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NOVEL APPROACHES IN DEVELOPMENT OF CELL PENETRATING PEPTIDES

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ABSTRACT
Therapeutic cargos which are impermeable to the cell can be delivered by cell penetrating peptides (CPPs). CPP-cargo complexes accumulate by endocytosis inside the cells but they fail to reach the cytosolic space properly as they are often trapped in the endocytic organelles. Here the CPP mediated endosomal escape and some strategies used to increase endosomal escape of CPP-cargo conjugates are discussed with evidence. Potential benefits can be obtained by peptides such as reduction in side effects, biocompatibility, easier synthesis and can be obtained at lower administered doses. The particular peptide known as cell penetrating peptides are able to translocate themselves across membrane with the carrier drugs with different mechanisms. This is of prime importance in drug delivery systems as they have capability to cross physiological membranes. This review describes various mechanisms for effective drug delivery and associated challenges.

INTRODUCTION

The lipid bilayer is hydrophobic in nature so it is impermeable for most hydrophilic molecules and renders protection from extracellular matrix. Internalization of large molecules such as protein occurs via endocytosis [1]. Some viral and non-viral delivery vectors have been developed to increase transport of active agent into the cells. Limitations of viral vectors are problem in production and immunogenicity [2]. These can be overcome by non-viral vectors but they also have some limitations such as cellular toxicity and cargo-vector complex instability [3]. In recent years some short, amphipathic or cationic peptides called cell penetrating peptides (CPPs) and protein transduction domains (PTDs) being able to translocate into mammalian cell by energy and receptor independent mechanism gained more attention as they have been used successfully to transport peptides, protein, siRNAs, antisense oligonucleotides, plasmids and large particles like liposomes into the cell both *in-vivo* and *in-vitro* [4,5]. CPPs were

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discovered in 1988 from Human immunodeficiency virus type 1(HIV-1) encoded on trans-activator of transcription (Tat) peptide [6,7] which can cross cell membranes. Few years later, small exogenous peptide was delivered using penetratin [8–11]. One Study suggest that the cellular uptake is mainly due to the small domains in these peptides so shortening of these translocation sequences without losing cellular uptake efficiency could be possible [12]. As the selection of CPP mainly depend on the application type, the most commonly used peptides are Tat, Pep-1, penetratin, polyarginine, transportan which can deliver small bio- molecules [13], nucleic acids [14], proteins [15–17].

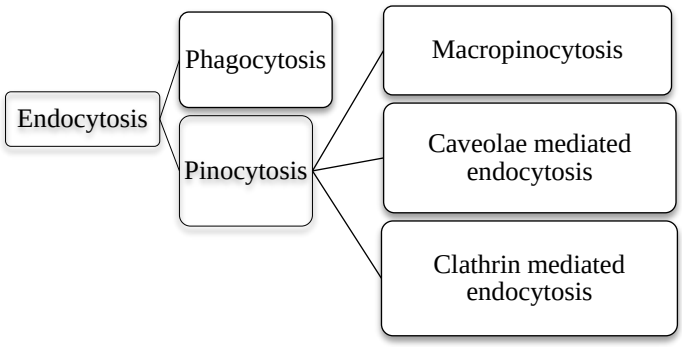

Figure 1 Types of endocytosis mechanisms

CLASSIFICATION

Based on linkage with cargo: [18][19]

Covalent bonding between CPP and cargo:

The covalent linkage present between CPPs and cargo made by chemical cross linking or cloning with expression of fusion protein of CPP [20] such as polyarginine Arg8 sequence, Tat peptide , transportan ,antimicrobial peptides SynB and buforin I, VP22 protein from Herpes Simplex Virus(HSV) [17,21,22].

Non-covalent bonding between CPP and cargo:

To improve intracellular delivery, CPPs are non-covalently bonded with biomolecules having polar and nonpolar domains.

Based on physicochemical property of CPP [18]

Cationic

This class has positive charge and polyarginine groups in primary sequence. E.g. Tat in HIV-1 contains lysine and arginine residues [23].

Amphipathic

This class has amphipathicity because of lysine residues. E.g. Transportan.

Hydrophobic

This class has very less importance as carriers and has only non-polar/hydrophobic sequences E.g. SG3, Pep-7

Based on origin of CPPs

Chimeric CPPs

They are made up of two or more motifs from different peptides. Example, Transportan derived from mastoparan and galanin.

Protein derived CPPs

Transactivators of gene transcription [24], viral envelope protein [25,26], antimicrobial peptides [27,28], DNA /RNA binding proteins [29], plant skeletal proteins [30] which can cross plasma membranes are natural proteins. E.g. Penetratin and Tat are derived from natural proteins.

Artificial CPPs

They are synthesized and designed on the basis of structure of naturally derived CPPs [31,32]. Example, polyarginine which mimics with arginine and is made artificially by many arginine residues as it helps in transduction mechanism [33].

STRUCTURAL REQUIREMENTS FOR CPPS

For the cellular entry, the first step is electrostatic interaction between proteoglycans charged negatively and phospholipids present on the surface of cell with basic CPPs [34][35–39]. Membrane binding and insertion can lead to direct translocation or endocytic pathways especially for amphipathic CPPs [40–42]. Hydrophobic or electrostatic interaction is affected by positive charge, hydrogen bonds and density [18,43,44].

