Review on: Cell Penetrating Peptide-enabled Drug Delivery into a Targeted Cell

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Cell penetrating peptide-enabled drug delivery into a targeted cell" submitted by Afrina Naznin (17146046) of Spring, 2021 has been accepted as satisfactory in partial fulfillment ofthe requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not involve any human or animal trial.

Dedication

Dedicated to my parents

Abstract

In the past two to three decades, researchers have looked into a variety of perspectives and methods for cell penetrating peptides-mediated drug delivery, which has formed the basis of a brilliant method for delivering drugs. Their capacity to break through cellular membranes and transport a wide variety of contents into the cells led to their selection as an ideal delivery system. Preclinical studies suggest that these chemicals may be useful in treating neurodegenerative diseases like Parkinson's and Alzheimer's since they may relatively easily breach the blood-brain barrier. Amidst that, no cpp-cargo has been approved by the FDA as of yet due to specific restrictions. However, scientists are continually attempting to overcome these constraints in order to obtain more accurate results.

Keywords: cell penetrating peptides, cellular uptake mechanism, drug delivery, translocate Nano particles.

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Table of Contents

List of Tables

List of Figures

List of Acronym

PTDS- Protein transduction domain

CPP- Cell penetrating peptide

FDA- Food and drug administration

TAT-Tran's activator of transcription

HSV-1 - Herpes Simplex Virus type-1

SLN- Solid lipid nanoparticles

NLC-Nano structured lipid carriers

LN- Lornoxicam

ivit- Intravitreal

CNV- Choroidal neovascularization

MMP- Matrix metalloproteinase

RS- Rasagiline

MAP-Amphipathic model peptide

DA -Dopamine

TP- Transportan

PD- Parkinson disease

MPTP - 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine

COMT-catechol-O-methyltransferase

AD- Alzheimer disease

RU- Ruthenium

PEG-Polyethylene glycol

AB-Amyloid beta

PF-Pepfects

BBB-Blood brain barrier

CNS- Central nervous system

Chapter 1

1.1 Introduction

The use of biologically active molecules like protein, peptide and oligonucleotides has been emerging in the therapeutic field from a few decades now. These bioactive compounds were chosen because they are highly selective for target cells and well tolerated, whereas a wider variety of bioactive molecules have trouble reaching the target due to their hydrophilic nature. Many therapeutic trials have investigated small molecules such as peptides and oligonucleotides, but due to their poor membrane permeability, it has been challenging to get the drug concentration of interest. Therefore, a new strategy is urgently needs for delivering the macromolecules, like liposomes nanoparticles etc.

Normally, the endocytosis pathway allows hydrophilic macromolecules to enter the cell. Then, to get around these problems, some peptides were found that can pass through the plasma membrane and bring hydrophilic macromolecules into the cell through a pathway that doesn't need energy (Gupta et al, 2005). These peptides are now recognized as cell penetrating peptides, and they are also known as protein transduction domains (PTDS). CPPS is currently extensively researched and used in the detection and treatment of diseases such as cancer, neurological disorders, inflammation, and others. This review investigates the cellular uptake of these cell-penetrating peptides (CPPS) and their therapeutic applications.

1.2 Rationale of the study

CPPS are capable of transporting Cargos (biologically active conjugates) insides cells e.g./including protein, siRNA, small drugs molecules. CPPs have been utilized therapeutically more frequently because targeted drug delivery increases drug distribution and lowers dosage and toxicity. Nanoparticle CPPs are used to deliver various therapeutics; nevertheless, the FDA has not yet given its approval to any CPPs or CPP/cargo complexes. Although preclinical assay with CPP mediated therapeutics shows efficient results in various models, some even reached phase 1, phase 2 and phase 3 also. The results of preclinical studies have also widened the possibilities for the development of CPP-based human therapeutics.

