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EXPERT REVIEW

Targeted Delivery of Nano-Therapeutics for Major Disorders of the Central Nervous System

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ABSTRACT Major central nervous system (CNS) disorders, including brain tumors, Alzheimer's disease, Parkinson's disease, and stroke, are significant threats to human health. Although impressive advances in the treatment of CNS disorders have been made during the past few decades, the success rates are still moderate if not poor. The blood–brain barrier (BBB) hampers the access of systemically administered drugs to the brain. The development of nanotechnology provides powerful tools to deliver therapeutics to target sites. Anchoring them with specific ligands can endow the nano-therapeutics with the appropriate properties to circumvent the BBB. In this review, the potential nanotechnology-based targeted drug delivery strategies for different CNS disorders are described. The limitations and future directions of brain-targeted delivery systems are also discussed.

KEY WORDS brain tumor · central nervous system · cerebrovascular disease · neurodegenerative disorder · targeted delivery

INTRODUCTION

At present, CNS disorders, including brain tumors, neurodegenerative diseases, and cerebrovascular diseases are serious threats to human health. The blood–brain barrier (BBB) is a major obstacle to treating disorders of the CNS (1). BBB is composed of several kinds of cells including endothelial cells, astrocytes, microglial cells and pericytes (2). The exchange of substances between brain tissue and blood is restricted by both physical (tight junctions) and metabolic (enzymes) barriers (3). Normally, nutrients, such as hexoses, amino acid and neuropeptides are transported through specific receptors. Beside nutrients, only small lipophilic molecules (<500 Da) are able to cross the BBB (4). Almost 100% of the macromolecular drugs and over 98% of the small-molecule drugs cannot penetrate this barrier (5). Intracranial drug delivery is a useful but inconvenient method with poor compliance, which is overshadowed by the risk of infection and edema.

Brain targeted delivery can be achieved through receptor-mediated, transporter-mediated and adsorptive-mediated BBB transportation, intranasal delivery to bypass BBB and pharmacological disruption of BBB (6). Targeted drug delivery systems are promising modes of treating CNS diseases due to several distinguishing characteristics. First, systemically administered targeted delivery systems that envelope drugs can convey them into the brain. Second, producing targeted delivery systems with excellent traits is possible due to the rapid developments in materials science and nanotechnology. Third but not last, progress in biology and etiology has provided strategies that allow targeted delivery systems to enter the brain and reach the sites of disease. Many nano-therapeutics have been developed and used to treat CNS disorders, including liposomes, micelles, polymer nanoparticles, dendrimers, inorganic nanoparticles, carbon nanotubes, and fullerenes (7).

In this review, recent advances in targeted delivery systems for the management of CNS diseases are discussed. The limitations and the future directions of brain-targeted delivery systems are also discussed.

BRAIN TUMOR

Brain tumors are the most common CNS disease, with an incidence of approximately 6/100,000 for malignant brain tumors (8–10). Among them, gliomas accounts for approximately 80% (11). Their surgical resection is necessarily restricted because of the important functions of the brain.

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Moreover, infiltration of tumor cells into normal tissues makes complete surgical resection impossible (12). Invasive growth of brain tumors leads to a poor prognosis and frequent recurrence (9). The median survival time of patients suffering from brain tumors is approximately 10 months. Although temozolomide chemotherapy in combination with surgical resection can improve the median survival time to 15 months (13), the outcome is still far from desirable.

BBB is the first barrier to managing brain tumors. Although some argue that the BBB is complementary in brain tumors (14), the complement exists only in the beds of brain tumors. The BBB is complete in the invasive part of brain tumors, particularly the part distanced from the tumor bed (11). In a study of the distribution of erlotinib in the U87 brain tumor model, the drug concentration in the brain around the tumor core is 73% lower than the concentration in the tumor core (15).

Blood-tumor barrier (BTB) is another obstacle to delivering drugs into brain tumors (16,17). Compared with the BTB of peripheral tumors, the BTB of brain tumors is stricter terms of the trans-endothelial fenestrations, transporter expression and inter-endothelial cell gaps (18,19). The pore cutoff size of subcutaneous tumors ranges from 200 nm to 1.2 μm (20). However, the pore size is dramatically smaller in cranial microenvironments. The pore cutoff size of the U87 brain tumor model is 7–100 nm, while this number is even smaller in the RG2 brain tumor model (21,22), leading nanomedicine hard to access brain tumors through blood circulation. The drug efflux pumps on brain tumor cells further restrict drug uptake. There are several such pumps including P-glycoprotein, breast-cancer resistance protein and multidrug-resistance associated proteins (16,23,24). Taskar *et al.* evaluated the lapatinib distribution in breast tumors and brain metastases (25). The lapatinib concentration in brain metastases (672 ng/g) was much lower than that in peripheral metastases. For example, the drug concentration in lung metastases was 3,464 ng/g. This is why most chemotherapeutics are failed in treatment of brain tumors.

Drugs can be encapsulated in nano-therapeutics to escape recognition by drug efflux pumps and increase their penetration of the BBB and BTB. Many targeting strategies have been developed to penetrate the BBB or the BTB or both.

Brain Targeting Delivery

Many receptors are highly expressed on the BBB, including transferrin receptor (TfR), low-density lipoprotein receptor, insulin receptor, insulin-like growth factor receptor, diphtheria toxin receptor, nicotinic acetylcholine receptor (nAChR) and scavenger receptor class B type (6). Thus, delivery systems can be decorated with ligands of these receptors to mediate their penetration of the BBB.

Polymersomes functionalized with transferrin, a specific ligand for TfR, was used for targeted delivery (26). In this study, the transferrin-modified polymersomes (Tf-PO) delivered significantly more cargo to brain tumors than did unmodified polymersomes. Treatment with doxorubicin-loaded Tf-PO significantly increased the median survival time of brain tumor-bearing rats, which was 70% longer than that of rats treated with a doxorubicin solution. However, endogenous transferrin may inhibit the internalization of Tf-PO mediated by the TfR. Coupled antibodies directed against these receptors can avoid this problem. OX26, an antibody for TfR, has been used for brain-targeted delivery (27).