Magic arginine and positive charge

Studies suggest that the residues of arginine were more effective for internalization than lysine residues, so by replacing the residues of lysine with arginine enhanced the uptake rates [45,46]. But the uptake efficiency is not only because of positive charge but also because of guanidinium head group of side chain of arginine and also number of residues of arginine. This is important as with 7-15 residues, it gives optimal uptake [7,45,47,48].

Hydrophobicity and ‘‘tryptophan power’’

Translocation across the plasma membrane bilayer can be increased by presence of hydrophobic residues in CPPs [49–51]. Studies suggest that by adding fluorescein isothiocynate (FITC) or tryptophan residue to the Tat peptide will increase translocation but caution must be observed because the deep

insertion in lipid bilayer membrane may decrease internalization as peptide will be stuck in the plasma membrane [52–54].

CELLULAR UPTAKE MECHANISM OF CPPS

For cellular uptake, it has been reported that positively charged amino acids such as arginine and lysine interact with acidic motifs containing proteoglycans of plasma membrane in receptor-independent manner [19,55–57]. After this interaction, peptides undergo internalization which will be dependent on type of CPPs, size and charge of cargo [58,59]. One study has indicated that Tat and Antp CPP internalization can be mediated by caveolin-dependent endocytosis, clathrin-dependent endocytosis, macro pinocytosis and direct intracellular translocation [60].

Endocytosis

Receptor mediated endocytosis

In this type of mechanism, the cell surface receptors first recognize the ligand and uptake mechanism is mediated by invagination of plasma membrane to from vacuole which is the energy dependent process and depends on clathrin for mediation of invagination process and also involves actin and microtubule filaments.

Pinocytosis

In this mechanism ions or molecules gain entry in the cytoplasm but only small molecules can be engulfed by this mechanism which occurs continuously.

Macropinocytosis

Larger molecules can be engulfed into the cell without formation of endocytic vesicle using RhoGTPases, form the irregular, large vesicles at the site of membrane ruffling because of the closure of lamellipodia. The ruffling of membrane in this process is predominantly actin-driven and so there is no need of clathrin.

Potocytosis / caveolae mediated uptake

Flask shaped regions of the plasma membrane characterized by the filamentous caveolins coat lining the inner surface known as caveolae used to transport both large and small molecules. It is not associated with the clathrin and can transport the molecule into the cell bypassing the lysosome in that way it differ from endosomes [61].

Receptor independent uptake

In this mechanism, specific cell surface receptors are not required but the overall charge of peptide is important [62]. Arg residues are important in mediating translocation which was demonstrated by the structural studies of Tat PTD [34,45].

Direct penetration

Direct penetration can be possible only when the concentration of CPPs is high, while endocytosis is possible in almost all the cases [31,63–65]. It is energy independent process so occurs only when there is high concentration of CPPs and even in the presence of endocytosis inhibitors and at low temperature (4^0C). It involves interaction of negatively charged cell membrane components and positively charged CPPs.

Barrel stave model

It requires CPP with helix conformation where hydrophilic residue forms the internal environment of pore and hydrophobic residue bind with hydrophobic tail of lipid bilayer. Such as alamethicin, which forms the trans-membrane pores having 3-11 parallel helical structure [66].

Toroidal pore model

It also dependent on the helix conformation of CPP but differs in the mechanism of pore formation. Pore formation occurs because of peptides associated with the polar head groups of the lipid inside the cell membrane. The hydrophilic core of the toroidal pore is lined with the inserted peptides and hydrophilic head group of the phospholipid cell membrane [67]. Magainins, protegrins and melittin induce the formation of toroidal pores [66].

Carpet like model

It is based on the cell membrane destabilization and reorganization because of electrostatic interactions between cationic charged particles and anionic head groups of phospholipids [68]. The concentration of CPPs must be high to form the carpet like membrane coating then only permeation across the membrane occurs. Main difference is absence of peptide internalization into the hydrophobic core which was shown in barrel stave model [69].

Inverted micelle formation

When the lipid bilayer is converted into a micelle, it will lead to formation of transient hole. The interaction between hydrophobic part of cell membrane and hydrophilic residue of CPP and also interaction between cationic charged CPPs and anionic charged membrane is responsible [70]. Octa-arginine is internalized effectively with inverted micelle formation.

Endosomal escape of CPPs

The drawbacks of endocytosis include endosomal accumulation and degradation in the endosome. The CPP-cargo complex can interact with the phospholipid known as Bis (Monoacylglycero) Phosphate (BMP), which is a part of endosomal membrane.