Chapter 2

Methodology

Firstly, I searched for the scientific articles and papers relevant to my topic over the Internet. About 80 papers were gone through on Elsevier, Science Direct, pub med, Research gate, MDPI and pharmaceutics. Lastly 40 papers were chosen as they‘re more relevant to the topic and had most recent years of publication for getting the updated information. Using the available information on the papers I made an outline and gathered them altogether and assembled them. All the information was re written and references were added using APA referencing system. The review entitled “Cell penetrating peptide-enabled drug delivery into a targeted cell” was conducted in this manner.

Chapter 3

Overview on cell penetrating peptide

3.1 What is CPP?

Short (4–40 aa) peptides that can enter cells through endocytosis, by enhancing intracellular activity, or by interacting with covalently or non-covalently linked bioactive passengers, known as cell-penetrating peptides (Langel 2021). Because of their unique characteristics such as water solubility, cationic or amphipathic nature, or both, etc., CPP is a good drug delivery vector. An essential factor regarding CPPs is that they can move across the plasma membrane both in vivo and in vitro at lower micromolar concentrations without doing much damage to the membrane. For a drug to reach its target cell, it has to go through several difficult steps. These steps include wide circulation, invasion of biological barriers, cellular uptake by cells, and endosomal escape towards intracellular region upon endocytosis. In addition, plasma membranes function as biochemical barriers that prevent external entry, making it difficult for many bioactive chemicals to enter cells and have the desired therapeutic effect. In fact it's been found in various extensive therapeutic studies that peptides and oligonucleotides have relatively low Tran's membrane efficiency as a result the therapeutic site cannot achieve desired drug concentration. Mostly the delivery of macromolecules like nanoparticles, liposomes, microinjections has poor specificity, immunogenicity and high in toxicity which results low delivery efficiency and efficacy (Jing et al., 2020). Therefore, the advantage of adopting CPPs for therapeutic drug delivery is that they are less toxic or completely non-toxic as especially in contrast to some of the other intracellular delivery systems such liposomes, polymers, etc (Sonia et al., 2020).

3.2 An overview on the history of CPP

The protein/peptide transduction domains (PTD), often referred to as CPPS, were first identified in 1988 by two different groups. They said that the HIV-1 TAT (Tran's activator of transcription) protein could get into cells when tested in a lab (Guidotti et al., 2017). Together with Lowenstein and Green M, they created an assay to produce the complete-length, 86-amino-acid TAT protein and show that it is biologically active. The 86-amino-acid HIV-1 TAT protein was chemically produced, and TAT-86 transactivated HIV-LTR and was very effectively absorbed by cells from the cell medium. Later on the Alain prochinatz introduced the home domain of Antennapedia and its penetratin peptide. According to Langel u, it contributed to the leaping point for research into the CPP. Currently, the website CPP site 2.0 (http://crdd.osdd.net/raghava/cppsite/) contains a dataset of some 1700 unique CPPS, with many additional awaiting confirmation (Langel 2021).

3.3 Classification of CPP

Three distinct groups of cell-penetrating peptides are (a) cationic, (b) amphipathic, and (c) hydrophobic. Cationic CPPs are made up of a short set of amino acids (arginine, lysine, and histidine), and amphipathic CPPs have tails that are both lipophilic and hydrophilic. The translocation is primarily mediated by amphipathic CPPs (Sonia et al., 2020).

3.3.1 Cationic CPP

Peptides with highly positive net charge fall under the Cationic class category. They are derived mostly from arginine and lysine, two fundamental short strands of protein. Examples are penetratin, TAT derived peptides, polyarginines (Guidotti et al., 2017). Cationic CPPs have a stronger affinity towards cytoplasmic membranes under situations where the pH is normal for the physiological environment. They are electrostatically attracted to the negatively charged glycoprotein on the cell membrane, where they merge and are then transported inside the cell. (Xie et al., 2020). Additionally, a hydrogen bond is formed between the negatively charged phosphate and sulfate groups on the cell membrane and the positively charged guanidine head group in the arginine. This causes the arginine to move throughout the cell (koren et al., 2012). The activity of cationic CPP is affected by things like how many positively charged arginines are in the CPP structure and where they are located (Xu et al., 2019). Tunnemann et al., in their assessment found that the arginine-rich peptides had the high transduction efficiency (Koren et al., 2012).

