Published in final edited form as:

J Control Release. 2007 October 8; 122(3): 345–348. doi:10.1016/j.jconrel.2007.04.001.

Blood-brain barrier delivery of protein and non-viral gene therapeutics with molecular Trojan horses

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Abstract

The products of biotechnology, recombinant proteins, monoclonal antibodies, antisense, RNA interference, or non-viral gene transfer, cannot be developed as pharmaceuticals for the brain, unless these molecules are re-formulated to enable transport across the blood-brain barrier (BBB). Large molecule drugs, and plasmid DNA, can be delivered across the BBB with receptor-specific molecular Trojan horses. Trojan horse BBB delivery systems, coupled with one of 3 different technology platforms (fusion proteins, avidin-biotin, or Trojan horse liposomes), can enable the BBB transport of virtually any large molecule drug or plasmid DNA.

Keywords

blood-brain barrier; drug targeting; insulin receptor; fusion protein

1. Introduction

Large molecule therapeutics for the brain include recombinant proteins, monoclonal antibodies (MAb), antisense drugs, short interfering RNA (siRNA), and non-viral gene medicines. None of these products of biotechnology can be developed as neurotherapeutics, because these large molecules do not cross the brain capillary endothelial wall, which forms the blood-brain barrier (BBB) in vivo. Certain endogenous large molecules, such as insulin or transferrin, do cross the BBB via a process of receptor-mediated transcytosis (RMT) [1]. The insulin receptor or the transferrin receptor (TfR) is expressed on the plasma membrane of the brain capillary endothelial cell and serves to transport endogenous insulin or transferrin from blood to brain. Similarly, certain peptidomimetic monoclonal antibodies undergo RMT across the BBB on the endogenous peptide receptor transporters. The peptidomimetic MAbs bind exofacial epitopes on the BBB receptor, which triggers transport across the BBB. Since the MAb binding site is different from the binding site of the endogenous ligand, there is no interference of endogenous ligand transport [2]. The peptidomimetic MAbs may be used as molecular Trojan horses (MTH) to ferry large molecule therapeutics, including non-viral plasmid DNA, across the BBB via the endogenous RMT systems.

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1.1 Species-specific molecular Trojan horses

The MAb-based MTHs are species-specific (Table 1). For drug delivery to the mouse, the rat 8D3 MAb to the mouse TfR is used [3]. For drug delivery to the rat, the murine OX26 MAb to the rat TfR is used [4]. The murine 83-14 MAb to the human insulin receptor (HIR) is used for drug delivery to Old World primates such as the Rhesus monkey [5]. The HIRMAb is not active in New World primates such as squirrel monkeys. Drug delivery across the human BBB employs genetically engineered chimeric or humanized forms of the HIRMAb [6,7]. The MTH activity of these peptidomimetic MAbs have all been validated with in vivo pharmacologic reduction to practice. In addition, the MAb-based MTHs optimize the plasma pharmacokinetics (PK) of the drug. The plasma area under the concentration curve (AUC) of a drug is increased following attachment to the MAb-based MTH. Cationic import peptides are effective MTHs in in vitro cell culture models. However, in vivo, the cationic import peptides are rapidly cleared from the blood and have very low plasma AUCs. Attachment of drugs to cationic import peptides actually results in a reduction in the plasma AUC in vivo [8]. Since brain uptake of the therapeutic is directly proportional to the plasma AUC, any MTH that results in reduction in the plasma AUC of the drug will most likely be an ineffective MTH in vivo.

2. Blood-brain barrier transport of recombinant proteins and antisense agents

The MAb-based MTHs have been reduced to practise in vivo in CNS experimental systems, including brain ischemia, brain cancer, Parkinson's disease, and brain amyloid (Table 2). Vasoactive intestinal peptide (VIP) is a potent cerebrovasodilator when applied topically to brain blood vessels [9]. However, the infusion of VIP into the carotid artery does not result in an increase in cerebral blood flow (CBF) [10], owing to lack of transport of the VIP across the BBB. Conjugation of VIP to the TfRMAb results in a 65% increase in hemispheric CBF in the conscious rat following intravenous administration of low doses (10-20 µg/kg) of the VIP-MAb conjugate [11]. Brain derived neurotrophic factor (BDNF) is a potent neuroprotective agent in global brain ischemia when injected directly into the brain [12]. However, the intravenous administration of BDNF in rats subjected to transient forebrain ischemia and isoelectric electroencephalogram does not result in any neuroprotection [13]. Intravenous BDNF is not neuroprotective because (a) the BDNF does not cross the BBB [14], and (b) the BBB is intact in the first six hours following brain ischemia when neuroprotection is still possible [15]. Conjugation of BDNF to the TfRMAb results in 100% neuroprotection of the pyramidal neurons of the CA1 sector of the hippocampus following delayed intravenous administration [13]. BDNF is also neuroprotective in regional brain ischemia following direct intra-cerebral injection [16]. However, the intravenous administration of BDNF does not result in neuroprotection in regional brain ischemia such as induced by middle cerebral artery occlusion (MCAO) [17], because the BBB is intact following ischemia [18], and because BDNF does not cross the BBB. The conjugation of BDNF to the TfRMAb results in a 65-70% reduction in stroke volume in rats subjected to either permanent or reversible MCAO [17,19]. Reduction in stroke volume is correlated with a >200% improvement in motor function as determined by rotarod testing [20]. Fibroblast growth factor (FGF)-2 is a potent neuroprotective agent. However, the intravenous administration of FGF-2 in focal brain ischemia does not result in a reduction of stroke volume [21], because FGF-2 does not enter brain in the absence of BBB disruption [22]. Conjugation of FGF-2 to the TfRMAb results in an 80% reduction in stroke volume in the permanent MCAO model following delayed intravenous administration [21]. The Aβ¹⁻⁴⁰ peptide rapidly binds pre-existing amyloid plaque [23], and Aβ¹⁻⁴⁰ could be used as a peptide radiopharmaceutical for imaging brain amyloid with standard external detection modalities such as positron emission tomography (PET) or single photon computed emission tomography (SPCET). However, Aβ¹⁻⁴⁰ does not cross the BBB [24]. Conjugation of Aβ¹⁻⁴⁰ to the TfRMAb allowed for BBB transport of the peptide

