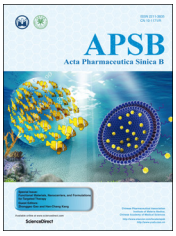
Acta Pharmaceutica Sinica B 2016;6(4):352–358

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



SHORT COMMUNICATION

Nose-to-brain delivery of macromolecules mediated by cell-penetrating peptides

Tingting Lin[a,b], Ergang Liu[a,b], Huining He[a], Meong Cheol Shin[c,d], Cheol Moon[e], Victor C. Yang[a,c,], Yongzhuo Huang[b,*]**

[a]*Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnosis, School of Pharmacy, Tianjin Medical University, Tianjin 300070, China*
[b]*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*
[c]*College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109-1065, USA*
[d]*College of Pharmacy and Research Institute of Pharmaceutical Sciences, Gyeongsang National University, Jinju, Gyeongnam 660-701, Republic of Korea*
[e]*College of Pharmacy, Sunchon National University, Suncheon, Jeonnam 540-950, Republic of Korea*

Received 27 January 2016; received in revised form 24 February 2016; accepted 7 April 2016

KEY WORDS

Intranasal protein delivery;
Brain targeting;
Cell-penetrating peptide;
Low molecular weight protamine;
Blood–brain barrier

Abstract Brain delivery of macromolecular therapeutics (*e.g.*, proteins) remains an unsolved problem because of the formidable blood–brain barrier (BBB). Although a direct pathway of nose-to-brain transfer provides an answer to circumventing the BBB and has already been intensively investigated for brain delivery of small drugs, new challenges arise for intranasal delivery of proteins because of their larger size and hydrophilicity. In order to overcome the barriers and take advantage of available pathways (*e.g.*, epithelial tight junctions, uptake by olfactory neurons, transport into brain tissues, and intra-brain diffusion), a low molecular weight protamine (LMWP) cell-penetrating peptide was utilized to facilitate nose-to-brain transport. Cell-penetrating peptides (CPP) have been widely used to mediate macromolecular delivery through many kinds of biobarriers. Our results show that conjugates of LMWP–proteins are able to effectively penetrate into the brain after intranasal administration. The CPP-based intranasal method highlights a promising solution for protein therapy of brain diseases.

*Corresponding author. Tel.: +86 21 20231000x1401; fax: +86 21 20231981.
**Corresponding author at: College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109-1065, USA. Tel.: +1 734 764 4273; fax: +1 734 763 9772.
E-mail addresses: vcyang@umich.edu (Victor C. Yang), yzhuang@simm.ac.cn (Yongzhuo Huang).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.04.001

1. Introduction

The blood–brain barrier (BBB) poses a formidable challenge to the central nervous system (CNS) delivery of macromolecular therapeutics. In some brain diseases pathological processes often associate with changes in BBB permeability. As a good case in point, leaky blood vessels are commonly found in brain cancers, thereby contributing to the enhanced permeability and retention (EPR) effect[1]. In most circumstances, however, the leakage is generally limited to insignificant amounts of active macromolecules that yield little therapeutic benefit. Moreover, neurodegenerative diseases do not induce angiogenesis and thus there is no significant change in BBB permeability.

Intracranial injection is the direct but difficult way to deliver drugs into the brain. Despite the high risk of surgical operations, intracranial administration remains the primary means of direct brain drug delivery; for instance, it is the only clinically employed method for biomacromolecular drugs due to their inability to penetrate the BBB. However, the BBB is not the only problem; the difficult problem of drug diffusion across CNS compartments must also be addressed. Although intraparenchymal or CSF administration can yield a high degree of targeting, the distribution of proteins is restricted within the injection sites due largely to the hydrophilic nature and large size[2]. For example[3], diffusion coefficients of proteins in the brain is estimated to be 10^{-6} cm^2/s. The delay or restriction of intra-tissue diffusion may compromise the desired pharmacological efficacy. Hence, the dual barriers (*i.e.*, BBB and intracerebral diffusion) render most efforts to deliver protein drugs into brain a failure. Therefore, the need for new techniques to overcome the BBB and deliver drugs into the CNS remains exigent.