Despite having the same net charge as arginine, lysine is less permeable to the plasma membrane because it lacks the guanidine head group. Most cationic CPPs have over five positively charged amino acids. Poly-arginine stretches are most capable of being absorbed by cells and have therapeutic potential. Another finding indicates that oligo-capacity arginine's for internalization rises with length. However, R8 to R10 is the ideal length for delivery. Higher values will harm the cells permanently and decrease the effectiveness of delivery as a whole.

3.3.2 Amphipathic CPP

This CPP class compared with a high degree of amphipathicity they contain both hydrophobic and hydrophilic region of amino acid. The charge contribution of this peptides primarily originates from the lysine residue but this peptides are also reached in hydrophobic residue, like alanine, leucine, valine (Guidotti et al., 2017). Examples include MAP, PEP-1, and transportan. PEP-1 was the first peptide to be used extensively to transport proteins into cells without a covalent link (Koren et al., 2012). According to Milletti F, amphipathic CPPS can be categorized into three sub category (a) primary amphipathic, (b) secondary amphipathic helical and CPPS (c) protein rich amphipathic CPP. The precise targeting of a NLS to a cell membrane is made possible by the chimeric shape of many amphipathic CPPs. The chimeric structure of these primary amphipathic in obtained by covalent bond with a hydrophobic domain. Most primary amphipathic CPPs come from proteins found in nature. Like PVEC and ARF (1-22). The reverse face of secondary amphipathic -helical CPPs may be cationic, anionic, or even polar. β Sheet amphipathic CPPs have one stretch of amino acids that is hydrophobic and one length is hydrophilic which is exposed to the solvent (Milletti et al., 2012). Proline-rich CPPS molecules are considered to be extremely peptides due to the fact that, although their sequence and structure can vary between families, all of the families share a similar proline pyrolidine template. And as a result of this peculiar pyrolidine ring, proline stands out among the other 20 genetically encoded amino acids with its peculiar characteristics. Examples are synthetic fragments of Bac7 (Guidotti et al., 2017).

3.3.3 Hydrophobic CPP

Compared to the other two peptides that have been discovered so far, hydrophobic CPPs are less common. Peptides like vascular endothelial-cadherin (pVEC) and MPG show that charged and hydrophobic residues are spatially segregated along the chain's length (koren et al., 2012). Hydrophobic peptides contain non polar residues which results in low net charge. Examples of hydrophobic CPPs are mostly based on natural amino acid; C10SY peptide and its PFVYL 1 c-terminal portion, pep-7 peptide. This class also has some chemically modified peptide (Guidotti et al., 2017).

3.4 Cellular uptake mechanism of CPP

The process through which these penetrating peptides enter the cell remains debatable. The mechanisms that are involved are still not completely understood. Although two mechanisms been recognized to be common in all research (a) energy independent also known as direct penetration or membrane transduction and (b) endocytosis. Initially, direct penetration was believed to be the primary method for CPP internalization but latter this misconception was cleared and after several investigations, endocytosis is the pathway that most CPP and its cargo molecules use for efficient cell internalization. Entry of the CPP or its cargo units depends on several factors, such as their size, purity level of the used CPP-cargo conjugate, physiochemical properties or the concentration gradient. These factor play a vital role weather cellular intake will be placed or not (Muller et al., 2008).