Strategy to improve endosomal escape

Multivalent CPPs enhance the endosomolytic activity by strong interaction with the BMPs in membranes, and they can escape efficiently from the endosomes than monomeric CPPs. This approach is based on increasing the local concentration of the CPP by presenting large number of copies of CPPs on a delivery vector where the peptide interacts with cellular components [71,72,81–84,73–80]. Multivalency can be achieved by chemical conjugation of CPPs to dendrimers, by conjugating a protein oligomerization domain to the CPP, or by attaching CPPs to the branched oligopeptides, such as the fork-like structure of glutamic acid or lysine. Limitations of this approach are higher risk of immunogenic properties and difficulty in chemical synthesis of multivalent CPPs. It is important to balance the number of branches of CPP, to obtain a strong but not too extensive reaction. Cyclization of CPPs rich in arginine led to efficient cellular uptake process and delivery to the nucleus and cytoplasm [65,85,86].

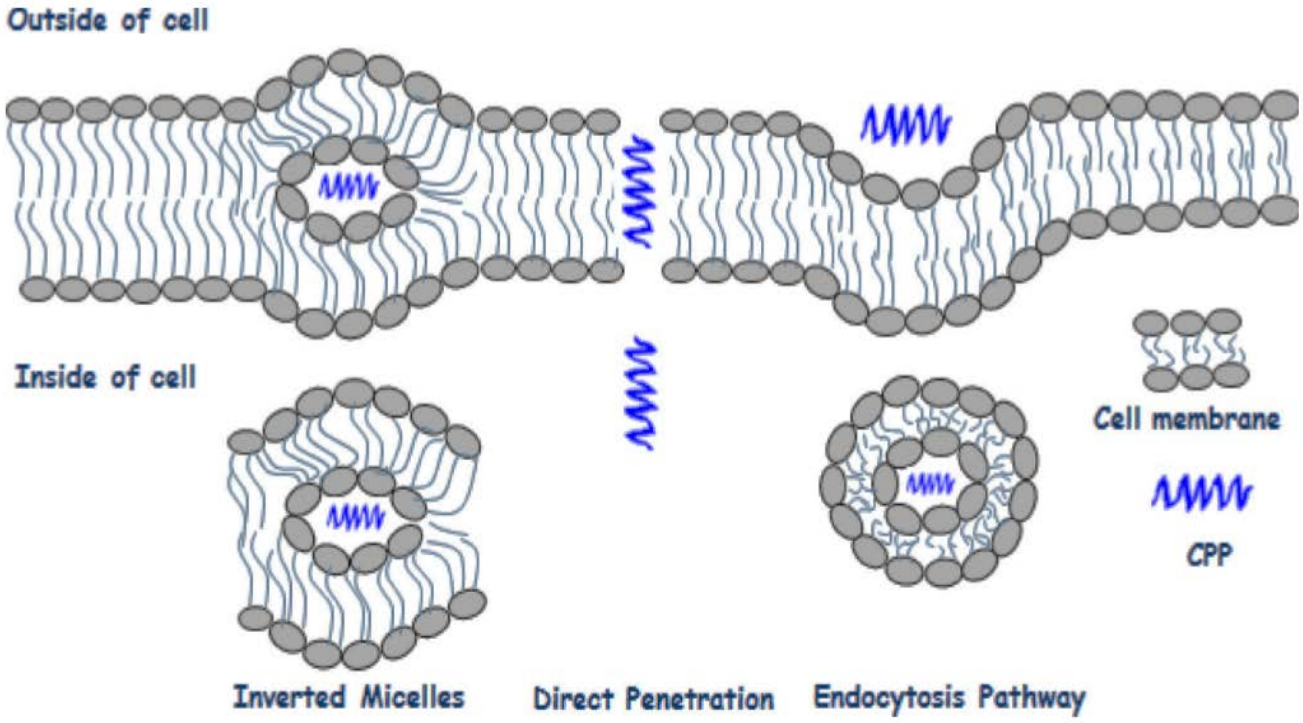

Figure 2 Mechanisms of CPP's entrance through cellular membrane

[Source of figure: Derakhshankhah H, Jafari S. Cell penetrating peptides: A concise review with emphasis on biomedical applications. Biomed Pharmacother 2018; 108:1090–6. https://doi.org/10.1016/j.biopha.2018.09.097.]

