International Journal of Pharmaceutics 500 (2016) 128–135

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Role of scavenger receptors in peptide-based delivery of plasmid DNA across a blood–brain barrier model

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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 31 December 2015
Accepted 5 January 2016
Available online 7 January 2016

Keywords:
Blood–brain barrier
bEnd.3
Plasmid transfection
Scavenger receptors
angiopep-2
LRP-1 receptor
Receptor-mediated endocytosis

ABSTRACT

Receptor-mediated transcytosis remains a major route for drug delivery across the blood–brain barrier (BBB). PepFect 32 (PF32), a peptide-based vector modified with targeting ligand (Angiopep-2) binding to low-density lipoprotein receptor-related protein-1 (LRP-1), was previously found to be a promising vector for plasmid delivery across an in vitro model of the BBB. Cellular uptake of PF32/plasmid DNA (pDNA) complexes was speculated the internalization via LRP-1 receptor. In this study, we prove that PF32/pDNA nanocomplexes are not only transported into brain endothelial cells via LRP-1 receptor-mediated endocytosis, but also via scavenger receptor class A and B (SCARA3, SCARA5, and SR-BI)-mediated endocytosis. SCARA3, SCARA5, and SR-BI are found to be expressed in the brain endothelial cells. Inhibition of these receptors leads to a reduction of the transfection. In conclusion, this study shows that scavenger receptors also play an essential role in the cellular uptake of the PF32/pDNA nanocomplexes.

1. Introduction

Delivery of drugs across the BBB remains a major challenge for central nervous system (CNS) drug development (Manich et al., 2013). Since the BBB restricts passage of approximately 98% of small molecules (MW > 500 Da), proteins, peptides, and 100% of macromolecules such as recombinant proteins or gene-based medicines (Pardridge, 2007). Hence, crossing the BBB is a major obstacle to develop therapeutic drugs for brain diseases. Various strategies have been developed to enhance the amount of therapeutic agents delivered into the brain (Gabathuler, 2010). Conventionally, invasive approaches for drug delivery to the brain by neurosurgery-based strategies such as intracerebro-ventricular infusion, convection-enhanced delivery, and intracerebral implants cause high risks of infection, pathological changes due to disruption of the BBB, and neuronal damages (Gabathuler, 2010; Scherrmann, 2002). Consequently, non-invasive strategies have been applied for CNS drug delivery particularly by utilizing physiological transport systems. The application of homing devices that use endogenous transport systems for site-specific delivery via receptor-mediated transcytosis (RMT) at the BBB has been successfully employed in the brain-targeted transport system (De Boer and Gaillard, 2007a,b).

RMT is an active physiological process requiring energy and employs vesicular trafficking. It occurs in three steps: receptor-mediated endocytosis at luminal (blood) side by the ligands binding to specific membrane receptors, transfer of endocytic vesicles through the cytoplasm, and exocytosis of the large molecules at the abluminal (brain) capillary endothelium (Brasnjevic et al., 2009; Gabathuler, 2010; Malcor et al., 2012). Currently, several receptors are known to be expressed on the BBB such as transferrin receptor (TfR), insulin receptor and insulin-like growth factor receptor, diphtheria toxin receptor, heparin binding epidermal growth factor-like growth factor receptor, LRP-1 and -2 receptors (De Boer and Gaillard, 2007a,b; Jones and Shusta, 2007), as well as scavenger receptor class A type I (SR-AI) and scavenger class B type I (SR-BI) (De Boer et al., 2003). RMT-based delivery has been achieved using brain-targeting nanoparticles (Ulbrich et al., 2009; Xin et al., 2011),

Abbreviations: PF, PepFect; BBB, blood–brain barrier; ANG, angiopep-2; CR, charge ratio; pGL3, luciferase-encoding plasmid; SRs, scavenger receptors; TAE, Tris–acetate-EDTA; TBE, Tris–borate-EDTA; EtBr, ethidium bromide.

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http://dx.doi.org/10.1016/j.ijpharm.2016.01.014
0378-5173/

peptides (Demeule et al., 2008a), and antibodies (Wu et al., 1997), therefore, the brain-targeted drug delivery has been developed to bind to the receptors which are expressed on the brain endothelial cells (Malcor et al., 2012).

The low-density lipoprotein receptor (LDLR) family is a group of cell surface receptors involved in the uptake of macromolecules via receptor-mediated endocytosis. A number of studies suggest that the LDL receptor and members of its superfamily play a vital role of cellular drug uptake (Chung and Wasan, 2004). LDLR-related protein (LRP), a member of LDLR family, is expressed in CNS (Ke et al., 2009), cerebellum, cerebral cortex, hippocampus, brain stem, and also on the BBB (Rip et al., 2009). Interestingly, LRP-1 was found not only to be over-expressed on BBB, but also expressed in malignant astrocytomas, especially in glioblastomas or glioma cells (Gabathuler, 2010; Xin et al., 2012b). Hence, LRP-1 has become a crucial target for the brain-targeting approaches. Recent studies reported that LRP-1 successfully mediated the uptake of angiopep-2 (ANG), a Kunitz domain-derived peptide from protease enzyme, across the BBB resulting in high BBB permeability and accumulation in parenchyma (Demeule et al., 2008a,b). A conjugation of ANG and anticancer drug, paclitaxel (PTX), was already used in clinical studies to evaluate the safety, tolerability, pharmacokinetic, and efficacy in patients. The studies showed that ANG-PTX has a potential in advanced solid tumors treatment (Xiao and Gan, 2013). In addition, ANG was also modified by conjugation with 3 molecules of PTX in order to increase survival of mice with intracranially implanted tumors. This drug is now in clinical trial phase I for recurrent malignant gliomas and for brain metastases in advanced cancer (Gabathuler, 2010). Hence, LRP-1 has been suggested an involvement of ANG-modified nanoparticle transcytosis across the BBB and the cellular uptake in glioma cells both in vitro and in vivo (Huang et al., 2011; Ren et al., 2012; Sun et al., 2012; Xin et al., 2012a,b, 2011).