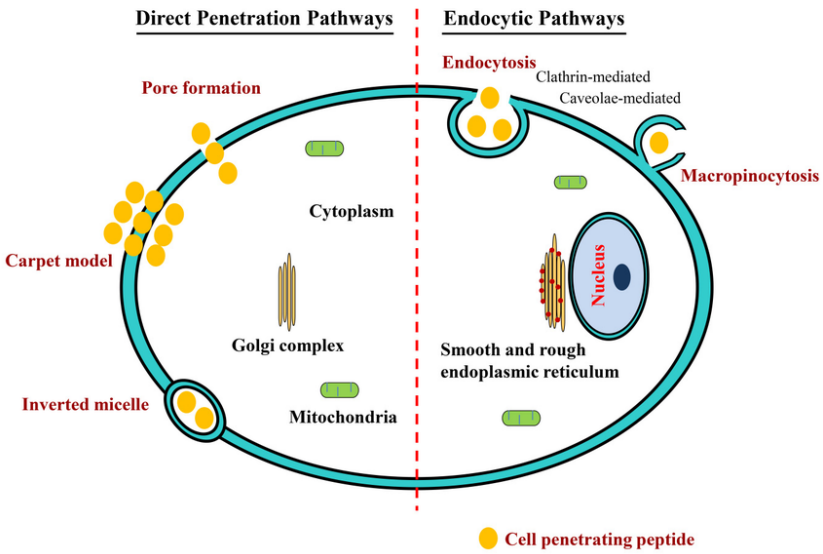

Figure 1: Illustratration of the comparison between endocytosis and energy independent / direct penetration pathways of CPP (Layek et al., 2015).

3.4.1 Energy independent pathway

Direct penetration or direct translocation of CPP across the plasma membrane can occur at low temperature without utilizing any energy or receptor. In this process, which has several entry points, the CPP is first taken inside the cells by making the membranes more porous and unstable. There are currently three distinct model types available for porosity generation and membrane destabilization mechanism (a) barrel-stave pore model, (b) carpet like model and (c) torodial pore model (Prakash et al., 2018).

Barrel-stave model

The hydrophilic area of the peptide remains perpendicular to the interfacial plane. When enough peptides (at least three) build up on the inner membrane's effect at high pH, the combination

formed between the fatty acids of the lipid bilayer and the guanidium group of the peptide results in the creation of a pore (Borrelli et al., 2018).

Carpet-like model

The outer layer of the membrane contains phospholipids, which have negative charges, and alpha-helical cationic CPP, which has positive charges. These two types of CPP interact with one another. The conversation that takes place between these two is concealed by a carpet. Then, when the concentration reaches a critical point, the peptides that are still parallel to the surface of the membrane begin to rotate, which causes a phospholipid redirection. This results in an increase in the fluidity of the membrane as well as the development of micelles and pores inside it.

The toroidal model consists of two distinct steps. This theory states that the peptide's transition from an inert to an active state depends on the peptide's intensity. If CPP is in an active form and present in large concentrations, it will orient perpendicular to the lipid bilayer and breach the hydrophobic area. These three models quite efficiently explain the internalization of large cell penetrating peptides (Borrelli et al., 2018).

3.4.2 Endocytosis pathway

Endocytosis is currently recognized as the primary method by which CPP and CPP-cargo complexes are ingested by cells (Ramsey et al., 2015). Several methods, including macropinocytosis, clathrin, or caveolin-mediated endocytosis, are used by this energy-dependent process to ingest CPPs (Borrelli et al., 2018). In this pathway CPP or CPP-cargo conjugate will choose its pathway according to its size and physiochemical properties. CPP must escape to the