However, the application of proteins and antibodies are restricted by their instability and immunogenicity. Small molecules, such as peptides and aptamers, are better choices. CDX is a peptide redesigned from loop II region of candoxin, a ligand for nAChR (28). CDX- functionalized micelles accumulated in brain tumors at significantly higher levels than did unmodified micelles. Paclitaxel-loaded targeting micelles prolonged the median survival time of brain tumor-bearing mice to 27 days, which was significantly longer than that obtained with unmodified micelles (a median survival time of 20 days).

In addition to receptors, transporters on the BBB can also be used for brain-targeted delivery, including amino acid transporters, hexose transporters and monocarboxylate transporters (6). The transporter for glutathione is highly expressed at the BBB. G-Technology is a glutathione conjugated liposomes based delivery system developed by the company of ToBBB (Netherlands) (29). Several drugs were successfully delivered into brain by G-Technology. One of them, glutathione pegylated liposomal doxorubicin was under evaluation of clinical phage I/II, which may become the first targeted nanomedicine approved in the world because the well defined system, favorable pharmacokinetics and safe and human applicable ligand.

Due to electrostatic forces, positively charged delivery systems interact with the negatively charged BBB through adsorption-mediated endocytosis. Cationized albumin is an example of this. Lu *et al.* conjugated cationized albumin to nanoparticles (CBSA-NPs) to deliver the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene and aclarubicin to the brain (30,31). The aclarubicin concentrations in the tumors of CBSA-NP-treated mice were 2.6- and 3.3-fold higher than those of mice treated with unmodified NPs and a solution of aclarubicin 1 h post-injection and were 2.7- and 6.6-fold higher after 24 h, respectively. Four cycles of treatment with aclarubicin-loaded CBSA-NPs significantly increased the median survival time of brain tumor-bearing mice. Repeated injections of TRAIL gene-loaded CBSA-NPs also provided a better anti-brain tumor effect than did the unmodified NPs. However, poor selectivity is the predominant problem of adsorptive-mediated targeting.

Brain targeted delivery could enhance the distribution of drugs in brain, however, the poor selection between normal brain and diseased brain restricted the application of this strategy. Further, the distribution of drugs in normal may cause serious CNS side effects. Thus, brain targeted delivery may be more suitable for delivering of active or nutritional compounds rather than cytotoxic drugs.

Brain Tumor-Targeted Delivery

Using brain-targeted delivery, the therapeutic outcome indeed improved, accompanied by the increased access of drugs to the brain and brain tumors. However, the distribution in the brain, particularly the brain tumor/brain ratio, is very important because the chemotherapeutics or genes generally do not exhibit cell-type selection. Distribution in the normal brain tissue may cause serious side effects. Most recent studies have focused on brain tumor-targeted delivery.

In high-grade brain tumors, the BBB is modest due to the rapid amplification of brain tumor cells and the formation of neovasculature (8,11). Consequently, nano-therapeutics can reach the brain tumor bed directly *via* the enhanced permeability and retention (EPR) effect and display a brain-tumor treatment effect (32). Brain tumor cells overexpress several receptors, including epidermal growth factor receptor, matrix metalloproteinase-2 (MMP-2), integrins, interleukin 13 receptor, nucleolin, TfR and low-density lipoprotein receptor (19,33,34). Studies of brain tumor-targeted delivery were generally based on these receptors (8,35–37).

Recently, several technologies were developed to screen for peptides or aptamers that possess high binding efficiency and high specificity. Phage display and SELEX screens are examples, and redesigning the existing ligands is another useful approach. AS1411 aptamer that binds nucleolin was discovered by SELEX (38). AS1411 modified nanoparticles (AsNPs) displayed approximately 2-fold higher uptake by and localization in brain tumor cells compared with unmodified nanoparticles. *In vivo*, paclitaxel-loaded AsNP effectively slowed tumor growth (81.68% slower than controls) and prolonged the median survival time of brain tumor-bearing mice (72% longer than controls), which was significantly better than the values obtained using unmodified NPs (39). The GMT-8 aptamer that selectively binds U87 cells was also used for brain tumor therapy and it exhibited an elevated anti-brain tumor effect (40).

To further decrease localization in the non-target sites, ligands can be coated while circulating in the blood, then uncoated in specific microenvironments. Low molecular weight protamine (LMWP), a cell-penetrating peptide (CP), was coated with a short cationic peptide through a linker, PLGLAG, which can be cleaved by MMP-2 (41,42). This molecule is called active cell penetrating peptide (ACP). ACP-modified nanoparticles displayed significantly lower distribution in the liver, spleen, heart and lungs, but much higher distribution in brain tumors. Consequently, paclitaxel-loaded ACP-modified nanoparticles prolonged the median survival time of brain tumor-bearing mice 39% longer than did LMWP-modified nanoparticles. Alternatively, activatable ligands can be coated with PEG *via* pH-sensitive, esterase-sensitive or reduction-sensitive chemical bonds (43–46).

Co-delivering chemotherapeutics with genes or proteins can synergistically enhance the antitumor effect. Guo *et al.* encapsulated TRAIL protein and doxorubicin in liposomes for brain tumor therapy (47). Low-dose doxorubicin sensitizes brain tumors cells to TRAIL protein. Incubating U87 cells for 12 h with either 37 ng/ml of TRAIL protein or 1.0 μg/ml of doxorubicin did not significantly inhibit cell growth (inhibition effect <25%); however, the combination of these two drugs effectively inhibited cell growth (inhibition effect >50%). The median survival time of mice treated with the co-delivery liposomes was 50% and 23% longer than that of mice treated with TRAIL protein-loaded or doxorubicin-loaded liposomes, respectively.

Utilizing targeting ligands may further improve the antitumor effect. Zhan *et al.* encapsulated the TRAIL gene in CDX-modified micelles and encapsulated paclitaxel in RGD-modified micelles, which bind nAChR and integrin $\alpha_v\beta_3$, respectively (28,48,49). The median survival time of the brain tumor-bearing mice treated with these co-delivery therapeutics (33.5 days) was significantly longer than those of mice treated solely with paclitaxel micelles (25.5 days) or TRAIL gene micelles (24.5 days). Co-delivery of the TRAIL gene and paclitaxel in liposomes anchored to angiopep-2 also showed promising anti-brain-tumor effects (50).

