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Cell Penetrating Peptides: A Challenge for Drug Delivery

Aroui S* and Kenani A

Department of Cellular Signaling and Pathologies", Faculty of Medicine of Monastir, Tunisie

***Corresponding Author:** Sonia Aroui, Unité de recherche UR 12ES08 Department of Cellular Signaling and Pathologies", Faculty of Medicine of Monastir, Tunisie. Fax: +216 73 46 07 37. E-mail: sonia_aroui2002@yahoo.fr

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1. ABSTRACT

Cell Penetrating Peptide (CPPs) is a term that describes relatively short amphipathic and cationic peptides (7 to30 amino acid residues) with rapidly translocation cell membrane activity. They can be used to deliver molecular bioactive cargos due to their efficacy in cellular internalization and also to their low cytotoxicity. In this review we provide an overview of the current approaches and describe the potential of CPP-based drug delivery systems and indicate their powerful promise for clinical efficacy.

2. KEYWORDS: Cell penetrating peptides; Drugs.

3. INTRODUCTION

A novel approach to overcome cell membrane impermeability and to deliver a large variety of particles and macromolecules into cells has been recently emerged, which is called Cell-Penetrating Peptides (CPPs), also known as Protein Transduction Domains (PTDs) [1-2]. CPPs are generally short (up to 30a mino acids in length) water soluble, cationic, and/or amphipathic peptides which make them as promising vectors for therapeutics delivery leading to a considerable amount of research focused on the intracellular delivery of drugs [3,4,5]. There are two principal types of CPPs that have been utilized for this purpose: i) Cationic CPPs composed of short sequence of amino acids (arginine, lysine and histidine). These amino acids are responsible for the cationic charge of the peptide that allowed the interaction of the peptide with anionic motifs on the plasma membrane by a receptor-independent mechanism. ii) Amphipathic peptides, which have lipophilic and hydrophilic tails that are responsible for a direct peptide translocation mechanism across the plasma membrane [6].

The most important characteristics of CPPs is that they are able to translocate the plasma membrane at low micromolar concentrations in vivo and in vitro without using any receptors and without causing any significant membrane damage [7,8]. Another benefits of using CPPs for therapeutic delivery is the absence of toxicity as compared to other cytoplasmic delivery devices, such as liposomes, polymers, etc [6]. The mechanism for the CPP facilitated cellular uptake remains not clear and depends on cargo and cellular type [9]. Due to its high density of basic amino acid residues (Arg and Lys), the large charge at physiological pH excludes the passive diffusion of CPPs across the lipid bilayer. Furthermore, it seems that classical uptake mechanisms such as protein-based receptors and transporters are not involved. In contrary, endocytosis was shown as a common uptake mechanism but is controversial at the same time. For example, in a number of reports, CPP uptake was not inhibited at 4 °C or in the presence of inhibitors of endocytosis; in contrast, capture of CPPs in endocytotic vesicles was observed when soluble heparin sulfate was added [9,10]. Many other studies indicate that aggregation of the cell surface glycosaminoglycan heparan sulfate (HS) is an important element in the uptake mechanism [2]. The challenge of the strategy using CPPs should take under consideration size, stability, non-specific versus specific associations and potency versus toxicity that all play an important role for selection of delivery systems [11].

4. HISTORY AND ORIGIN OF CPPS:

The CPPs are initially discovered in 1965 when it was observed that histones and cationic polyamines such as polylysine stimulate the uptake of albumin by tumor cells in culture. It was shown that the conjugation of polylysine to albumin and other proteins enhance

their transport into cells. Moreover, a comparison study of different homopolymers of cationic amino acids demonstrate that medium-length polymers of arginine enter cells more effective than similar-length polymers composed of lysine, ornithine or histidine [12]. In 1988, it was discovered that the human immunodeficiency virus type 1 (HIV-1) encoded Trans-acting activator of transcription (Tat) peptide which also translocate cell membranes and gaining intracellular mammalian cells [13-14]. Covalently conjugation of Tat peptide to proteins or fluorescent markers allowed these molecules to gain into the cell. A few years later another discovery was followed when polycationic peptide of natural (VP22 and AntP) and synthetic origin (transportan) were used for the delivery of genes, proteins, small exogenous peptide or even nano-particles. Furthermore, it was demonstrated that small domains in these peptides are often responsible for cellular entry [15]. Thus, these translocation sequences could be shortened to a few amino acids in comparison with the first Tat peptide, without affecting cell penetration efficiency [14]. Since that time, the list of synthetic CPPs has increased sharply and the number continues to rise (Table 1). In the last decade, another peptide was described named Maurocalcine (MCa), a 33 amino acid residue peptide that has been isolated from the venom of the Tunisian chactid scorpion Scorpio maurus palmatus. It folds according to an 'Inhibitor Cystine Knot' (ICK) motif and contains three disulfide bridges connected by the following pattern: C1-C4, C2-C5 and C3-C6 [16]. MCa acts on ryanodine receptors resulting in pharmacological activation. These receptors are calcium channels located in the membrane of the endoplasmic reticulum. They control Ca_2+ release from internal stores and therefore a large number of cell functions [17-18].