cytosol in order to reach its destination location and avoid inactivation by the endosomal lysosome (Prakash et al., 2018). The greatest barrier to effective cellular uptake of functional macromolecules appears to be escaping from the endocytic vesicle (Guidotti et al., 2017). Several theories have been proposed on endosomal escape. According to one mechanism, a lower pH enhances CPPs' capacity to interact with endosomal membranes, which results in greater intracellular delivery. If the endocytotic vesicles increase, it might help in the endosomal escape (Borrelli et al., 2018). Employing PePFects(PFs), ulo Langel and coworkers introduced a new method of endosomal escape . Peptide fragments (PePFects) are a family of transportan10-derived peptides. The N-terminus of TP10 changed when it was sterilized. This made it easier for it to get out of endosome, which made intracellular delivery work better . Examples of PePFects are PF6, PF14 (Guidotti et al., 2017). Choloroquine also shows to be slowing down the endocytosis thus leaving more time for endosomal escape (Muller et al., 2008). Figure1 shows the illustrated comparison between endocytosis pathway and energy independent pathway (Buddhadev et al., 2015).

Chapter 4

Applications of CPP

4.1 Disease treatment

4.1.1 Central Nervous system disorders

The main disadvantage in managing these central nervous system conditions is how inadequate therapeutics passes the BBB. The most widely recognized CNS disorders that are challenging to treat include cancer, Parkinson's, and Alzheimer's disease. To find whether CPP can deliver cargos across the BBB, Many studies have been conducted. In vivo experiments on CPPS selectively crossing the BBB were carried out by Stalmans et al. This research was carried out with the use of five different distinct chemical CPPS, namely PVEC, SYNBB, TAT 47-57, TP 10, and TP 10-2. In comparison to synBB and TAT 47-57, the transparency across the BBB of PVEC was found to be excessive, according to the findings of the study. This experiment indicates that the brain influx behavior and cell penetration properties of CPPS are not connected. TP-10 showed the maximum response but the weakest BBB inflow attributes (Stalmans et al., 2018). This review will highlight on the studies of CPPs on CNS diseases that has been conducted in the most recent years.

CPP on Alzheimer's disease

CPP-derived peptide therapies have been thought to be a possible treatment for Alzheimer's disease, neurodegenerative impairment. Cognitive and memory impairments are the markers of the clinical presentation of this condition. pathological hallmarks for recognition of this disease

are cerebrovans cular amyloid (CVA) deposit consist of amyloid beta(AB) proteins, accumulation of extra cellular amyloid plaques, intraneuronal tangles.(Yin et al., 2016). Yin et al, conducted their experienced with penetrating peptide, A powerful characteristic of penetrating peptide is its capability to cross the BBB barrier and transport cargo without resulting in a significant amount of cytotoxicity. In order to prevent the generation of AB, they created PEG stabilized AUNS complexed with pen peptide (pen @ PEG-AUNS), that has high permeability across the BBB (Yin et al., 2016). As (PEN@ PEG-AUNS) lacks fluorescent property that is why the scientists used Ru (ii) complex which exhibits fluoresces properties. They investigated whether Ru@pen@PEG-AUNS inhibited the formation of amyloid beta aggregation. The study's findings demonstrated that RU@PEN@PEG AUNS, even in low concentrations, considerably suppressed AB protein aggregation, suggesting the complex could be used in AD treatments. (Yin et al., 2016). In another study researchers conjugated CPP K16APOE-targated Nano particles. This complex showed that they can significantly accumulate in the brain and recognize amyloid plaque (Ahlschwede et al., 2018).

CPPs on Parkinson disease

Parkinson's disease is yet another typical form of neurological dysfunction. Dopaminergic neurons in particular regions of the brain can be selectively lost, which can lead to the development of Parkinson's disease (PD). As TP10, syn, Tat can efficiently penetrate the cell membrane including BBB (stalman et al., 2015) so they're gained great interest to use them as as a vehicle in Parkinson's therapeutic strategies. Rusiecka and her associates conducted an experiment where they combined the dopamine molecule with TP10. They considered dopamine

as a treatment because dysfunction in the D1 and D2 receptors is likely responsible, according to its pathology. Changes in intracellular signal transduction pathways happen when DA can't bind to or reach these receptors. This makes the disease get worse.