However, the efficacy of brain tumor targeting delivery systems is restricted by poor access to infiltrated tumor cells where the BBB is intact. Consequently, the survival time is indeed prolonged, but recurrence cannot be prevented. Moreover, brain tumor-targeted delivery is useless for low-grade brain tumors, in which the EPR effect is absent.

Dual Targeted Delivery

Dual targeted delivery systems can target both the BBB and brain tumor cells, fully conquering the two treatment barriers. Ideally, the first ligand should be dissociated after penetration of the BBB to minimize the unfavorable effects of this ligand on the targeted diseased cells.

TGN peptide is a BBB-specific ligand discovered through phage display and AS1411 aptamer is a specific ligand for nucleolin that is highly expressed on tumor cells (38,51). We recently functionalized nanoparticles with these two ligands (52), and they showed high brain tumor selectivity and accumulation (Fig. 1). Docetaxel-loaded dual modified

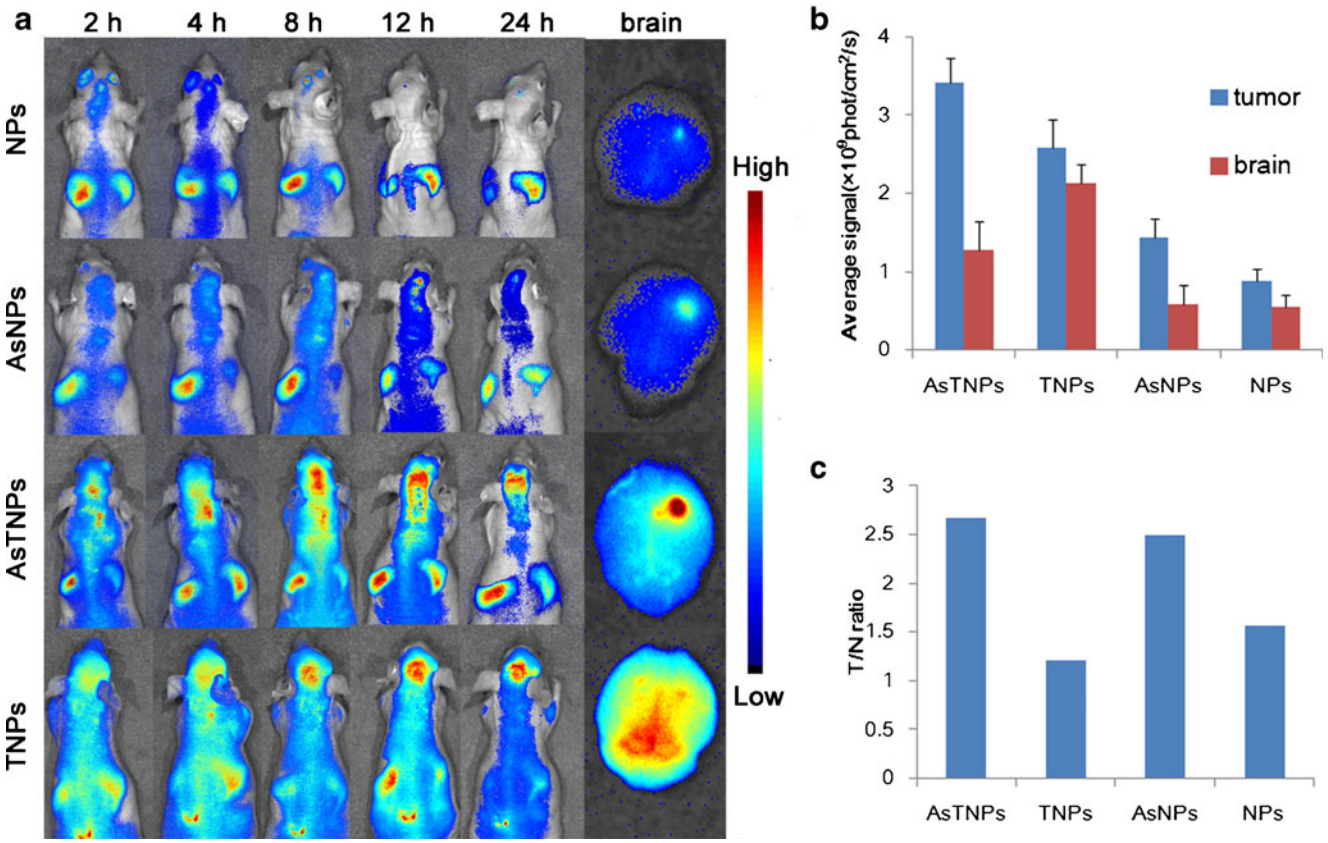

Fig. 1 (**a**) The *in vivo* imaging of DiR-loaded NP, AS1411 modified nanoparticles (AsNP), AS1411 and TGN dual modified nanoparticles (AsTNP) and TGn modified nanoparticles (TNP) in the brain glioma bearing nude mice at several time points with ex vivo imaging of the brain at 24 h. (**b**) Brain and glioma fluorescent intensity at 24 h. (**c**) The tumor/normal brain (T/N) ratio of the brains 24 h after treatment with different formulations. Reprinted from (52) with permission of the copyright holder, Elsevier, Amsterdam.

nanoparticles provided the best treatment effect in brain tumor-bearing mice. The median survival time of dual targeted nanoparticle-treated mice was 28% longer than that of mice treated with solo targeted nanoparticles. However, the TGN peptide could not be disjunctive after entering brain, which may lead to the normal brain distribution.

Other dual targeting strategies have enhanced the brain tumor-targeting effect, for example, RGD and transferrin co-modification to target neovessels and brain tumor cells (53), transferrin and tamoxifen co-modification to target the BBB and inhibit MDR (54), transferrin and RGD co-modification (55), TfR-targeted peptide B6 and RGD co-modification (56), p-aminophenyl-α-D-mannopyranoside and transferrin co-modification (57), transferrin and wheat germ agglutinin (WGA) co-modification(58), tamoxifen and WGA co-modification (59) and co-modification of murine 83–14 monoclonal antibody targeted to the human insulin receptor and rat 8D3 monoclonal antibody targeted to the mouse TfR (60). Most studies claimed that dual modified systems had a stronger antitumor effect than did solo modified systems.