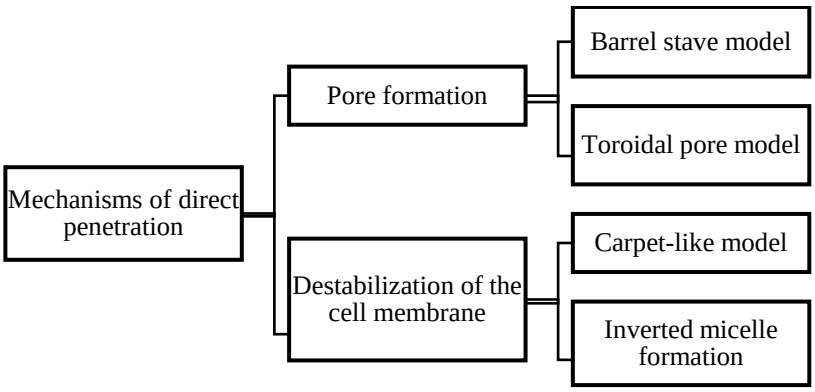

Figure 3 Mechanisms of direct penetration

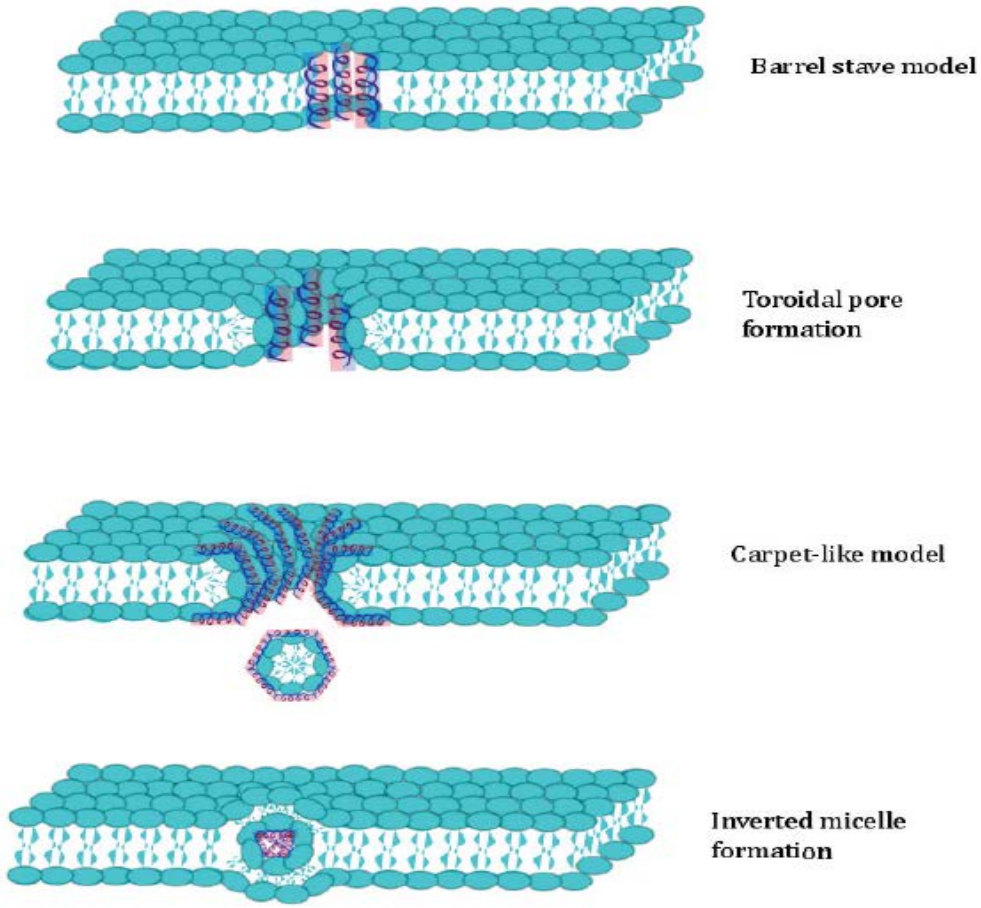

Figure 4 Schematic models of direct penetration of CPPs through cell membrane. The hydrophilic parts of the peptides are red colored and the hydrophobic parts of the peptides are blue colored

[Source of figure: Böhmová E, Machová D, Pechar M, Pola R, Venclíková K, Janoušková O, et al. Cell-penetrating peptides: A useful tool for the delivery of various cargoes into cells. Physiol Res 2018;67:s267–79.]

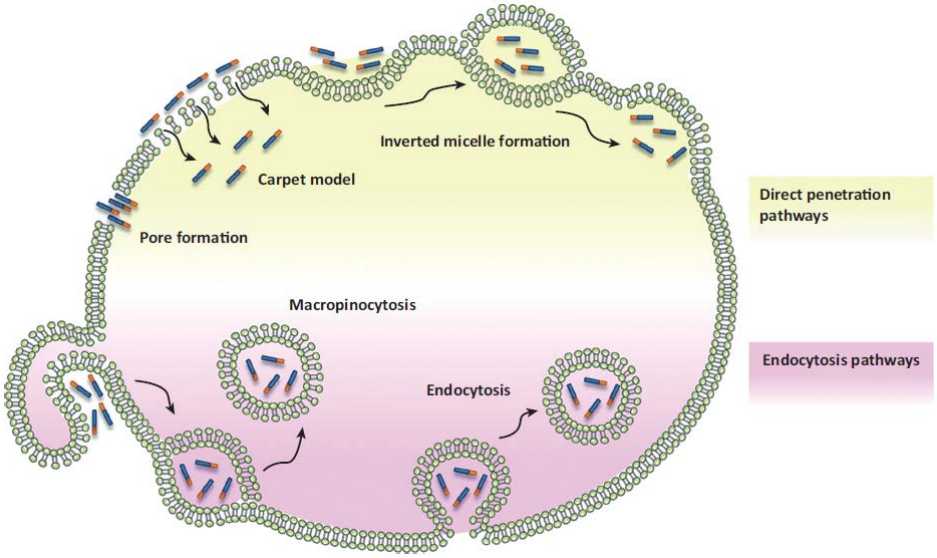

Figure 5 Schematic Representation of Proposed Mechanisms for Cell-Penetrating Peptide (CPP) Internalization

[Source of figure: Guidotti G, Brambilla L, Rossi D. Cell-Penetrating Peptides : From Basic Research to Clinics. Trends Pharmacol Sci 2017;38:406–24. https://doi.org/10.1016/j.tips.2017.01.003.]