The pharmacokinetics and pharmacodynamics functions of the TP10-Dopamin complex were preferable to those of dopamine. Because of TP10's ability to penetrate cells, conjugates of this compound rapidly cross the brain tissue, exhibit a high affinity for D1 and D2 receptors, and are less susceptible to the COMT's O-methylation response. The preclinical animal model of PD mediated by MPTP showed the anti-parkinsonian impact (Rusiecka et al., 2019).

Rasagiline, a highly specific propargylamine of monoamine oxide B (MAO-B) inhibitor that is suggested for the treatment of symptoms associated with PD, was conjugated with MAP (amphipathic model peptide) in a more recent investigation. RAS-MAP conjugate treatment was done to evaluate its effect on syn (Alpha-syncline). Increased expression of syc protein leads to neurodegeneration which is linked with development of PD. At lower concentration the RAS-MAP conjugate was shown to be more efficient and more readily entered into the cell (vale et al., 2020).

4.1.2 CPPs application on Cancer Treatment

Peptides that are able to penetrate cells offer a potential answer to the issues that are associated with the administration of drugs in conventional chemotherapeutic treatment for cancer. CPP-based delivery involves transporting cargo into the cells and compares favorably to standard chemotherapeutics and gene-based drug delivery (Regberg et al., 2012). Both the absence of selectivity toward tumor cells and the low anti-tumor efficacy of chemotherapy are downsides to the treatment. Using these targeted moieties, chemotherapy drugs could improve their

characteristics. Imaging compounds and drugs combined with CPP have shown promise in a variety of preclinical studies for use in cancer detection and therapy. (prakash etal., 2018). Though there are now some CPP-based medications in the clinical trial stage, none are yet available. TAT, HSV-1, penetratin, transportan, MPG, and PEP peptides are CPPs used in the therapy of cancer. To address their efficacy and stability problems, synthetic CPPs with changed residues are being developed in addition to natural CPPs for use in therapy. In 2011, the first clinical trial utilizing P28 CPP to treat resistant solid tumors was completed. (NCT00914914). This was the first case to used CPPs as therapeutics in human subject with advance solid tumor. The pharmacokinetic result indicated no significant adverse events and high p28 tolerance.

In 2015 the second clinical study on the use of CPP in cancer treatment was conducted (NCT01975116). Subject of the study was younger patients with brain tumor. Once again, P28 has been employed to determine the least harmful dose of azurin-derived P28 CPP and any potential adverse effects (Timur et al., 2021).

4.1.2.1 CPPs on cancer imaging and diagnosis

It's crucial to visualize malignant tissue during surgery for breast cancer. A suitable strategy is to perform fluorescence image-guided surgery utilizing an in vitro system of a fluorescent cancer imaging agent and imaging camera systems. A clinical phase investigation for the promising drug pegloprastide (AVB-620) has been completed. In radiometric imaging and the process of detecting breast cancer, the material serves as an imaging agent that contains a fluorescent dye. It operates on the principle of an activable cell-penetrating peptide (ACPP). It is administered intravenously before 3-29 hours of surgery and has proven excellent results in excluding malignancy from margin specimens. AVB-60 is been shown to be safe and tolerable in the first

human phase I research for surgically treated primary non-recurrent breast cancer patients (Malijass et al, 2021).

4.1.3 CPPs application on Inflammation

Inflammation is an innate immune response in various diseases. Many diseases are associated with Inflammation. It's an essential part of the disease response process. There are already many anti Inflammation drug are available in the market but reason behind choosing CPP mediated anti Inflammatory delivery is to get an efficient delivery.