Fusion proteins that combine the active domains of two ligands can also be used for this purpose. However, most studies using them have focused on neurodegenerative disorders, which will be discussed in the next section. Alternatively, if receptors are highly expressed on both the BBB and diseased brain cells, such as the low-density lipoprotein receptor-related protein (LRP) (34,61,62), the corresponding ligands could serve as dual targeting ligands to conquer both barriers with only one ligand. Angiopep-2 is a peptide derived from the Kunitz domain that possesses high binding affinity for LRP (63). Angiopep-2 modification enhanced the BBB penetration of nanoparticles and led to higher gene expression in the brain (64). Angiopep-2 modified nanoparticles encapsulating paclitaxel effectively prolonged the median survival time of brain tumor-bearing mice by 20% compared with unmodified nanoparticles (65).

Compared with brain-targeting delivery systems and brain tumor-targeting delivery systems, the dual targeting delivery systems improved the access of drugs to brain tumors and the consequent anti-brain tumor effect. However, these systems are still not intelligent enough, which is due not only to the un-cleavage of the ligands. Cargo release is not controlled and environment-response in these systems, leading to the unfavorable release of the drugs in normal tissues and the consequent side effects.

NEURODEGENERATIVE DISORDERS

Neurodegenerative disorders are chronic progressive neuropathies characterized by selective and generally symmetrical loss of neurons in the motor, sensory, or cognitive systems. The most common examples are Alzheimer's disease (AD) and Parkinson's disease (PD). These two diseases are increasing threats for human beings because the population is aging. Although developments in biology and medical science have enriched our knowledge of these two diseases, the currently available treatments can only temporarily alleviate their symptoms (7).

Alzheimer's Disease

AD is a progressive disorder with the pathological hallmarks of extracellular amyloid-β (Aβ) plaques and neurofibrillary tangles of intraneuronal hyperphosphorylated tau protein. As the leading cause of dementia, AD currently affects more than 24 million people worldwide and is projected to affect 115 million people by 2050 (66,67). Aggregations of Aβ and tau protein are both neurotoxic and are the most important cause of dementia (68,69). Concomitant pathological features also contribute to the AD pathology, including elevated oxidative stress, increased levels of metal ions and the death of many neuronal subsets (7).

The most common treatment for AD is inhibition of Aβ plaque formation. Aβ plaques rapidly disaggregate after intracerebral injection of an anti-Aβ antibody (70). To enable the antibody to penetrate the BBB, an antibody directed against the TfR was fused with the anti-Aβ antibody (71). After intravenous injection of the fusion protein, 3.5% of the injected dose/gram of brain tissue was attained. The concentration of $A\beta_{1\text{-}42}$ in the brain was reduced by 40% after treatment with the fusion protein, without any elevation in the plasma $A\beta_{1\text{-}42}$ concentration. However, the efficacy of the fusion protein was overshadowed by its poor stability and immunogenicity. Conjugating the anti-Aβ antibody with WGA would enable intranasal delivery of the antibody to the brain because WGA recognizes sugar molecules and binds to glycosylated membrane components, thus increasing uptake *via* the olfactory mucosa (72). Compared to the unmodified antibody, intranasal administration of the WGA-conjugated antibody significantly reduced the plaque size and the cerebral Aβ40/42 content of 5XFAD transgenic mice.

Encapsulating anti-Aβ therapeutics in nano-therapeutics can increase their bioavailability and extend their blood circulation time. Curcumin is a potential therapeutic for AD because it inhibits $A\beta_{1\text{-}42}$ oligomer formation and reduces amyloid levels *in vivo* (73,74). However, this compound displayed poor bioavailability after injection. Attaching curcumin to liposomes or nanoparticles overcomes this drawback and increases its affinity for $A\beta_{1\text{-}42}$ (75,76). Curcumin-decorated liposomes inhibited the formation of fibrillar and oligomeric Aβ *in vitro* (77). To allow them to penetrate the BBB, nanoparticles were decorated with apolipoprotein E (ApoE), a ligand for the low-density protein receptor expressed on the BBB (78). The targeted nanoparticles reduced the $A\beta_{1\text{-}42}$-related cell toxicity by 40%. Retrograde axonal transport is a hopeful alternative to bypassing the BBB and targeting neurons (79). Anila *et al.* coupled curcumin-incorporating nanoparticles with Tet-1 peptide, which has affinity for neurons and possesses retrograde transport properties (76). The targeted nanoparticles destroyed amyloid aggregates and exhibited antioxidative activity. However, *in vivo* evaluation has not been conducted.

The use of antioxidants is another tactic in AD therapeutics because oxidative damage is an early outcome of AD pathology (7). Several antioxidants, including glutathione, ferulic acid, fullerenes and nanoceria, inhibited the fibrillization of Aβ peptide and/or neuronal oxidative stress (80). Chelating metal ions are also useful for reversing Aβ plaque formation (7). D-penicillamine, a copper chelator, dissolves pre-existing Aβ aggregates *in vitro*. Nanoparticles covalently linked to D-penicillamine also induced the solubilization of Aβ-copper aggregates *in vitro* (81). Although nanoparticles may allow this hydrophilic drug to cross the BBB, there is no *in vivo* evaluation of this strategy.

An important pathologic aspect of AD is deficient cholinergic neurotransmission, which contributes to the learning and memory impairments (80). Therefore, enhancing cholinergic activity with cholinesterase (AChE) inhibitors or acetylcholine is an effective strategy (7,80). The limitations of acetylcholine, including its short half-life and poor BBB penetration, can be overcome by enveloping it in nano-therapeutics. Single-walled carbon nanotubes were used for this purpose. Interestingly, the nanotubes crossed the BBB and were taken up by brain cells (82). Coating nanoparticles with polysorbate-80 facilitates their penetration of the BBB. The reason for this is that polysorbate-80 absorbs ApoE in the blood stream (83). Compared to unmodified nanoparticles, polysorbate-80-coated nanoparticles delivered 2.12-fold more rivastigmine to the brain (84). Polysorbate-80-coated rivastigmine-loaded nanoparticles improved the spatial learning and memory of mice with scopolamine-induced memory loss more than did uncoated nanoparticles and a rivastigmine solution (85).