METHODS TO STUDY MECHANISM OF CPPS

Various biophysical and biological methods are used to quantify CPPs and cargos inside the cell and to study the internalization mechanism.

In cellulo approaches

These methods are indirect and used to detect the biological activity of the cargos by fluorescence [87]. Direct method had been developed for quantification of intact CPPs bound to the cellular membranes or inside the cells, based on matrix-assisted laser desorption-time of flight mass spectrometry(MALDI-TOF MS) [88,89]. Other biophysical methods are also used in the living cells, like electron microscopy to study the distribution of peptides and membrane structures induced by CPPs [90,91] and in cell Raman spectroscopy to know the secondary structure of peptide in the cellular compartments [92].

Fluorescence-based protocols

These are the most commonly used methods. In fluorimetry the fluorophore is covalently attached with the peptides and the measurement of fluorescence will directly quantify the peptides. In the confocal microscopy there is localization of the probes inside the living cells. It is convenient but has some limitations such as quenching of the fluorescence because of the accumulation in the subcellular compartments therefore may give inaccurate results [93].

Functional assays in cells

These methods are used to detect the biological activity of cargoes or conjugated molecules [94,95]. These approaches are very useful for the biotechnological and therapeutic applications [96,97].

APPLICATIONS

Cell-penetrating peptides as delivery vectors

Major challenge in the drug delivery is often the inability of drug to cross the lipid membrane of the cell but CPP can transport different cargos across the lipid membrane.

Peptides as cargo

Use of small peptide is better than the full length protein in several ways, such as purification of peptide is easy as can be synthesized daily while purification of protein is money and time consuming process. Peptides have great therapeutic potential in the treatment of several diseases such as diabetes, cancer, influenza, neurodegenerative disorders with less side effects [16,98]. Therefore, in future impermeable bioactive peptides can be used for therapy both in vivo and vitro using CPPs for the delivery [17,99].

Delivery of other cargo by CPP

Liposomes have been used to enhance the solubility and half-life and reduce the toxicity but the cell penetration is very slow which limits their use. Conjugation of Antp or Tatp on the surface of the liposomes improves the cellular delivery and show efficient and fast translocation into cytoplasm [100–102]. Fluorescent microscopic observation of the markers trapped inside the liposomes showed the liposomes remain intact for few hours in the cytoplasm and then migrate towards the nucleus slowly and release the contents into cytoplasm. Tatp-liposomes used as vectors for gene delivery, result as with high *in-vitro* transfection efficiency and are less cytotoxic [102]. Peptide-based imaging agents OxorheniumV and Oxotechnetium V can be delivered by CPPs into the cellular compartments to achieve high intracellular concentrations to carry out radio therapy and imaging [103]. The intracellular uptake of the paramagnetic nanoparticles can be significantly improved by Tatp which can be detected easily through magnetic resonance imaging (MRI) [34,104,105].

Cell penetrating peptides in biopharmaceuticals

The membrane of the cell prevents the entry of peptides, proteins and drug carriers into the cell unless transported by an active transport [106]. So CPPs are used to promote the delivery of biopharmaceutical agents into the Cell which includes SiRNA delivery, Antisense oligonucleotide delivery and delivery of drug carriers.

SiRNA delivery

CPPs and siRNA can be conjugated non-covalently or covalently easily. Covalently linked siRNAs to Penetratin or Transportan associated with the silencing response have high reproducibility [107]. Non-covalent complexes with siRNA have net positive charge [108]. However the non-covalent strategy is more efficient for delivery of siRNA [109,110].

Antisense oligonucleotide delivery

Antisense technology is based on the use of oligonucleotides (ONs) specific to sequence that can hybridize with the complementary mRNA strands lead to mRNA degradation by activation of the cellular enzymes belongs to the RNaseH family or translational arrest and prevent the gene expression [111]. The therapeutically potential ONs include aptamers, ribozymes, antisense ONs, triplex-forming ONs, immunostimulatory CpG motifs. CPPs can be used for the delivery of ONs with the

therapeutic agent by either a non-covalent or covalent linkage [111,112].

Delivery of drug carriers

Nanoparticles, liposomes, and other different types of nanocarriers have been used to modulate their biodistribution and pharmacokinetics, improve the drugs stability, decrease side-effects and increase efficacy [106]. However, the main challenge is intracellular delivery of these large molecules because of their hydrophobic/hydrophilic nature and three-dimensional structure [113]. CPPs have been used to deliver the therapeutic molecules which are 200 times larger than the current bioavailability size restriction [114].