A NSAID called lornoxicam is used to treat a number of painful and inflammatory conditions. Oral administration of LN causes dyspepsia, ulceration, anorexia as it's poorly soluble in the acidic condition of the stomach. The first pass metabolism of LN shows some other side effects as well, i.e. anemia, hepatitis also injections as inapplicable for its poor safety and compliance in chronic condition patient. To overcome these side effects transdermal delivery route could be used for avoiding first pass effects and gastrointestinal damages for NSAID drug delivery.to get an efficient drug delivery via transdermal rote several chemical and physical approaches has been developed for skin permeation of drug i.e. micro needles, iontophoresis, magnetophoresis. But this application has side effects like toxicity and therapeutic feasibility. So other methods wrere introduced to enhance drug permeation through skin like solid lipid nanoparticles (SLNs), Nano emulsion, liposomes and Nano structured lipid carriers (NLCs). CPPs like polyarginine (R8, R7 AND R11) peptide, tat peptide, penetratin, and polylisine (K9) has been used for improving skin permeability of the drugs. Gao et al., conducted an study where they developed LN-NLCs modified withR11 peptide to see if the drug s anti-inflammatory effects increases. In carrageenan-induced paw edema test, LN-NLC-R11 gel's anti-inflammatory potential was

studied. The LN-NLC-R11 gel was determined to be stable, and cytotoxicity revealed reduced cell viability. Additionally, the coating of CPP R11 that was applied to the exterior of the LN-NLCs demonstrated increased transdermal accessibility of the LN-NLCs, which resulted in a more potent anti-inflammatory action (Gao et al., 2020).

4.1.4 CPPs application in ocular disease

Classifying the eye's front and back is important for determining where to administer medications. The front part includes the conjunctiva, cornea, two aqueous humor-filled chambers, the iris, and the lens; Topical medication is administered to treat this segment's infections and diseases. The other segment includes retina, posterior sclera, vitreous humor and disease of this section are treated by local injection drug delivery. Efficient drug delivery to eye is challenging because of it's several static and dynamic and metabolic barriers. CPPS mediated ocular drug delivery can be useful for drug moieties to reach at the target site crossing different ocular barriers. TAT, penetratin, PEP1 are the most commonly used CPPs for the delivery of intraocular drug (pescina et al., 2018).

Jain et al., conducted an study where they conjugated natamycin with a cell penetrating peptide(TAT 2) to found if the drug s poor penetrating ability decrease or not. Natamycin is an anti-fungal drug treated with fungal keratitis. It is very poorly penetrable through the corneal epithelium. As a result of conjugation, the medication was shown to be more soluble in aqueous media in addition to having improved cellular penetration. Therefore, an effective drug delivery system for the prevention of acute fungal keratitis can be achieved by a clinical formulation of the conjugated complex (Jain et al., 2015).

Another study evaluated the effectiveness of anti-VEGF medications administered intravitreally (ivit) versus utilizing CPP to treat choroidal neovascularization (CNV). Their analysis revealed that topical administration of CPP with anti-VEGF suppressed CNV in vivo as effectively as a mono-dose i.v of anti-VEGF. CPP +anti VEGF was nontoxic to ocular cells and cleared out from retina of the studied animal within 24 hrs (Cogan et al., 2017).

4.2 CPP-conjugated therapeutics under clinical development

Several preclinical and clinical studies are currently under development. Though there are many CPPs have been discovered but only few of them are currently in use. Some of the clinical have successfully cleared phase3 and some were discontinued after phase1. Instances of cpp-conjugated medicaments that are currently undergoing clinical trials are included in Table 1.

Table 1: Lists examples of CPP-conjugated medicaments that are currently undergoing clinical studies (Guidotti et al., 2017; Falenga et al., 2020).