radiopharmaceutical, and for imaging brain amyloid in a transgenic mouse model of Alzheimer's disease (AD) [25].

Sequence-specific antisense radiopharmaceuticals could be used to image gene expression in the brain, should these large molecules be made transportable through the BBB. The peptide nucleic acid (PNA) is an antisense agent suitable for imaging, because hybridization of the PNA to the target mRNA does not result in RNase activation and degradation of the target transcript. Sequence-specific PNAs have been synthesized with dual modifications of incorporation of a diethylenetriaminepentaacetic acid (DTPA) moiety, for chelation with ^{111}In , and a single biotin residue for high affinity attachment to a conjugate of a BBB MTH and either avidin or streptavidin (SA) [26]. Avidin or SA bind biotin with extremely high affinity with a K_D of 10^{-15} M and dissociation half-time of 89 days [27]. Therefore, attachment of an antisense radiopharmaceutical to a BBB MTH via a biotin-SA bridge results in a highly stable complex for in vivo applications. The caveolin (CAV)-1 α gene is up-regulated and the glial fibrillary acidic protein (GFAP) gene is down-regulated in experimental brain gliomas [26]. The modulation of the CAV gene in brain cancer could not be detected in vivo with a PNA specific for the CAV mRNA, because the PNA does not cross the BBB, and because the BBB is intact in brain cancer until the very terminal stage. However, conjugation of the CAV-specific PNA to the TfRMAb allowed for delivery of the PNA into brain, and for imaging of the up-regulation of the CAV gene in experimental brain cancer [26]. Similarly, conjugation of the GFAP-targeted PNA to the TfRMAb allowed for in vivo imaging of the down-regulation of GFAP gene expression in experimental brain cancer [26]. The GFAP gene is also down-regulated in the first 24 hours following regional brain ischemia and this was verified by in vivo imaging of GFAP gene expression in rat brain 24 hours after reversible MCAO with a GFAP-specific PNA that was attached to the TfRMAb [28].

Lysosomal storage disorders are treated with enzyme replacement therapy (ERT) using the respective recombinant lysosomal enzyme for the given disorder. There are over 40 different lysosomal storage disorders and the majority of these affect the brain [29]. However, ERT is ineffective in the brain because the enzyme does not cross the BBB. β -galactosidase was used as a model lysosomal enzyme. In vivo studies in the mouse showed that β -galactosidase does not cross the BBB [30]. However, conjugation of the β -galactosidase to the TfRMAb resulted in a rapid increase in brain uptake of the 116 kDa enzyme [30]. Therefore, MTHs can be used to deliver even large recombinant proteins across the BBB.

3. Delivery of protein therapeutics across the human blood-brain barrier with genetically engineered fusion proteins

The HIRMAb has been developed for brain drug targeting in humans, because the BBB transport activity of this MTH is nearly 10-fold greater than that of the TfRMAb [1]. The murine HIRMAb cannot be used in humans. Therefore, genetically engineered forms of the HIRMAb have been produced, and both chimeric and humanized forms of the HIRMAb undergo rapid transport across the primate BBB in vivo [6,7]. A fusion protein of the HIRMAb and BDNF has been genetically engineered, and shown to retain the bi-functional properties of high affinity binding to both the HIR and to the BDNF receptor, trkB [31]. The HIRMAb-BDNF fusion protein binds with high affinity to the HIR in the human BBB and undergoes rapid transport across the primate BBB in vivo [31]. A genetically engineered fusion protein of the HIRMAb and a single chain Fv (ScFv) antibody against the A β amyloid peptide of AD has been genetically engineered, expressed, and validated [32]. The fusion protein is a tri-functional molecule that binds the HIR, to enable transport across the BBB in the blood to brain direction, binds the neonatal Fc receptor (FcRn) on the BBB to mediate BBB transport in the brain to blood direction, and binds and disaggregates A β fibrils [32].