These peptide posses vector properties when coupled to fluorescent streptavidin. This complex was shown to enter various cell types within minutes and in all cell types tested, a common feature of CPPs. A variety of mutants of MCa were then designed in order to unravel the most active residues for its pharmacological and penetration activities (figure1) [19-20].

5. THERAPEUTIC APPLICATIONS OF CPPS

Chemotherapy used for treatment of cancer has a lot of defects because of the toxicity of the drugs to normal healthy cells and also to resistance developed by tumor cells to the anti-cancer drug [21]. The major inconveniences with used cancer chemotherapy are the absence of specificity target to tumor cells and thus poor anti-tumor effect. The challenge in cancer therapy is to know how to deliver a drug intact to the cytosol of every cancer cell, sparing healthy cells.

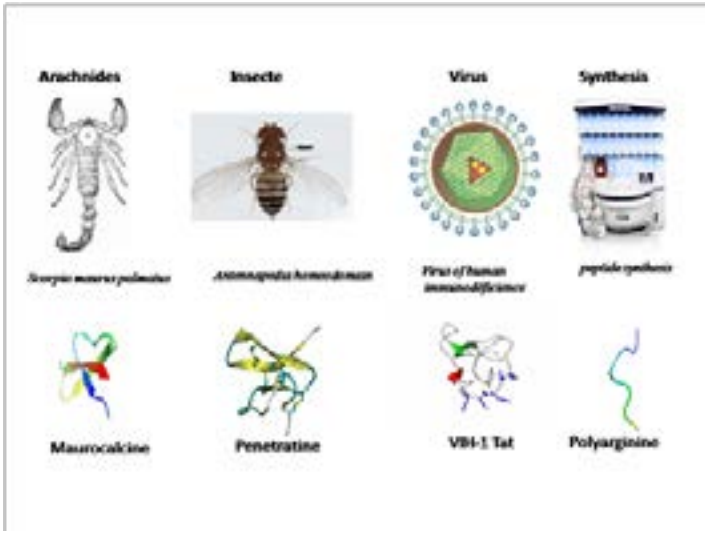

Figure 1: Exemple of origin of four CPPs: Maurocalcine, Penetratin, Tat and polyarginine. Maurocalcine, Penetratine and Tat derived from natural sequences but polyarginine was produced by de novo conception in order to obtain a good cellular penetration.

It was shown that polyarginines carry cargoes that exceed the 500 Da by molecular electroporation across the cell membrane wich may solve part of the drug-delivery problem [22]. However, the use of well chosen linkers and anions can help target cancer cells and contribute to successful of the conjugation process. For example, the CXC chemokine receptor 4 (CXCR4) is over-expressed in different types of cancer, including prostate, breast, colon and small-cell lung cancer. Snyder et al. linked the CXCR4 receptor ligand, DV3 to two transducible anticancer peptides: a p53-activating peptide (DV3-TATp53C′) and a cyclin-dependent kinase 2 antagonist peptide (DV3-TAT-RxL). Treatment of tumor cells expressing the CXCR4 receptor with either the DV3-TATp53C′ or DV3-TAT-RxL targeted peptides resulted in an enhancement of tumor cell killing compared with treatment with nontargeted parental peptides [23]. Furthermore, Hypoxia-Inducible Factor-1 (HIF) the transcription factor central to oxygen homeostasis is regulated via the Oxygen-dependent Degradation Domains (ODD) of its α isoforms (HIFα). The amino- and Carboxyl-terminal sequences of ODD (NODD and CODD) was fused to TAT and injected into sponges implanted subcutaneously (s.c.) in mice by William et al. They demonstrated that this injection causes a markedly accelerated local angiogenic

response and induction of glucose transporter-1 gene expression and thus opening additional therapeutic avenues for ischemic diseases [23].

In some cancer cells, such as uveal melanoma (common eye cancers in adults), p53 seems to be inhibited by over-expressing of HDM2. A transducible peptide that inhibit HDM2 and Bcl-2 for their ability to induce tumor-specific apoptosis in these cells was tested [24]. In this study it was demonstrated that the anti-Bcl2 peptide induced apoptosis in tumor cells but also caused variable levels of toxicity in normal cells and tissues. Contrary, the anti-HDM2 peptide induced apoptosis in tumor cells, with little effect on normal cells in a therapeutic dose range. This peptide also caused regression of retinoblastoma in rabbit eyes, with minimal damage to normal ocular tissues. They conclude that inhibition of HDM2 may be a promising strategy for the treatment of uveal melanoma and retinoblastoma and that strategy may be an effective technology for local delivery of anticancer therapy to the eye.