CPP	CPP-cargo	Compound name	Pharmaceutical company name	Application	Clinical trial status	Clinicaltrials.gov.ID
TAT	TAT-JBD20 (D-JNKI-1)	AM-111	Auris medical	Hearing loss	Phase II completed 2014	NCT00802425
					Phase III Recruiting 2016	NCT02561091 NCT02809118
TAT	TAT-JBD20 (D-JNKI-1)	XG-102	Xigen SA	Inflammation	Phase I completed 2012	NCT01570205
					Phase III completed 2016	NCT02235272
TAT	TAT- PKC Inhibitor	KAI-9803	KAI pharmaceuticals	Intraocular pain and inflammation	Phase III completed 2016	NCT00785954

		KAI-1678		Myocardial infarction	Phase II completed 2011	NCT01106716 NCT01135108 NCT01015235
PTD4	HSP20	AZX100	Capstone Therapeutics	Postoperative pain, Spinal cord injury	Phase IIa completed 2012	NCT00451256 NCT00892723 NCT00811577
MTS	MTS-botulinumtoxin-A	RT001	Revance therapeutics,inc.	Duchenne muscular dystrophy	Phase II completed 2013	NCT01064518 NCT00968942 NCT00968825 NCT00884234 NCT01124552 NCT01124565 NCT00907387 NCT00888914 NCT01940991
MTS	Trans MTS-botulinumtoxin-A	RT002	Revance therapeutics, inc.	Crows feet and facial wrinkles Glabellar lines	Phase I & II Completed 2016	NCT02303002

In order to address hearing loss, Auris Medical created the biocompatible and biodegradable ointment (AM-111). The drug's clinical study was finished in 2014, and it provided patients with hearing loss with well-tolerated, clinically significant, long-lasting benefits. (NCT00802425). Phase3 clinical development is currently ongoing (Falenga et al., 2020).

Xigen SA created XG-102 for pain and inflammation management. In 2012 the conducted the first clinical trial to found its tolerability, safety and pharmacokinetics. Volunteers who taken the XG-102 showed no such adverse effects that were associated with the compound. Phase3 of the compound was treated patients with intraocular inflammation and pain which was completed in 2016 and the results will publish soon (Guidotti et al., 2017).

Chapter 5

Limitation associated with CPP based drug delivery

Major drawbacks of CPPs as therapeutics are lack of cell and tissue specificity, cytotoxicity due to endosomal degradation and Cellular uptake efficiency (Falanga et al., 2020). Widespread tissue delivery caused by the lack of selectivity frequently has serious side effects and renders them useless in many in vivo circumstances. Since the cargo gets accumulated in endosomes and is degraded by certain enzymes, the distribution of therapeutic agents and their bioavailability are both hindered for cationic CPPs. This is why the cystoic delivery of cargo is poor for cationic CPPs. This is a serious limitation for the membranotopic CPP, which avoids endosomal trapping and subsequently enables the intracellular release of cargo (Falanga et al., 2020).

Before a CPP/CPP-cargo may be used as a drug delivery vector, one must have a thorough understanding of the toxicity window of them in cells. The Cationic nature of CPP is responsible for the toxicity. A specific interaction between CPP and the cytoplasmic membrane or even one component of the membrane is responsible for the toxicity that results from the interaction (langel 2021). Despite of being a potential drug delivery median CPPs use is limited because of their chemical instability. They are susceptible to destruction by extracellular and intracellular enzymes as a result of their inherent chemical instability, which lowers the concentration of CPP (kristensen et al., 2016).

Chapter 6

Future aspects and concluding remarks

Though the cellular entry process of CPP is still debatable but with passing time the scientists are getting more and more information and having a better understanding. Clear understanding of CPP mechanisms to mediate cargo entry into cell can give new ideas to design platforms for efficient drug delivery systems. Nano platforms can improve the cystoic delivery efficiency, tissue specificity of the CPP - cargo conjugate. Over the last few decades, a great number of preclinical and clinical trials of CPPs were carried out, and the findings of these studies have led to the opening of a previously uncharted arena for the development of pharmaceuticals focusing CPPs. Although no CPP-conjugated therapeutics been approved by FDA yet. But a few has cleared clinical trial phase3 with acceptable results. So we can now hope that within the next few years CPP based drug will be approved and placed in the market.

Chapter 7

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