Neuroprotective agents can be used for the treatment of AD because they block AD-related neuron death and improve neuronal activity. S14G-humanin is a neuroprotective agent that exhibits activity *in vivo* (86). Yu *et al.* packaged S14G-humanin in polymersomes to protect it from digestion by peptidase (87). The polymersomes were decorated with lactoferrin to mediate BBB transport. Lactoferrin-modified polymersomes delivered 3.32-fold more cargo to the brain

than did unmodified polymersomes. Consequently, the modified polymersomes reversed the decrease of choline acetyltransferase (ChAT) activity caused by intracranial injection of $A\beta_{25-35}$. NAP is an active peptide identified as an activity-dependent neuroprotective protein, which has been reported to be a promising candidate for AD therapy. TGN-functionalized nanoparticles were utilized to deliver NAP into the brain of the AD rat model created by intracerebroventricular injection of $A\beta_{1-40}$ (88). The TGN-modified nanoparticles proved to be effective brain targeting systems, delivering approximately 4-fold more cargo to the brain than did unmodified nanoparticles (51). The improved treatment effect of the TGN-modified nanoparticles was demonstrated by the improved spatial learning and elevated levels of AChE and ChAT activity (88).

Intracranial administration of NC-1900, a vasopressin fragment analog, improved the spatial memory impairment of scopolamine-lesioned rats (89). Although oral and subcutaneous administration was effective, encapsulation in polymersomes improved its bioavailability (27). To enhance their BBB penetration, the polymersomes were modified with OX26, an antibody directed against TfR. Compared to unmodified polymersomes, 1.26-fold more OX26-modified polymersomes accumulated in the brain. The effect of the elevated brain delivery was demonstrated by the performance of scopolamine-lesioned rats in the Morris water maze test. The rats treated with NC-1900-loaded OX26-modified polymersomes showed significant improvements in learning and memory compared to rats treated with unmodified polymersomes.

Intranasal drug delivery is a strategy to bypass the BBB and effectively deliver therapeutics to the brain (90). Vasoactive intestinal peptide (VIP), a neuroprotective agent, was encapsulated in nanoparticles for intranasal delivery (91). Modification with WGA increased the brain delivery by approximately 2 folds (92). The WGA-functionalized nanoparticles improved the spatial memory of dementia mice in a dose-dependent manner, significantly better than did the unmodified nanoparticles. The greater effect of the WGA-modified nanoparticles was confirmed by the alleviated decrease in acetylcholinesterase activity (Fig. 2). Reducing the size of the ligands decreased the local toxicity and immunogenicity caused by lectin. Odorranalectin is the smallest lectin that can be used for brain-targeted delivery (93). Odorranalectin-decorated cubosomes delivered 3.46-fold more cargo to the brain than did undecorated cubosomes. Consequently, S14G-humanin-loaded odorranalectin-decorated cubosomes also increased the spatial memory and acetylcholinesterase activity similarly to WGA-modified nanoparticles (94). However, the intranasal delivery is only suitable for high activity compounds because of the relative low dose can be administered through nasal cavity.

Most researches on treatments for AD have focused on strategies to deliver therapeutics to the brain, including the systemic delivery of BBB-targeted ligand-modified systems and local delivery that bypasses the BBB, such as intracranial and intranasal administration. However, few studies considered the distribution of therapeutics in the brain. The distribution of certain therapeutics, for example, neuroprotective agents, in the normal brain may not be a problem because of their low toxicity. However, selective distribution is required for toxiferous agents. Dual targeting strategies using systems anchored to BBB-targeting ligands and targeted cell-binding ligands could be used to improve selective brain distribution.

Parkinson's Disease

As the second most common neurodegenerative disorder, PD affects 1–2% of the population over the age of 65. The hallmarks of PD are the selective loss of dopaminergic neurons in the substantia nigra and brainstem accumulation of α-synuclein aggregates, leading to difficulty controlling movement (7,66). Enhancing the level of dopamine in the brain is the most common treatment to improve the symptoms in PD patients; however, this treatment does not alter the progression of the disease or restore the affected dopaminergic neurons (95). Although there are several treatment strategies for PD, including cognitive-behavioral therapy, electroconvulsive therapy, stem cell therapy, gene therapy and exercise or physical therapy (96–99), this section will focus on targeted delivery of therapeutic agents rather than therapeutic strategies.

Glial-derived neurotrophic factor (GDNF) is potential therapeutic for PD due to its neuroprotective effect (100). However, the application of GDNF is limited by its poor penetration of the BBB. A monoclonal antibody directed against TfR (cTfRMAb) was fused with GDNF to enable the protein to penetrate the BBB (101). The fusion protein was rapidly taken up by the brain; the brain uptake was 3.1% of the injected dose/g of brain tissue 60 min after intravenous injection of a 1 mg/kg dose of the fusion protein. However, the rapid blood clearance was the limiting factor for the application of the fusion protein. For example, the $t_{1/2(\alpha)}$ and $t_{1/2(\beta)}$ of cTfRMAb-GDNF were only 0.92 min and 71.4 min.

Urocortin, a cytoprotectant, arrested the development of PD-like features in the rat bearing 6-OHDA and lipopolysaccharide paradigms of PD when administered intracerebrally (102). However, intravenously injected urocortin did not penetrate the BBB. Lactoferrin-modified nanoparticles deliver 1.98-fold more cargo to brain than do unmodified nanoparticles (103). Hu *et al.* encapsulated urocortin in lactoferrin-modified nanoparticles for PD treatment (104). The urocortin-loaded targeting nanoparticles attenuated the striatal lesions in rats treated with 6-hydroxydopamine (6-