Nasal administration using the olfactory axonal pathway from the epithelium into cerebral tissue for drug delivery has attracted scientific attention for its circumvention of BBB and ease of administration. The research focus of nose-to-brain delivery, however, has been most prominently focused on small drugs, because the tight intercellular junctions in the nasal mucous membranes normally prevent the passage of drugs with molecular weights greater than 1000 Da. Apart from the tight junction barrier, migration along olfactory axons presents an additional obstacle for the delivery of proteins because of their poor cell penetration ability. Some viruses[4] and phage-display peptide sequences[5] have been reported to reach the CNS *via* olfactory axonal transport, but so far little is known about the feasibility of the olfactory axonal delivery of macromolecular therapeutics.

Cell-penetrating peptides (CPPs), also known as protein transduction domains (PTDs), have been extensively explored for their potential application in mediating biomacromolecular drug delivery[6]. The CPP-based intracellular delivery has been shown to be cell-type independent[7], and able to penetrate across various biobarriers (*e.g.*, retina and neurons[8,9], blood brain barrier[10,11], intestine wall[12–14], and skin[15,16]) that otherwise constitute great impediments to conventional approaches of macromolecular drug delivery. Therefore, CPPs are a powerful tool for mediating protein delivery.

Low molecular weight protamine (LMWP) is a nature-sourced CPP with the sequence of VSRRRRRRGGRRRR, firstly identified by our laboratory from enzymatically-digested fractions of protamine. In our previous studies the ability of LMWP-mediated transcutaneous delivery has been demonstrated[16,17]. More interestingly, LMWP was also found to be able to mediate nose-to-brain delivery of nanoparticles[18]. Therefore we were motivated to develop a nose-to-brain protein delivery system by conjugating a CPP to a protein molecule (Fig. 1).

2. Experimental

LMWP was prepared by digestion of native protamine using an enzymatic method we reported previously[19]. Three model proteins, bovine serum albumin (BSA, Sigma–Aldrich), peroxidase (HRP, Sigma–Aldrich) and β-galactosidase (β-gal, Invitrogen) were used in the investigations. The proteins were conjugated to LMWP *via* a covalent bond. In brief, LMWP in PBS (5 mg/mL) was activated by *N*-succinimidyl-*S*-acetylthioacetate (SATA, Pierce, Fisher) at a reaction ratio of 1:3. After a 2-h incubation at room temperature (RT), the excess SATA was removed using a heparin affinity column (HiTrap, GE Healthcare). Deacylation to generate a sulfhydryl at the N-terminal of LMWP was accomplished using dydroxylamine · HCl. A maleimide-activated protein (10 mg/mL in PBS) was produced by reaction with a 3-fold molar excess of succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, Pierce) for 30 min, and purified using a desalting column. The thiolated LMWP and maleimide-activated protein were mixed (5:1, mol/mol) and incubated for 2 h at r.t. to produce LMWP-conjugated protein *via* a thioether bond. Further purification of the LMWP-conjugated proteins was conducted by dialysis and heparin column as described in our previous report[16]. The elution obtained from the heparin column with a gradient elution of 2 mol/L NaCl

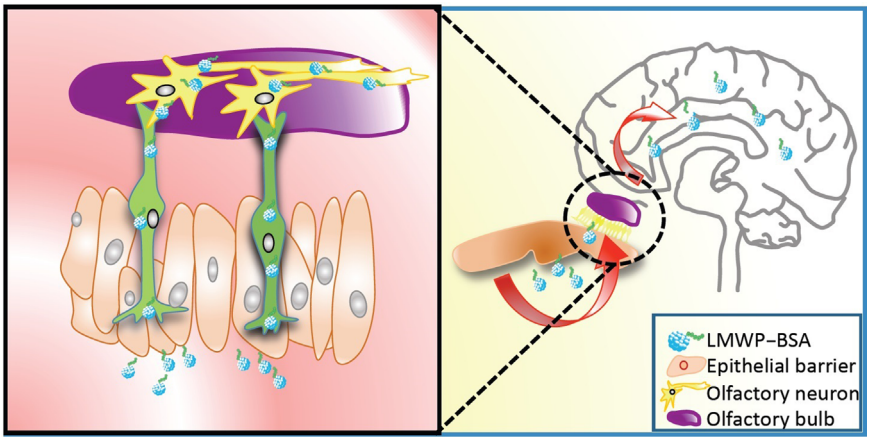


Figure 1 The cell-penetrating LMWP peptide-mediated protein drug from nose to brain delivery.