Imaging applications

The technology is similar to that used for cancer therapeutics. CPPs having peptide with the transduction ability labeled fluorescently and attached with the cleavable linker for example, proteases expressed by tumor tissue recognize the cleavable site present in the linker. The neutralizing peptide is cleaved off when exposed to tumor tissue and its associated proteases, giving high concentration of CPP locally which leads to increased uptake by the tumor tissue [115,116]. Quantum dots (QD) are photostable, semiconductor nanocrystals, having diameter of 1–6 nm, brightly fluorescent, used mainly for biological imaging. Benefits of QDs over the traditional dyes are narrow emission peak, high quantum yield, resistance to the photo-bleaching and dependent on the size, broad photoluminescence. But major limitation is their inability to cross the plasma membrane. To overcome this limitation, CPPs, most commonly Tat, has been used. Dynamic confocal imaging studies suggested that Tat-QD conjugates were internalized by macropinocytosis which was triggered by the binding of the conjugate to the negatively charged cell membranes [117–119].

Application of CPPs in gene therapy

CPPs offer many advantages for cellular delivery, for example *in-vivo* efficacy, applicability in various types of cell, favorable nuclear targeting, no restriction for cargo size, non-immunogenic [62]. CPPs can also able to deliver nucleic acids, peptides into the bacterial cells. For treatment of genetic disorders therapeutically active genes are incorporated to cure the mutation. The major challenge is DNA delivery across the biological membranes- plasma membrane and nuclear membrane with minimum cell toxicity. Viral gene delivery vectors have efficient capability of gene transfer but some drawbacks which limit their use are oncogenicity, pathogenicity and stimulate ions of immunological responses in the host [111]. Non-viral gene delivery vectors are safe but the limitation is their inefficiency. With the use of CPP based delivery systems problems with non-viral gene therapy can be solved and also there is improvement in the viral gene therapy to some extent [120–123].

Delivery of DNA to the intracellular environment through synthetic CPPs

Oligonucleotides have been modified in many ways, such as with modification of chemical group, changes in the sequence, and the use of analogues of nucleotide show varying antisense activity. Modification in the sugar-phosphate backbone of the oligonucleotides is very important as it plays a role in gene silencing and membrane translocation [120]. Improvements to ON modifications are constantly being developed [124]. The degradation issues of naturally occurring oligonucleotides can be overcome by using ONs containing nucleotide analogues. For example, morpholinos, which are modified ONs, having standard nucleic acid bases, but are bound to the morpholine rings instead of deoxyribose rings and attached through the phosphorodiamidate groups instead of phosphates [125]. These changes make them resistant to the nuclease degradation and prevent immune responses. Synthetic CPPs have been developed to overcome the problem of inefficient gene transfer by non-viral vectors. Synthetic CPPs have been designed in the way that they can condense the DNA and transport it into the cell through the bilayer of lipid, either via endosome where CPP destabilize the endosomal lipid bilayer at low pH and mediate the plasmid release or directly in the case of amphipathic CPPs [126].

Suicidal gene therapy approaches

It is widely used for treating hyperproliferative disorders and cancer. It is based on introduction of gene into the target cells which encode the enzyme that converts inactive prodrug to the potent cytotoxic agent. Various prodrug /enzyme combinations have been developed, but the most commonly used is HSV-1 thymidine kinase (TK)/ganciclovir (GCV) combination [34,127].

Transdermal delivery with CPPs

Skin act as barrier for the macromolecules to deliver across the skin [128]. The barrier function of skin is because the stratum corneum has highly organized structure [129]. It protects the body from the outside environment, but also acts as epidermal

permeability barrier for the delivery of therapeutic agents for treatment of skin diseases. The drugs for the treatment of primary cutaneous disease are administered systemically because of poor absorption of drug through skin and very low topical bioavailability [130]. Topical delivery of peptides has been studied because these compounds are important in the treatment of skin diseases and improvement in the skin properties in case of cosmeceuticals. Administration of several peptides by topical route would be better, such as TGF-b, IGF-1, leptin for wound healing, interferon as antiviral, bacitracin for the skin infection, cyclosporine for the treatment of autoimmune diseases [130,131]. Several peptides have been studied as antigens by applying to the skin for the development of topical vaccines [132].

Anti-inflammation therapy

Antisense peptide nucleic acids (PNAs) have been demonstrated to prevent the growth of E. coli and gene expression and are good anti-inflammatory agent. For the efficient delivery of PNAs, PNAs are conjugated with the CPPs (CPP-PNA complex) [133]. For example, administration of acpP-targeting PNA conjugated with the CPP into E. coli K-12-infected BALB/c mice enhanced survival of the infected mice, prevented the fatal infection and reduced bacterial blood counts [134].

Tumor therapy

Conventional chemotherapy cause severe side effects because of lack of specificity to the tumor cell and has low concentration of drug at the local tumor areas. Efficient strategies for targeting tumor have been developed to overcome these limitations. Conjugation of antitumor agents with the CPPs has increased the efficiency of tumor therapy. CPPs can be used in tumor therapy as the conjugated antitumor therapeutics have increased permeability through the cellular membrane so targeting of tumor cells can be possible [135]. Bleomycin (BLM) is extensively used as an anticancer agent, but its effect is dependent on the cytosolic accumulation. The artificial R8-DOPE-BLM conjugate can enter into cytosol resulting in strong induction of tumor cell death and in vitro DNA damage compared to BLM. Similar results have been obtained by combination of CPP with Taxol, doxorubicin, methotrexate [136–138]. These data indicate that CPP conjugated antitumor agents can improve the treatment by increase in the concentration of drug at the tumor tissue.