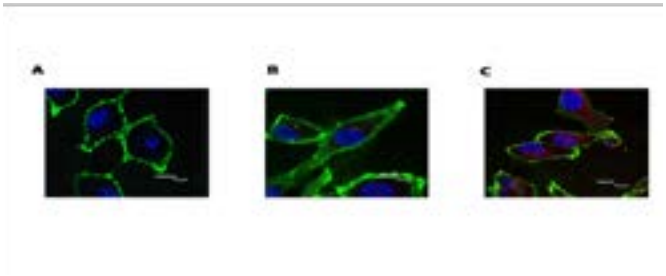

Figure 2: Cellular internalization of Dox by MCa. MDA-MB231 cells treated with (A) RPMI. (B) Dox alone (red). (C) Dox coupled to Dox at the same concentration (red).

Most of patients with sporadic Renal Cell Carcinomas (RCCs) exhibit mutation of the Hippel-Lindau (VHL) tumor suppressor gene. Conjugation of the protein transduction domain of HIV-TAT protein to the amino acid sequence (104-123) in the beta-domain of the VHL gene product (pVHL) arrested and then reduced proliferation and invasion of 786-O renal cancer cells in vitro. Besides, daily i.p. injections with the conjugate put off and, in some cases, caused partial regression of renal tumors that were implanted in the dorsal flank of nude mice [25].

The tumor suppressor gene p16INK4A, an inhibitor of cdk3 4, is often inactivated via intragenic mutation, homozygous deletion, and methylation-associated transcriptional silencing in a large number of human cancers, mainly in pancreatic cancer. Treated animals with the p16-derived synthetic peptide coupled with the Antennapedia carrier sequence, which we designated as Trojan p16 peptide reduced AsPC-1 and BxPC-3 s.c. tumors, respectively. Thus we conclude that Trojan p16 peptide system, a gene-oriented peptide coupled with a peptide vector, functions for experimental pancreatic cancer therapy [26].

Recently, it was shown by Sonia et al that coupling doxorubicin (Dox) to three cell penetrating peptides Tat, penetratin and Maurocalcine (Dox-CPPs) is a good strategy to overcome Dox resistance in MDA-MB 231 breast cancer cells and CHO cells (figure2) [3, 27]. We also reported that, Dox-CPPs were found to induce apoptotic death in MDA-MB 231 cells at a lower dose that needed for unconjugated Dox. Cell death induction was associated with Bax oligomerisation, release of cytochrome c, caspase activation, chromatin condensation and internucleosomal degradation. However, whereas Bcl-2 overexpression was very potent in inhibiting apoptosis triggered by Dox, this anti-apoptotic protein was largely inefficient in preventing Dox-CPPs-induced apoptosis. These observations suggest that mitochondrial disruption is the main event in Dox-induced apoptotic signaling but that Dox-CPPs are probably able to trigger additional apoptotic pathways independent of mitochondrial events. Thus, the higher efficacy of Dox conjugated to CPPs in apoptosis induction might not be due exclusively to increased drug accumulation but also to the activation of multiple apoptotic pathways. These pathways include death receptors and activation of the JNK pathway [4, 28].

Another study led by LeslieWalker et al conjugated Dox to ELP and to SynB1 in order to inhibit tumor growth in mice. Tumor inhibition under hyperthermic conditions with SynB1–ELP–Dox was 2-fold higher than under therapy with free doxorubicin at the equivalent dose, and thus becomes a promising candidate for optimizing thermally responsive drug polymer conjugates [29].

Anticancer activity of Dox was also enhanced when constructed a drug delivery system by developing 25 nm gold nanospheres (GNSs) conjugated to four α-helical CPPs [30].

A thermally sensitive quantum dots that exhibit an “on-demand” cellular uptake behavior via temperature induced “shielding/deshielding” of CPP on the surface was synthesized. Poly(N-isopropylacrylamide) (PNIPAAm) and CPP were biotinylated at their terminal ends and co-immobilized on to the surface of streptavidin-coated quantum dots (QDs-Strep) through biotin–

streptavidin interaction. The cellular contact of CPP was sterically hindered due to hydrated PNIPAAm chains below the Lower Critical Solution Temperature (LCST). In contrast, above the LCST, grafted PNIPAAm chains were collapsed to make CPP moieties resurfaced, leading to increased cellular uptake of QDs. The temperature-controlled "shielding/deshielding" of CPP was further applied for a thermally triggered siRNA delivery system, where biotinylated siRNA was additionally conjugated to the surface of TSQDs. The level of gene silencing was significantly enhanced by increasing temperature above the LCST due to the surface exposure of CPP [31].

6. CONCLUSION

The progressive and continuous use of CPPs indicates their potential as effective delivery vectors. Due to the need for development of safe and efficient vectors for drug delivery, an increasing number of CPP-based applications have been described recently. In this review, the current trends in drug delivery by CPPs are summarised. Conjugation with CPP increases cell-surface affinity and eventual cellular uptake of bioactive molecules.

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