4. Brain delivery of non-viral plasmid DNA with Trojan horse liposomes

Non-viral plasmid DNA can be delivered across the BBB with MTHs using liposome technology and Trojan horse liposomes (THL) [33]. The non-viral plasmid DNA is encapsulated in a 100 nm liposome and the surface of the liposome is conjugated with several thousand strands of polyethylene glycol (PEG). The tips of 1-2% of the PEG strands are conjugated with the MTH. Pegylated liposomes, per se, are relatively biologically inert and do not cross the BBB. However, conjugation of the MTH to the surface of the liposome induces RMT across the BBB and also induces receptor-mediated endocytosis across the plasma membrane of brain cells [34]. Both the insulin receptor and the TfR are highly expressed in both the BBB and the brain cell membrane. Plasmid DNA encoding reporter genes such as luciferase or β -galactosidase have been delivered to mouse, rat, and primate brain with THLs [34-36]. Following the intravenous injection of THLs carrying non-viral plasmid DNA, there is global expression of the transgene throughout the primate brain [34]. THLs enable adult transgenics in 24 hours. The only other technology that could provide this experimental result is the pro-nuclear injection of the β -galactosidase transgene into a Rhesus monkey embryo and growth of the embryo into an adult animal.

Therapeutic transgenes have been delivered across the BBB with THLs in models of experimental Parkinson's disease (PD) and experimental cancer. A tyrosine hydroxylase (TH) expression plasmid was encapsulated in TfRMAB-targeted THLs and injected intravenously to rats with apomorphine-confirmed experimental PD. The THL transfer of the TH gene results in a 100% normalization of striatal TH enzyme activity, as well as an 80% reduction in abnormal motor behavior associated with experimental PD [37]. In experimental brain cancer, survival of mice with intra-cranial human brain cancer was increased 100% following weekly intravenous injection of THLs encapsulating an expression plasmid encoding a 700 nucleotide RNA that was antisense to a specific sequence of the human epidermal growth factor receptor (EGFR) [38]. Similarly, a 90% increase in survival time of mice with intra-cranial human brain cancer was observed following weekly intravenous administration of THLs encapsulating an expression plasmid encoding a short hairpin RNA (shRNA) against a defined sequence of the human EGFR mRNA [39]. The THL technology enables intravenous RNA interference (RNAi) of the brain.

5. Summary

The BBB MTHs can be used with 3 different technology platforms to deliver virtually any large molecule therapeutic across the BBB (Figure 1). The fusion protein (FP) technology can be used to deliver recombinant proteins and monoclonal antibody-based therapeutics. In the avidin-biotin (AB) technology, a fusion protein of the MTH and avidin is produced in parallel with monobiotinylation of the therapeutic, which might be an oligopeptide, an antisense agent, or a siRNA. Non-viral plasmid DNA can be delivered across the BBB by formulating liposomes with MTHs and production of Trojan horse liposomes.

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Figure 1.

Molecular Trojan horses are incorporated into 1 of 3 technology platforms, depending on the type of pharmaceutical to be delivered to the brain. Recombinant proteins or monoclonal antibodies are delivered with the fusion protein technology [31,32]. Peptides, antisense, or short interfering RNA (siRNA) are delivered with the avidin-biotin technology, wherein the drug is mono-biotinylated in parallel with the production of a fusion protein of avidin and the Trojan horse. Plasmid DNA, which can encode short interfering RNA (shRNA), is delivered across the BBB with Trojan horse liposomes [33,39].

Table 1

Species-specific peptidomimetic monoclonal antibody molecular Trojan horses for blood-brain barrier delivery

Species	Targeted receptor	Trojan horse
Mouse	TfR	rat 8D3 MAb to mouse TfR
Rat	TfR	murine OX26 MAb to rat TfR
Rhesus monkey	insulin receptor	murine 83-14 MAb to HIR
Human	insulin receptor	chimeric or humanized HIRMAb

TfR=transferrin receptor; MAb=monoclonal antibody; TfR=transferrin receptor; HIR=human insulin receptor

Table 2

Delivery of protein, antisense, and non-viral gene therapeutics across the BBB with molecular Trojan horses

Class	Therapeutic	Indication	reference
Peptide	VIP	cerebral blood flow	11
	BDNF	global brain ischemia	13
	BDNF	focal brain ischemia	17, 19
	FGF-2	focal brain ischemia	21
	EGF	imaging brain cancer	40
	A β ¹⁻⁴⁰	imaging brain amyloid	25
Antisense	PNA-CAV	brain cancer imaging	26
	PNA-GFAP	brain ischemia imaging	28
Enzyme	β -galactosidase	lysosomal enzyme	30
Plasmid DNA	luciferase	reporter enzyme	34
	β -galactosidase	reporter enzyme	34
	Antisense	brain cancer	38
	RNAi	brain cancer	39
	TH	PD	37

VIP=vasoactive intestinal peptide; BDNF=brain derived neurotrophic factor; FGF=fibroblast growth factor; EGF=epidermal growth factor; PNA=peptide nucleic acid; CAV=caveolin; GFAP=glial fibrillary acidic protein; RNAi=RNA interference; TH=tyrosine hydroxylase; PD=Parkinson's disease