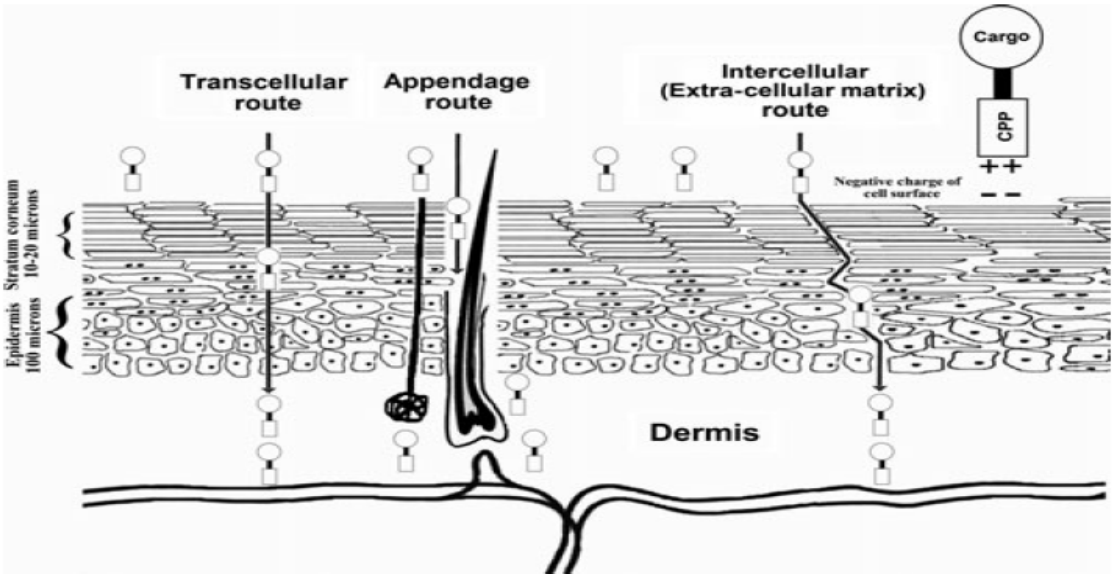

Figure 6 Schematic diagram of routes for topical delivery of cell-penetrating peptides (CPP)/Cargo complexes via human skin *[Source of figure: Nasrollahi SA, Taghibiglou C, Azizi E, Farboud ES. Cell-penetrating Peptides as a Novel Transdermal Drug Delivery System. Chem Biol Drug Des 2012;80:639–46. https://doi.org/10.1111/cbdd.12008.]*

Protein and nucleic acid delivery

Large macromolecules, such as proteins and nucleic acids are not able to penetrate the plasma membrane so are unable to enter into the cells. CPPs can be used as a delivery tool for proteins and nucleic acids as they enhance the cellular uptake of the large molecules. For the treatment of infectious diseases, cancer and genetic disorders, siRNA can be used for gene silencing [139]. CPPs can overcome the problem of low permeability and may

lead to the internalization of siRNA [140]. A CPP-siRNA complex was synthesized by disulfide shown to efficiently decrease the expression of reporter transgenes in several mammalian cells [141].

Biomedical applications of CPPs

The cell membrane act as barrier for peptides, proteins and drug carriers and prevent them from entering into the cells except an active transport mechanism is involved. CPPs can easily deliver the drugs intracellularly as they can carry cargos without injury to the cell. CPPs can also be used in biomedical applications such as direct action as antifungal, antimicrobials, imaging, and anti-parasitic and as a carrier to deliver the drugs, nucleotides, small interfering RNA (siRNA), peptides and proteins [114,142].

Antifungal and antimicrobial action of CPPs

From studies it is found that the CPPs have capability to disrupt the cell membranes of fungi and bacteria. Antimicrobial peptides and CPPs have similar structural features such as positive charge and size which increases their interactions with anionic bio-membranes. Antimicrobial activity of CPPs and their derivatives is because of arginine residue in the peptide sequence. The antimicrobial activity and uptake of CPPs can be enhanced in the presence of multiple guanidinum groups. Number of studies has been investigated for the antibiotic activity of penetratin and Tat against Gram-negative and Gram-positive bacteria [143].

CPPs improving intracellular delivery of anti-parasitic drugs

Protozoan parasites cause serious human infections such as leishmaniasis, malaria. The therapeutic application of anti-parasitic drugs is limited by increasing levels of resistance and poor intracellular access. Here, CPPs have been used to carry the active compounds across the parasite membranes, for improving the efficacy of anti-parasitic drugs. Miltefosine was the first orally active leishmanicidal drug but its clinical application is limited because of resistance mechanisms. But leishmaniasis resistance to miltefosine can be treated by conjugation to Tat in which the conjugate was internalized into the R40 Leishmania strain efficiently where Miltefosine alone was not permeable, resulting in fast killing of parasites [144,145].