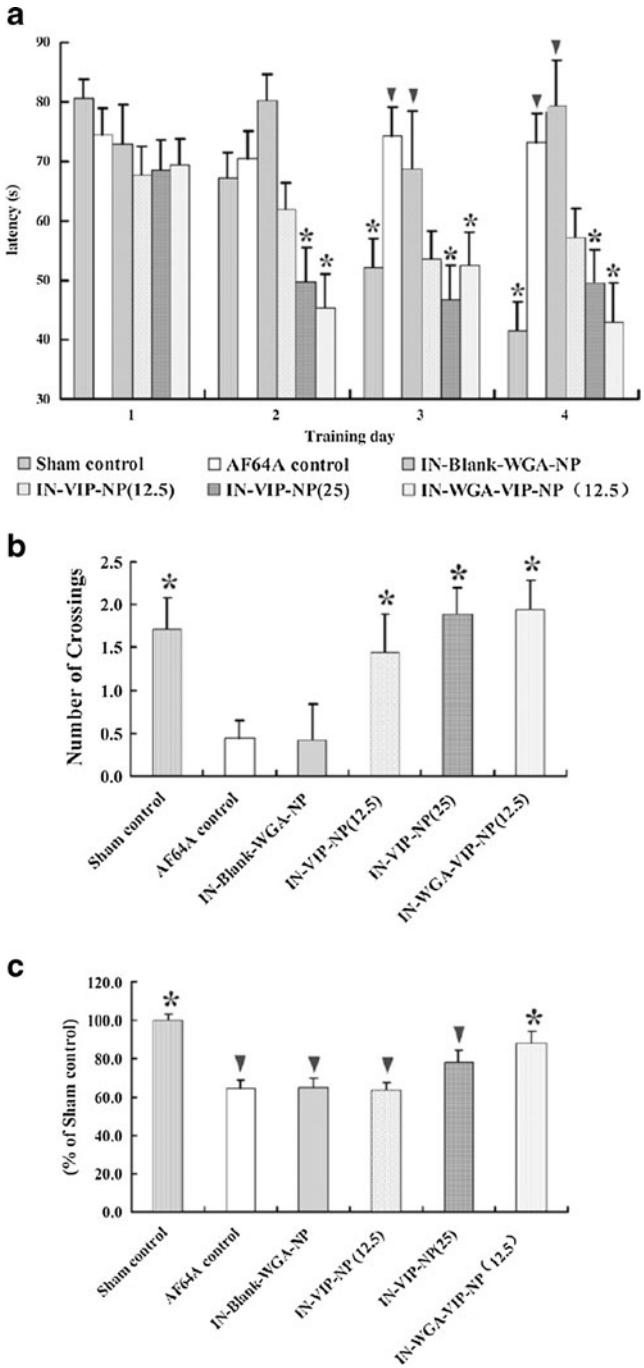

Fig. 2 (**a**) Neuroprotection effects of nasal administration of VIP solution, VIP loaded nanoparticles and VIP-loaded WGA-modified nanoparticles on the impairment of water maze learning in rats with AF64A-induced lesions. Training began after 7 days of recovering and daily drug application. Data represented the mean±S.E.M; (**b**) Effects of nasal administration of VIP solution, VIP-loaded nanoparticles and VIP-loaded WGA-modified nanoparticles on the number of times crossed the area where the platform had been located. Day 5 of testing, a spatial probe test performed with the platform removed. The animals allowed swim for 90 s, and the mean number of times the animals crossed the area where the platform had been located recorded. Data represented the mean±S.E.M; (**c**) Intranasal application of WGA-modified-VIP-loaded nanoparticles prevented reduction in acetylcholinesterase activity in AF64A-treated rats. Results were calibrated against sham control (100%). Data represented the mean±S.E.M. Sham control (n = 13), given artificial cerebrospinal fluid instead of AF64A and received daily applied vesicle; AF64A control (n = 11), daily applied vesicle; IN-Blank-WGA-NP, intranasal administration of blank WGA-NP (n = 8); IN-VIP-NP (12.5), intranasal administration of VIP-loaded nanoparticles at the dose of 12.5 μg/kg VIP (n = 10); IN-VIP-NP (25), intranasal administration of VIP-loaded nanoparticles at the dose of 25 μg/kg VIP (n = 10); IN-WGA-VIP-NP (12.5), intranasal administration of WGA-modified-VIP-loaded nanoparticles at the dose of 12.5 μg/kg VIP (n = 10). *p < 0.05, significant different with AF64A control; q p < 0.05, significant different with sham control. Reprinted from (91) with permission of the copyright holder, Elsevier, Amsterdam.

OHDA), as indicated by the results of behavioral tests, immunohistochemistry and a striatal transmitter assay. Intranasal administration was also used for targeted delivery of urocortin *via* odorranalectin-modified nanoparticles (105). Administration of odorranalectin-modified nanoparticles significantly reduced the rotational behavior and alleviated the reduction of neurotransmitters and the loss of dopaminergic cells caused by 6-OHDA.

Gene therapy has distinct advantages over drug therapy because of its potential to reverse the progression of PD, restore dopaminergic neurons or increase the level of dopamine synthetic enzymes (95). Restoring tyrosine hydroxylase (TH) in the striatum is one of the goals of PD therapy. TH-encoding plasmids were encapsulated in liposomes that were targeted *via* OX26 (106). Following their administration, the striatal TH activity was normalized, increasing from 738 to 5,486 pmol/h per milligram of protein. This treatment also reversed the apomorphine-induced rotational behavior.

The GDNF gene has also been used for PD therapy. Neurotensin polyplex was the first nonviral vector using for GDNF-gene delivery that provided effective expression of GDNF in the brain (107). Interestingly, neurotensin is the natural ligand of neurotensin receptor 1 (NTSR1). NTSR1 mediated the uptake of neurotensin by the cells in the human and rodent brain that have a high-affinity neurotensin receptor (108). This explains why neurotensin polyplex can effectively deliver genes to the brain without additional ligands.

Lactoferrin-modified nanoparticles were also used to deliver genes to the brain, and provided 4.2-fold higher gene expression in the brain than did unmodified nanoparticles (109). Five injections of lactoferrin-modified nanoparticles loaded with the GDNF gene effectively improved locomotor activity, reduced the dopaminergic neuronal loss and enhanced the levels of monoamine neurotransmitters in the unilaterally 6-OHDA-lesioned rat model (110). However, PD is a progressive disease that is not recapitulated in the unilaterally 6-OHDA-lesioned rat model (111). The rat model created by chronic, continuous exposure to rotenone reproduces most of the features of PD, including Lewy body formation in the nigral neurons, making this model more useful for evaluating the potential of targeted delivery systems for PD treatment (112). Gene therapy with lactoferrin-