Scavenger receptors (SRs) are a family of cell surface glycoproteins presented as transmembrane and soluble proteins able to bind modified LDL such as oxidized and acetylated LDL (oxLDL and acLDL) (Peiser and Gordon, 2001). SRs are divided into 8 different classes, class A, B, C, D, E, F, G, and H, encoded by unrelated genes. SRs are multiligand receptors with ability to bind a wide variety of endogenous and exogenous ligands (Kzhyshkowska et al., 2012; Peiser and Gordon, 2001; Stephen et al., 2010). Particularly, SR-BI was found in bovine and porcine brain capillary endothelial cells (BCECs) (De Boer et al., 2003) and also expressed in murine brain (Srivastava, 2003). The rodent SR-BI was studied and showed the identity to human SR-BI (De Boer et al., 2003). Additionally, SR-AI is also involved in the cellular uptake of Alzheimer amyloid-beta in human BCECs (De Boer et al., 2003; Mackic et al., 1998). SRs bind specifically to polyanionic ligands (Ezzat et al., 2012); for example, naked pDNA has been reported to be taken up via the scavenger receptor-mediated mechanism in bovine brain microvessel endothelial cells (BMEC). This uptake was significantly inhibited by polyanions such as polyinosinic acid and dextran sulfate, but not by polycytidylic acid and EDTA (Nakamura et al., 1998).

To understand the route of the endocytic pathway of peptide/pDNA nanocomplexes across the brain endothelial cells, PF32, a modified peptide coupled with targeting peptide ANG (Fig. 1), was non-covalently complexed with pDNA in comparison with the parent peptide PepFect 14 (PF14). In a previous study, we found that PF32 was a potent vector for pDNA delivery across the in vitro model the BBB and able to efficiently transfect in glioblastoma cells (Srimanee et al., 2014). In this study, we demonstrate that scavenger receptors (SCARA3, SCARA5, and SR-BI) are also involved in the uptake of the complexes into the brain endothelial cells (bEnd.3) and also mediate the transport of PF32/pDNA nanocomplexes across the BBB model. Since we had believed that there was only LRP-1 receptor involved in the brain delivery of the nanocomplexes due to ANG-modified cell-penetrating peptide (CPP), this study points out that scavenger receptors both class A and B also play a crucial role in the receptor-mediated endocytosis of PF32/pDNA nanocomplexes.

Stearyl-LLOOLAAAALOOLL-TFFYGGSRGKRNNFKTEEY-NH_2

PepFect 32: modified PepFect 14-angiopep-2

Fig. 1. Structure of PepFect32 (PF32). PF32 is comprised of truncated PF14 and LRP-1 targeting ligand, angiopep-2 (ANG).

2. Materials and methods

2.1. Materials

All amino acids were purchased from Iris biotech GmbH, Germany. bEnd.3 cell lines were purchased from American Type Culture Collection (ATCC), USA. Lipofectamine™ 2000 and RNAiMax were obtained from Life Technologies, Sweden. All the chemicals for peptide synthesis were purchased from AGTC bioproducts, UK and Sigma–Aldrich, Germany. Maxima SYBR green qPCR master mix was purchased from Thermo Fisher Scientific Biosciences GmbH, Sweden.

2.2. Peptide synthesis

All peptides were synthesized in a stepwise manner using a solid-phase peptide synthesis strategy on an Syro II automated peptide synthesizer (MultiSynTech, Germany) using Chemmatrix Rink-amide resin (0.20 mmol/g) (PCAS BioMatrix, Canada) to obtain C-terminal amidated peptides. Amino acids were activated by using 5 equivalents of 2-(6-Chloro-1H-benzotriazole-1-yl)-1, 1,3,3-tetramethylaminium hexamethylaminium hexafluorophosphate (HCTU)/6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt) and 10 equivalents of diisopropylethylamine (DIPEA). N-terminally stearylated peptides were treated with 5 equivalents of stearic acid (Sigma–Aldrich), 5 equivalents of HCTU/Cl-HOBt, and 10 equivalents of DIPEA in dichloromethane (DCM) for 2–4 h. The peptides were cleaved from resin using a cleavage mixture of trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/H_2O (95/2.5/2.5% v/v) for 2 h at room temperature. Peptides were precipitated and washed in cold diethyl ether and then purified by reversed phase high performance liquid chromatography (RP-HPLC) on a C_8 column with 20–100% acetonitrile (0.1% TFA) gradient. The peptides were analyzed by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Voyager-DE STR, Applied Biosystems) using α-cyano-4-hydroxy-cinnamic acid as a matrix.

2.3. Cell culture

bEnd.3 cells were cultured at 37 °C under an atmosphere containing 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) with glutamax (Invitrogen, Sweden) supplemented with 10% fetal bovine serum (FBS), 3 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Sweden).

2.4. Identification of receptor expression on bEnd.3 cells

bEnd.3 cells (5×106) were extracted to obtain total RNA using RNeasy Mini Kit (Qiagen, Sweden). Total RNA (1 μg) was reverse transcribed by using RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific Biosciences GmbH, Sweden) according to manufacturer's instructions. Briefly, total RNA was treated with 100 pmol non-specific primer (oligo $(dT)_{18}$) and nuclease-free