio of peptides exhibiting promising therapeutic abilities to the ones showing success in clinical trials is very low. Hence, there is a significant requirement of new peptides and modification of existing ones to completely reap the potential of the field. Membrane active peptides (MAPs) are oldest group of biologically active peptides that majorly comprise of cell-penetrating (CPPs) and antimicrobial peptides (AMPs). These peptides are unique in a way that their initial step of mechanism involves interaction with biological membranes. AMPs selectively act on microorganisms and subsequently result in their killing and on the other hand CPPs traverse biological membranes and are frequently employed as non-viral drug delivery vectors.

CPPs and AMPs possess overlapping characteristics and hence both the groups of peptides might have an ability to exhibit dual functions as antibiotic and/or cargo delivery vehicles. The attributes of certain CPPs to specifically target microbial membranes makes them ideal candidates of antimicrobials and an attempt has been made to evaluate the antimicrobial properties of CPPs in the present work. Two well-established CPPs- Cytoplasmic Localizing Peptide-1 (CyLoP-1) and Latarcin derivatives (LD) have been evaluated for AMP activity. Two microorganisms have been used as model organisms-Methicillin resistant Staphylococcus aureus (MRSA) and Fusarium solani, with an aim to evaluate AMP activity of CyLoP-1 and LD.
First objective of the study aims at evaluating the antimicrobial activity of the CyLoP-1 against representative plant and mammalian pathogens, and in-depth evaluation of the antimicrobial action of the peptide against MRSA. It has been observed that CyLoP-1 possess significant antimicrobial properties against many of representative microorganisms tested. It was observed that CyLoP-1 exerts its antimicrobial effect in MRSA within one hour of treatment and exhibited selective killing of MRSA, without affecting the health of HeLa cells. PI uptake studies revealed that CyLoP-1 kills MRSA by membrane damage. The study also revealed the importance of cysteine residues in exerting antimicrobial effect.

Second and third objective of the study aims at evaluating the antibacterial and antifungal effect of LD respectively. The study of LD against MRSA highlighted the antibacterial properties of LD. Two derivatives of Latarcins, LDP and LDP-NLS emerged as potential antibacterial peptides. LDP exhibited excellent antibacterial effect against both gram-positive and gram-negative bacteria, whereas LDP-NLS proved to be efficient against gram-positive bacteria. The cell-penetrating properties of the peptides in MRSA has also been evaluated in this objective. It was observed that LDP-NLS showed enhanced CPP activity than LDP in MRSA. It has been also observed that conjugation of NLS has reduced the cytotoxicity of LDP against HeLa cells apart from promoting the CPP activity. The study on mechanism of action showed that LDP-NLS exerted its antimicrobial effect by penetrating microbial membranes, whereas LDP exerted its antimicrobial effect by damaging the microbial membranes. The result was further reinforced by the ability of LDP-NLS to eliminate intracellular MRSA infection in HeLa cells.

On the similar lines, LD were evaluated for CPP and AMP activity in F. solani. It was observed that LDP and LDP-NLS exhibited antifungal property and killed F. solani in one hour of treatment. Uptake studies of LDP and LDP-NLS showed efficient cell-penetration in F. solani spores as well as hyphae. Dextran and PI uptake studies indicated that LDP-NLS formed pores
on *F. solani* membrane and subsequently killed the fungus. With an aim of testing the cytotoxicity of LDP and LDP-NLS in plant tissues, bean leaves were used as model systems and treated with peptides. Both LD and LDP-NLS did not exhibit cytotoxicity towards bean leaves. Thus, from the present work, it can be proposed that CyLoP-1 and LDP-NLS can act as efficient peptide antibiotics and drug delivery vectors.