Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Controlled Release 109 (2005) 77–85

journal of controlled release

www.elsevier.com/locate/jconrel

Present and future of cell-penetrating peptide mediated delivery systems: "Is the Trojan horse too wild to go only to Troy?"

Eric Vives *

INSERM EMI0227 Immunociblage des Tumeurs et Ingénierie des Anticorps Centre de Recherche en Cancérologie CRLC Val d Aurelle Paul Lamarque 34298 Montpellier Cedex 05, France

Available online 4 November 2005

Abstract

During the last decade, small peptides (10 to 15 amino acids) derived from the HIV-1 Tat protein and from the drosophila Antennapedia homeodomain have been used to internalize various types of molecules into the cells. The way these peptides enter cells is still under investigation and the object of strong controversy. The main discussions rely on whether these peptides are internalized or not in an energy-independent fashion, and, depending on the situation, whether they follow one pathway instead of another. At present, we find in the literature a very large number of data with, at times, some contradictory results. Indeed the diversity of employed peptide sequences, the cell type used, the attachment or not of a cargo molecule, the chemical nature of this cargo itself, and the followed protocol during the experimental process do not simplify the comparison and hence final conclusions about the mechanism of cell entry. However, one common feature emerges with these cell-penetrating peptides: most of them do not show any cell specificity. Despite their demonstrated efficiency in delivering biologically active molecules in in vitro experiments, their use for a therapeutic application in vivo has been the object of a relatively little number of studies, probably because of the quite important amounts of CPP–cargo that needs to be prepared for an accurate and complete in vivo study, but more likely, because of the massive spreading of the cargo all around the body. However, it appears from recent studies that an increased targeting ability of these CPPs is possible, making the use of CPP mediated delivery compatible with an in vivo therapeutic approach.

Keywords: Cell delivery; Cell-penetrating peptide (CPP); Targeting; Tat

1. Introduction

Small peptides with cell membrane translocation properties have been identified throughout the last decade. They are grouped under the acronyms of cell-penetrating peptides (CPP) or protein transduction domains (PTD). Two of them, the Tat- and the Antennapedia-derived peptides, were issued from

Abbreviations: CPP, cell penetrating peptide; GST, gluthation S-transferase; GFP, green fluorescent protein; FACS, fluorescence-activated cell sorter; PMO, morpholino oligomers; HPMA, *N*-(2-hydroxypropyl)methacrylamide.

* Tel.: +33 467 61 25 18; fax: +33 467 61 37 87.
E-mail address: evives@univ-montp2.fr.

0168-3659/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2005.09.032

natural proteins showing the ability to activate a nuclear biological response when simply added to the extracellular milieu [1–3]. The minimal sequences responsible for the native proteins translocating activity were defined in the mid nineties [4,5]. Since that time, other CPPs as for instance the transportan [6], a nonapolyarginine [7,8], a VP22 derived peptide [9] or Pep-1 [10] have been identified and used with various frequencies and with different efficacy by several research groups in order to induce the cellular delivery of a large panel of biologically active molecules. The first highlighting feature about these peptides is the huge diversity of the transported molecules in terms of size and biological nature. For instance, the Tat peptide has been used to internalize HPMA polymers [11], liposomes [12–14], adenovirus [15], plasmid DNA [16], phosphorothioate oligonucleotides [17], peptide nucleic acids [18], streptavidine [19], paramagnetically labeled DOTA [20], oligonucleotides [21,22] and several fusion proteins and peptides (for reviews, [23] and [24]). The second important point is the apparent cellular ubiquity of this translocating activity. To my knowledge, there is only one single cell type in which the Tat peptide was not able to enter [25], despite the presence at its cell surface of heparan sulfate receptors (see below). Otherwise, the number and the diversity of cell types successfully used for delivering biologically active molecules are very impressive (for recent reviews, [24] and [26]). Such ability to pass through the plasma membrane of all these cell types likely relies on a common mechanism of entry independent of a cell type specific receptor. That could be indeed an advantage for in vitro delivery systems but will certainly be a real problem for in vivo drug delivery applications towards a particular pathology, because of the expected high loss of a very valuable material (the CPP-drug) in untargeted tissues. Therefore, the development of various strategies to mask the strongly membrane sticky CPP sequence until its localization at the targeted cells is required. Otherwise CPPs would not be competitive enough compared to the "homing peptides" emerging in the literature for in vivo drug delivery applications and able to recognize a specific cell type and therefore to concentrate nearby the targeted cells any bound drugs [27–30]. The question will then be: is it better to administer a high level of drug bound to a peptide which goes efficiently to the targeted cells but enters weakly or to administer a large amount of drug bound to a peptide which enters more efficiently but goes everywhere? In a near future, we will probably have to compare the in vivo benefit of general CPP versus the "homing peptide" drug delivery strategies.

2. CPP, an efficient cell-delivery strategy

Examples of Tat peptide-mediated cellular delivery of various cargo molecules are still emerging almost weekly in the literature (for a recent review, [24]). Since the expected biological activity of these cargoes was retained upon coupling to the Tat peptide and to other CPPs, translocation mediated by these peptides is indeed considered as an attractive alternative to previously used cellular delivery systems. This strategy allowed number of in cellulo studies of various biological process by introducing into the cell, by an apparent "stressless" mean, biologically active peptides, proteins, oligonucleotides or drugs. As shown in the introduction, the diversity of the transported molecules is very large, and surprisingly, the size of the Tat peptide-associated cargo does not seem to be an important limiting factor. However a higher number of attached CPPs could be sometimes required to allow the cargo to be efficiently internalized.

In a very impressive review published some months ago [24], more than 300 different applications using different type of CPPs have been listed from the literature in the wide field of peptide-mediated delivery systems. If we look at the different CPPs reported in this review (Fig. 1), we can quantify that in almost 50% of cases, it is the Tat peptide which has been successfully used to deliver various molecules into the cells. An other important part of these applications uses the Antennapedia-derived peptide. It is noteworthy that these two peptides have been used in two thirds of all documented cases to deliver molecules into cells. Other studied CPPs include the family of the polycationic sequences such as poly-lysine and poly-arginine. Long poly-L-lysine polymers were evidenced 25 years ago for improving the cellular delivery of various proteins [31,32]. These polymeric