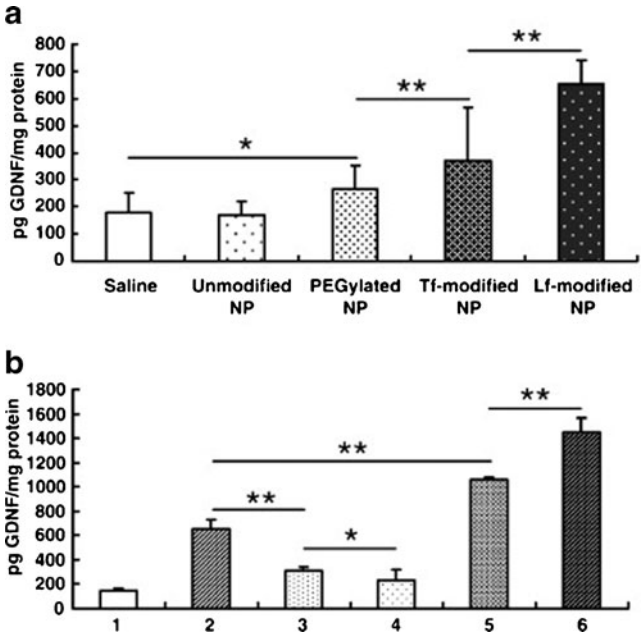

Fig. 3 Detection of GDNF content in rat brains by ELISA. (**a**) Rats were treated with a single injection of different NPs loading hGDNF, using saline as controls. GDNF expression was examined 2 days after treatments. (**b**) Rats were treated with a regimen of Lf-modified NPs loading hGDNF, using salineas controls. 1:GDNF expression 2 days post a single injection of saline; 2: GDNF expression 2 days post a single injection of Lf-modified NPs; 3: GDNF expression 6 days post a single injection of Lf-modified NPs; 4: GDNF expression10 days post a single injection of Lf-modified NPs; 5: GDNF expression 2 days post triple injections of Lf-modified NPs, one injection every other day; 6: GDNF expression 2 days post five injections of Lf-modified NPs, one injection every other day. Data are expressed as mean±S.E.M ($n=6$). Significance: *$p<0.05$; **$p<0.01$.Reprinted from (113) with permission of the copyright holder, Elsevier, Amsterdam.

modified nanoparticles was also performed using the rotenone-induced PD model (113). The lactoferrin-modified nanoparticles obtained higher GDNF expression, better improvement of locomotor activity, less dopaminergic neuronal loss and higher monoamine neurotransmitter levels compared with the unmodified nanoparticles (Fig. 3). These studies demonstrated that the targeted delivery of a gene through nano-therapeutics is a promising strategy for PD therapy.

Although many studies use viral vectors for gene therapy of PD, safety is a major concern of their application in gene therapy (95). As an alternative, non-viral vectors with better biocompatibility are being developed. Although their transfection efficiency is still far from desirable and lower than that of the viral vectors, non-viral gene delivery vectors are the direction of the future.

CEREBROVASCULAR DISEASE

Cerebrovascular disease is a group of brain disorders related to diseases of the blood vessels supplying the brain. Cerebrovascular disease is a leading cause of physical disability, the second commonest cause of mortality and the primary reason for the admission of a large proportion of hospital inpatients (114). The most common cerebrovascular disease is stroke; other causes include cerebral thrombosis, cerebral embolism and cerebral hemorrhage.

As the third leading cause of death in the industrialized world, stroke causes 15 million injuries and 5 million deaths each year (115). Ischemic stroke accounts for 80% of all the stroke insults that are precipitated by hypoperfusion, thrombosis or embolism. The remaining 20% of strokes are hemorrhagic in etiology and can be due to an underlying vascular lesion or hypertension (116).

The neuroprotective agents that are used for the treatment of stroke prevent the oxidative stress caused by the restoration of blood flow or reperfusion surgery (117). Brain-derived neurotrophic factor (BDNF), one of these neuroprotective agents, normalized pyramidal neuronal function after transient forebrain ischemia when intracerebroventricularly administered but not when intravenously administered (118). To improve its intravenous activity, BDNF was conjugated with polyethylene glycol (PEG), which reduced its hepatic clearance and prolonged its blood circulation time. The conjugated product was modified with OX26 for brain targeting. After daily intravenous administration, the neuronal density in the CA1 sector of the hippocampus was normalized in rats with transient forebrain ischemia (118). The OX26-modified BDNF-PEG conjugate was also effective in reducing the infarct volume in rats subjected to 24 h of middle cerebral artery occlusion, providing a treatment effect significantly better than that of unmodified the BDNF-PEG conjugate (119). This strategy could also be used for treatment of reversible middle cerebral artery occlusion (120). Correspondingly, other neuroprotective agents, such as VIP and bFGF, could be modified with OX26, resulting ischemia treatment effect through intravenous injection (121,122).

Neuroprotective effect is considered a benefit of administration of free radical scavengers or calcium channel blockers (117). Due to the higher oxygen carrying capacity, hemoglobin (Hb) was considered to be a therapeutic candidate in the early stage of stroke treatment. There are several methods to improve the oxygen-carrying capacity and reduce the toxicity of hemoglobin, including PEG-conjugation and diaspirin cross-linking, both of which provided beneficial effects for ischemic brain injury (123,124). SunBio1 is a PEG-conjugated bovine hemoglobin with a size of 30–50 nm. Its plasma half-life is expanded to 9.6 h in rodents, thus reducing the renal toxicity (125). Treatment with SunBio1 significantly reduced the size of brain infarctions and edema in rats occluded with a thrombotic blood clot (126).

An alternative therapeutic strategy is to deliver the native form of superoxide dismutase (SOD), a free radical

scavenger. However, the limitations of this strategy included the short half-life and poor BBB permeability. Local delivery of SOD-loaded nanoparticles provided a sustained protective effect, with a 65% reduction in infarct volume, while treatment with a solution of SOD increased the infarct volume by 25% (127). The better protective effect of SOD-loaded nanoparticles was further confirmed by the longer survival of rats treated with nanoparticles compared with that of saline-treated rats (75% *vs* 0% alive at 28 days).

