On The Biomedical Promise of Cell Penetrating Peptides: Limits Versus Prospects

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ABSTRACT: The cell membrane poses a substantial hurdle to the use of pharmacologically active biomacromolecules that are not *per se* actively translocated into cells. An appealing approach to deliver such molecules involves tethering or complexing them with so-called cell penetrating peptides (CPPs) that are able to cross the plasma membrane of mammalian cells. The CPP approach is currently a major avenue in engineering delivery systems that are hoped to mediate the non-invasive import of problematic cargos into cells. The large number of different cargo molecules that have been efficiently delivered by CPPs ranges from small molecules to proteins and even liposomes and particles. With respect to the involved mechanism(s) there is increasing evidence for endocytosis as a major route of entry. Moreover, in terms of intracellular trafficking, current data argues for the transport to acidic early endosomal compartments with cytosolic release mediated via retrograde delivery through the Golgi apparatus and the endoplasmic reticulum. The focus of this review is to revisit the performance of cell penetrating peptides for drug delivery. To this aim we cover both accomplishments and failures and report on new prospects of the CPP approach. Besides a selection of successful case histories of CPPs we also review the limitations of CPP mediated translocation. In particular, we comment on the impact of (i) metabolic degradation, (ii) the cell line and cellular differentiation state dependent uptake of CPPs, as well as (iii) the regulation of their endocytic traffic by Rho-family GTPases. Further on, we aim at the identification of promising niches for CPP application in drug delivery. In this context, as inspired by current literature, we focus on three principal areas: (i) the delivery of antineoplastic agents, (ii) the delivery of CPPs as antimicrobials, and (iii) the potential of CPPs to target inflammatory tissues. J Pharm Sci 97:144–162, 2008

Keywords: cell penetrating peptides; drug delivery; cellular translocation; endocytosis; tight junctions; cellular differentiation; Rho-GTPases; inflammatory model; review

Abbreviations used: CPP, cell penetrating peptide; CF, carboxyfluorescein; CLSM, confocal laser scanning microscopy; ECM, extracellular matrix; MDCK, Madine Darby canine kidney cells; FACS, fluorescence-activated cell sorting; hCT, human calcitonin; HIV-1, human immunodeficiency virus-1; NLS, nuclear localization sequence; PNA, peptide nucleic acid; SAP, sweet arrow peptide; SV40, simian virus 40; TJ, tight junctions; ZO-1, zonula occludens protein 1; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; GalR-1, galanin receptor-1; TGN, trans-Golgi network; BBB, blood brain barrier; PTD, protein transduction domain; EthD-1, ethidium homodimer-1; Tc, Technetium; IBD, inflammatory bowel disease; CsA, cyclosporine A.

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INTRODUCTION

The full therapeutic potential of peptide-, protein-, and nucleic acid-based drugs is frequently compromised by their limited ability to cross the plasma membrane of mammalian cells, resulting in poor cellular access and inadequate therapeutic efficacy.[1,2] Today this hurdle represents a major challenge for the biomedical development and commercial success of many biopharmaceuticals. Over the past decade, however, attractive prospects for a substantial improvement in the cellular delivery of such molecules have been announced that were supposed to result from their physical assembly or chemical ligation to so-called cell penetrating peptides (CPPs), also denoted as protein-transduction domains (PTDs). CPPs represent short peptide sequences of 10 to about 30 amino acids which can cross the plasma membrane of mammalian cells and may thus offer unprecedented opportunities for cellular drug delivery. In fact, in a widely recognized landmark study in mice, the intraperitoneal injection of a fusion protein conjugated to Tat(47–57), an oligocationic CPP derived from human immunodeficiency virus (HIV) Tat protein, was found to let the ligated protein, β-galactosidase, overcome numerous biological barriers, distribute into virtually every organ and even pass the blood brain barrier.[3] Nevertheless, the biomedical promise of CPPs is still far from clinical implementation. In fact, whereas most of the scientific motivation in the field of the CPPs derives from the appealing perspectives for their biomedical use, much of the actual research is still very much focused on more fundamental aspects as to their biochemical, biophysical, and cell biological assessment. So far, clinically relevant contributions appear to be rare.

To illustrate the biomedical promise of cell penetrating peptides, we revisit the performance of CPPs for drug delivery, cover both accomplishments and failures and report on new prospects. Besides a selection of successful case histories, we also point towards limitations in CPP mediated translocation. Major obstacles to CPP mediated drug delivery consist in (i) their metabolic degradation, (ii) the cell line and cellular differentiation state dependent uptake of CPPs, as well as (iii) the regulation of their endocytic trafficking as affected by selected Rho-family GTPases. In the final section, we aim at the identification of promising niches for CPP application in drug delivery. In this context, being inspired by recent literature, we focus on three principal areas: (i) the delivery of antineoplastic agents, (ii) the delivery of CPPs as antimicrobials, and (iii) the potential of CPPs to target inflammatory tissue.

Selected CPP Families and Cargo Molecules

Most of the currently recognized CPPs are of oligocationic nature and derived from viral, insect or mammalian proteins endowed with membrane translocation properties. One of the first CPPs, reported as early as 1994, was derived from the third helix of the Antennapedia protein homeodomain of *Drosophila*.[4] Today, this peptide is commonly referred to as penetratin and, together with oligopeptides of the Tat family[5] and the chimeric peptide transportan,[6] one of the most widely studied CPPs. As for the third α-helix (residues 43–58) of the Antennapedia protein homeodomain the minimal sequence responsible for the cellular translocation of the Tat protein has also been identified and is represented by the predominantly cationic residues 47–57.[7] In addition, a wealth of further oligocationic CPPs has been revealed and described in the literature.[8,9] Prominent examples are the peptides of the so-called MPG family,[10] antimicrobial-derived CPPs,[11–15] pVEC,[16,17] and VP22.[18–20] Expectedly, by the genomic approach more and more CPP sequences are likely to be uncovered.

Besides oligocationic CPPs, enhanced translocation of the cellular membrane has also been reported for weakly cationic peptides such as, for example, for a family of peptide sequences derived from the C-terminal domain of human calcitonin (hCT).[21–23] Our discovery of this CPP class stemmed from the observation that a C-terminal fragment of hCT was subject to endocytosis when exposed to excised nasal epithelium.[24] More recently, sweet arrow peptide (SAP), a linear trimer of moderately cationic, repetitive VRLPPP domains, and conceived as an amphipathic version of a polyproline sequence related to γ-zein, a storage protein of maize, has been identified as CPP.[25–27] A selection of names, origins and sequences of representative CPP families is shown in Table 1.

The large number of cargo molecules that have been efficiently delivered by the CPP approach includes biologics like peptides,[28–30] proteins,[31–34] antisense oligonucleotides,[35–38] siRNA,[39,40] plasmid DNA,[41–44] as well as model drugs.[45,46] Even particulate systems such as liposomes,[47] and