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Cell Penetrating Peptides (CPPs): A new era for localised drug delivery

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SUMMARY

This article focuses on the promising roles of cell penetrating peptides (CPPs) in local drug delivery. The challenges in determining the exact cellular uptake hinders further understanding of the fate of these peptides. These challenges include the lack of a reproducible way to ascertain the mechanism of entry. In addition, the in-vitro work on cell-lines did not end in definite answer to the cellular uptake mechanism. Indeed, the unreliability of such work seems logical due to different reasons, including the cell line-dependent uptake and the cell membrane heterogeneity of different types of cells. Although several CPPs reached the clinical trials, no one could reach the market, yet. Re-evaluation era should be started through moving forward for more researches on the roles of CPPs in Local delivery to homogeneous cell population of specific tissue or organ of the body. While this article demonstrates an example on variability in cellular uptake in different cell lines, it encourages more sophisticated work to be conducted on CPPs for ocular, nasal, transdermal, buccal and even inner ear delivery.

INTRODUCTION

Cell penetrating peptides (CPPs), which is known as membrane translocation sequences or protein transduction domains (PTDs), can be defined as groups of short polypeptides (less than 30 amino acid) with a capability to pass through the impermeable cell membrane. The mechanisms of cellular uptake and the intracellular trafficking of CPPs could be divided into non-endocytic or endocytic pathway. Measuring the uptake at 4°C and 37 °C represents the common way to distinguish the possible pathway as endocytosis is abolished at 4 °C. Concerning the endocytic pathway, endocytosis represents a process of uptake of macromolecules and solutes through the formation of membrane bound vesicles. The three main endocytic mechanisms that facilitate the uptake of CPPs include clathrin-mediated endocytosis, caveolae-mediated endocytosis and macropinocytosis. In order to distinguish a certain endocytic pathway from another, there is a list of pharmacological inhibitors that can obstruct one endocytic mechanism more meaningfully than others. The use of these inhibitors in identifying the specific endocytic pathway of entry is helpful although they suffer from two disadvantages: lack of specificity and cell type dependency.

The mechanisms involved in internalisation are varied and depend on several factors including cell line, the type of the peptide vector, the cargos physical and chemical properties and the peptide concentration (Futaki et al. 2007). Study the uptake of CPPs in different cell lines leads to dissimilar results. Therefore a comparison of the cellular uptake of a certain peptide into different cell lines is not possible. These differences might be due to the different membrane compositions of different cells. One of these composite is cholesterol, which has a role in biological membrane fluidity. In addition, the expression level of glycosaminoglycans varies from cell line to another and this has been linked with diverse cellular uptakes of CPPs.

This work aims to highlight the cellular uptake variability by demonstrating an example of involvement of more than one mechanism of cellular uptake of the CPPs (Tat-LK15) within different cell

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lines. In addition, it aims to highlight the roles of CPPs in local delivery.

MATERIALS AND METHODS

K562 cells were seeded on the day of the experiment, whereas HT29 and HeLa cells were seeded one day before the experiment. The cells were incubated at 37◦C in OptiMEM with TAMRA-TatLK15 or TAMRA-Tat peptides with doses equivalent to 1, 2 and 5 micromolar. After the incubation period, the medium was aspirated and the cells were rinsed with PBS, twice. The other steps were described in previous published work (Alkotaji et al 2014). Fluorescence analysis was performed using a fluorescence-activated cell sorter FACS (Becton Dickinson, Oxford, UK) (Ex: 490 nm, Em: 520 nm).

RESULTS AND DISCUSSION

For all cell lines examined, incubation at 4 °C was unsuccessful to completely abolish uptake of TAMRA-Tat-LK15 peptide (Fig. 1.). This indicates a limited involvement of an energy independent mechanism (non-endocytic) of entry in addition to the energy-dependent one (the endocytic one). This demonstrated that is the uptake of TAMRA-Tat-LK15 peptide may involve an additional non-endocytic mechanism. Indeed, the uptake among the different cell lines were not equal. This cell line dependent uptake has been reported before (Mueller et al. 2008) and represents a drawback of CPP uptake comparison studies. Consequently, conclusions about mechanisms of CPP uptake may depend on the cell line studied. Among the possible explanations for the observed different behaviour might be that HT29, HeLa and K562 cell lines may express different amounts of negatively charged constituents of the cell membrane such as the heparan sulfate proteoglycan, which is essential for the electrostatic interaction with highly cationic peptides and its internalisation into the cell. On the other hand, several works are on-going for using CPPs for enhancing local delivery to specific organs such as ocular, nasal and transdermal delivery. In 2017, CPP was succeeded in topical delivery of anti-VEGF Drugs to the ocular posterior segment (Cogan F. et al 2017). In addition, many CPPs were investigated for their roles in systemic and brain delivery through nasal delivery. Some anti-diabetic drugs nasal delivery was investigated. Moreover, CPPs were used as an enhancer for transdermal drug delivery system. And not surprisingly, CPPs were used for gene delivery into inner ear for hearing loss.

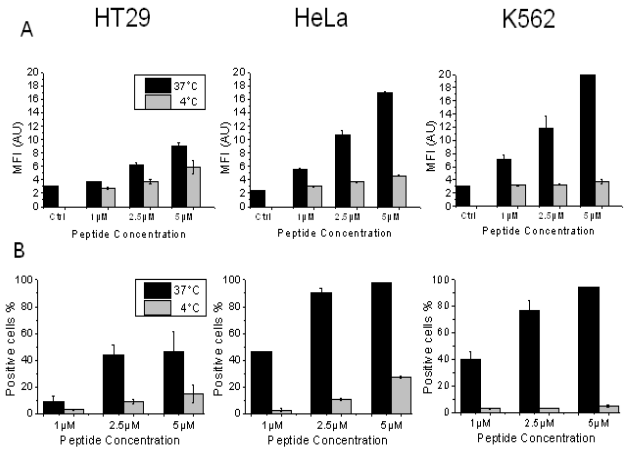

Fig. 1. Quantification of the cellular uptake of TAMRA-Tat-LK15 at 37 °C and 4 °C.

CONCLUSIONS

CPPs future could be in local or topical delivery to specific tissue were study of cellular uptake could give more precise results than using different cells of different origin.

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