Promoting tissue regeneration holds promise for functional repair after stroke (128). Neuroregeneration can be stimulated by endogenous growth factors. Delivery of growth factors to the subventricular zone, the neural stem-cell niche, is generally achieved by intrathecal injection (129). To minimize the invasiveness of the treatment, EGF was loaded into a hydrogel for epi-cortical delivery and sustained release, and EGF was conjugated with PEG to improve its diffusion (115,130). The PEG-EGF-loaded hydrogel significantly increased the stimulation of neural stem cells/progenitor cells. The epi-cortical delivery strategy was also successfully used to deliver erythropoietin, a glycoprotein that has neuroprotective and neuroregenerative activities, to the injured brain (131). Vascular endothelial growth factor (VEGF), an angiogenic growth factor, promotes neurogenesis and cerebral angiogenesis, which attenuates ischemic brain injury (132). For intravenous injection, VEGF-encoding plasmids were encapsulated in liposomes functionalized with transferrin to enable them to penetrate the BBB (133). Real-time PCR was used to determine the quantity of VEGF mRNA in the ischemic brain 24 h after injection of transferrin-modified liposomes or unmodified liposomes. Compared with unmodified liposomes, treatment with the transferrin-modified liposomes increased the expression of the VEGF mRNA approximately 5 folds. Western blot analysis showed that the rats administered transferrin-modified liposomes had increased levels of VEGF protein (48 h after administration) compared with the rats treated with unmodified liposomes or saline. Consequently, the cerebral infract size was significantly reduced in the rats treated with transferrin-modified liposomes. Neurological function was also obviously improved after treatment with the targeting liposomes, which was determined by two motor tests (head movement upon raising the rat by the tail and circling toward the paretic side upon being placing on the floor) and one sensory test (proprioception). In addition, treatment with the targeting liposomes significantly enhanced angiogenesis, as determined by the vascular density.

Gene delivery to reduce the oxidative damage is a potential method to protect the brain from ischemic lesions. High-mobility group box 1 (HMGB1) is a nonhistone DNA-binding protein that is released after ischemic insult and causes neuro-inflammation in the post-ischemic brain. siRNA-mediated HMGB1 downregulation reduced the infarct size and the microglial activation in the post-ischemic brain (134). Arginine-polyamidoamine esters (e-PAM-Rs) were used to deliver this siRNA to brain, (135). e-PAM-R effectively delivered the siRNA into H_2O_2-treated primary cortical cells, reducing the basal and H_2O_2-induced HMGB1 levels and suppressing neuronal death. *In vivo*, treatment with siRNA-loaded e-PAM-Rs successfully depleted the HMGB1 expression in more than 40% of the neurons and astrocytes in the normal brain. This treatment significantly reduced the infarct volume in the post-ischemic rat brain. Carbon nanotubes have also been used for gene delivery. Caspase-3 activation contributes to brain cell death after traumatic brain injury (136). Stereotactic administration of caspase-3 siRNA-bearing nanotubes downregulated the expression of caspase-3, leading to a neuroprotective effect on the ischemic brain (137). Combining the heme oxygenase-1 (an anti-oxidant enzyme) gene with dexamethasone (an anti-inflammatory agent) further reduced the injury caused by ischemia (138).

Intranasal administration is one of the effective noninvasive methods to deliver therapeutics to the brain. Many therapeutics, including recombinant human erythropoietin, bFGF, deferoxamine, BDNF, acidic fibroblast growth factor, ginsenoside Rb1 and HMGB1-binding peptide, have been delivered to the brain by intranasal administration, resulting in significant neuroprotective effects in rats with ischemia (139–146). Intranasal gene delivery also yielded promising results. As described above, intracranially administered HMGB1 siRNA-loaded e-PAM-R effectively transfected brain cells (135). The same research group delivered HMGB1 siRNA-loaded e-PAM-R *via* intranasal administration (147). Using fluorescence labeling, the siRNA was found to be delivered into the cytoplasm and processes of neurons and glia cells in many brain regions. More importantly, intranasal delivery of HMGB1 siRNA markedly reduced the infarct volume (by 42.8%) in the post-ischemic rat brain; this protective effect was further validated by their recovery from neurological and behavioral deficits. Although intranasal delivery provides a quick pathway for brain to access therapeutics, this strategy is restricted by the low administered dose. Furthermore, bioadhesive materials are often needed to improve the retention time in nasal cavity, which may cause serious local toxicity and immunogenicity.

PERSPECTIVE

Targeted delivery is a promising branch of nanotechnology that can be used to treat other CNS disorders, such as inflammation and psychosis (148–151). The potential benefits include noninvasive administration, optimized drug distribution, elevated treatment outcome, reduced systemic side

effects and improved compliance. To further improve the treatment outcome, researchers should understand the irregular conditions of CNS disorders, which are the basis of targeted delivery. Rather than organ-targeted delivery, diseased cell- and organelle-targeted delivery is direction of the future.

Although elevated treatment outcomes were generally observed in recent studies, the drug distribution is far from ideal. Most drugs (over 95%) are distributed in non-targeted sites. Modification with targeting ligands enhances the distribution in the targeted site; however, targeted delivery is passive rather than active because the interaction of ligands with targeted cells occurs only when the delivery system reaches the targeted cells *via* blood circulation. In other words, modification with targeting ligands increases the internalization by the targeted cells rather than drives the delivery system to the targeted site. Consequently, optimizing the pharmacokinetic behavior is a critical aspect of targeted delivery systems.

Translation from the laboratory to the clinic is the foremost challenge in applying targeted delivery systems. Currently, only a few nano-therapeutics are commercially available, including doxorubicin-loaded liposomes, paclitaxel micelles and nanoparticles albumin-bound paclitaxel. In addition to the complex production procedure for most nano-therapeutics, the safety of nano-materials is a major concern. Currently, only several materials are approved for injection, including polylactide, poly lactide-polyglycolide and poly ε-caprolactone. Most materials used for gene delivery and/or with good photo-electric properties cannot be used in human because of the systemic toxicity. Natural materials are superior in this regard. More research should focus on functionalizing natural materials with the required properties or reducing the toxicity of existing materials that have excellent characteristics.

The application of targeting ligands is also a big concern. Although many ligands were used in brain targeted delivery, the biocompatible of these ligands were not sure. For using in human being, ligands must be safe, effective and controllable.

Overall, the application of targeted delivery systems for CNS disorders depends on developments in CNS biology, nanotechnology and materials science. By combining the improvements made in these fields, targeted delivery systems could play a more important role in the management of CNS disorders.

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