CPPs-modified pH-sensitive delivery

The exploitation of the acidic pH can improve the cytoplasmic delivery of cargo molecules performed with CPPs. CPPs with nanocarriers have triggered exposure mechanisms, such as degradation of enzyme of the protective coat, acid degradable cross-links, allows their controlled effect at the site of tumor microenvironment, because of the presence of pH gradient between physiological environment and the tumor milieu. So the therapeutic efficiency of nanocarriers enhanced and reduced toxicity [146].

ADVANCES IN CELL PENETRATING PEPTIDE DEVELOPMENT

Chemical modification of CPP for enhanced delivery

Amino acid substitution

By the amino acid substitution in the cell penetrating peptides, desired properties of peptide like cationic nature or hydrophobicity can be achieved. This strategy has resulted in increased intracellular internalization by certain CPPs. Kaeko *et al*. conducted a comprehensive search for novel CPPs using an in vitro virus library of peptides consisting of 15 amino acids and reported improved intracellular translocation efficiency at low concentrations due to the effect of cationic amino acids. As the amino acid Arginine has strong affinity to the surface of the cell, substituting it with another amino acid in the peptide chain such as Lysine improved the intracellular translocation significantly even at low concentration[147,148].

Modification in functional group

It involves the formation of linkage or masking groups to the highly reactive sites in the peptide chain. The peptide bonds formed should be weak and so that can be easily broken by simple variation of physiological conditions for regeneration of the cell penetrating peptide [114,149].

CHALLENGES OF CPPS

Biosafety and cytotoxicity are the main challenges for CPPs. The studies have been found that CPPs are less cytotoxic; but it should be considered that everything can become cytotoxic in a certain dose threshold. CPPs generally show two types of cytotoxic effects: 1) cytotoxic effects arising from the specific interaction of CPPs with cellular components 2) cytotoxic effect on the cell and also organelle membranes [150,151].

LIMITATIONS AND FUTURE DIRECTION OF CELL-PENETRATING PEPTIDE-BASED STRATEGIES

CPPs based therapies pose three main limitations. The first limitation is the absence of specificity to the tumor cells over normal cells in case of anticancer therapy. Most anticancer drugs

interfere with the replication of cell therefore show similar effects on the proliferating tissues. The interaction with targeted tumor cells would result in therapeutic functions of the drug, but interaction with normal cells can cause toxic side effects. The second limitation is the rapid clearance of water soluble drugs having low molecular weight from the bloodstream, and immunogenicity and/or proteolytic degradation of large protein or nucleic acid -type drugs. The third limitation is the difficulty in penetrating through cell membrane. The first limitation of insufficient tumor selectivity can be overcome by attaching the drug to the targeting component such as a peptide ligand or an antibody. A combination of prodrug and targeted delivery system is a solution to this limitation, as drug remains in inactive form during the targeting and delivery process and then converted to the active form at the targeted site [112]. The second limitation, which is related to the pharmacokinetic properties of the drugs, can be managed. In the natural systems, the pharmacokinetic behavior of many small drugs is regulated by series of transport proteins [152]. So, binding of such drugs to the macromolecule or a carrier would prolong their circulation time [99].

CONCLUSION

CPPs can transport the wide range of therapeutic agents and macromolecules across the biomembranes, enabling their localization to the cell nucleus, cytoplasm and various tissues for execution of their different functions: permeation through the skin mucosa to develop percutaneous delivery of nucleic-acid and protein drugs for clinical use, penetration through intestinal mucosa to increase oral bioavailability and the rate of drug absorption. The penetration capacity of the CPPs can be used for the study of the functional effects and intracellular mechanisms of biomolecules.

The main limitations of CPPs are easy degradation by plasma proteases, and lack of specificity, which may lead to loss of membrane permeation ability of CPPs. Modified CPPs can facilitate the endosomal escape, increase drug permeating efficiency, improve the tumor targeting and stimulus responsive controlled release of drug specific for the tumor microenvironment. Use of CPPs will lead to convenient and effective multifunctional drug delivery system which is important in the clinical applications and promote the research of new drugs.

ABBREVIATIONS

as = Antisense
CPPs = Cell-penetrating peptides
HA = Hemagglutinin
HSV-1 = HERPES Simplex virus type 1
ON = Oligonucleotide
Pen = Penetratin
PG = Proteoglycan
PNA = Peptide nucleic acid
PTD = Protein transduction domain
siRNA = Small interferring RNA
TP = Transportan
ACPPs = Activatable CPPs
Antp = Antennapedia homeodomain
DNA = Deoxyribonucleic Acid
FITC = Fluorescein Isothiocyanate
MRI = Magnetic Resonance Imaging
mRNA = Messenger Ribonucleic Acid
RNA = Ribonucleic Acid
Tatp = Trans-activator of transcription (Tat) peptide
US = Ultrasound

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Vatsal Shah compiled and organized the data of the work and contributed to the drafting of the manuscript. Yamini Shah conceptualized the work and interpreted the data. Mansi Athalye contributed to the writing of the final manuscript. All authors read and approved the final manuscript.

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