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RESEARCH PAPER

Targeted Delivery of Proteins into the Central Nervous System Mediated by Rabies Virus Glycoprotein-Derived Peptide

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ABSTRACT

Purpose Delivery of therapeutic proteins across the blood-brain barrier (BBB) is severely limited by their size and biochemical properties. Here we showed that a 39-amino acid peptide derived from the rabies virus glycoprotein (RDP) was exploited as an efficient protein carrier for brain-targeting delivery.

Methods Three proteins with different molecular weight and *p*I, β-galactosidase (β-Gal), luciferase (Luc) and brain-derived neurotrophic factor (BDNF), were fused to RDP and intravenously injected into the mice respectively. The slices of different tissues with X-Gal staining were used to examine whether RDP could deliver β-Gal targeted into the CNS. The time-course relationship of RDP-Luc was studied to confirm the transport efficiency of RDP. The neuroprotective function of RDP-BDNF was examined in mouse experimental stroke to explore the pharmacological effect of RDP fusion protein.

Results The results showed that the fusion proteins rapidly and specific entered the nerve cells in 15 min, and the $t_{1/2}$ was about 1 hr. Furthermore, RDP-BDNF fusion protein showed the neuroprotective properties in mouse experimental stroke including reduction of stroke volume and neural deficit.

Conclusions RDP provides an effective approach for the targeted delivery of biological active proteins into the central nervous system.

KEY WORDS brain targeting · protein delivery · RDP fusion protein · stroke

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INTRODUCTION

The blood-brain barrier (BBB) regulates the passage of solutes between the central nervous system (CNS) and the blood (1). Although the BBB helps prevent damaging substances from entering the CNS,it also keeps helpful drugs out of the CNS. BBB is composed of endothelial cells, associated astrocytic end-feet processes, perivascular neurons, and pericytes. The endothelial cells are connected by tight junctions that form an almost impenetrable barrier to all compounds except small lipid-soluble molecules of less than 400 Da (2). The vast majority of therapeutic molecules either have a molecular weight >400 Da, or have high water solubility, which prevents free diffusion through the BBB (3). Therefore, neurodegenerative disorder, stroke and brain cancer become difficult diseases to treat because most medicines have trouble crossing the BBB. Some macromolecular proteins, such as neurotrophic factors, have been known the exact neuroprotective effect on brain cells, but unfortunately, they can not cross the BBB (4). Thus, researchers are trying to find ways to get potentially therapeutic molecules into the brain.

Although intracerebral injection of therapeutic proteins may be a reasonable approach for treatment of localized neural degenerative disorders that involve discrete anatomical structures within the brain, the treatment of many neurological disorders requires the delivery of a therapeutic protein to the whole CNS (5,6). Additionally, because the distance between capillaries in the brain is short, only about 40 μm, and a large molecule drug with a molecular weight of 50 kDa diffuses 40 μm in ~1 sec (7), the delivery of a protein across the BBB can result in an instantaneous and uniform increase in drug concentration throughout the brain once the drug crosses the BBB (3,8). Therefore,

the use of carrier-mediated delivery of a potentially therapeutic protein through vascular of brain would be a preferred method.

Among the various carriers, interest has been focused on peptide, particularly, cell-penetrating peptides (CPPs), because they are able to allow macromolecular translocation within multiple tissues *in vivo* upon systemic administration (9,10), and have the capability of inducing cell penetration within 100% of cells of a given cell culture population (11,12). Currently, CPPs have also been used to treat preclinical models of human disease (13). In the past years, we have reported that the one of the most popular CPPs (TAT 47–57, YGRKK RRQRRR) can carry the exogenous macromolecular proteins into brains for treatment of Alzheimer's disease, Parkinson's disease and depress (14–16).

While CPPs provide a means for brain delivery of exogenous proteins, they can not deliver them with cell specificity since CPPs are effective in a very large number of different cell types. Herein lays one of the major drawbacks of CPPs, their lack of cell specificity, and this limitation hampers the most promising applications of CPPs (17).

The use of peptides in the delivery of proteins would have a major relevance if they were able to target specific cells or tissues. There lie neurotropic organisms in the nature, such as rabies virus and *Clostridium tetani*. These naturally occurring peptide derived from neurotropic organisms seems to keep their ability of the highly cell specificity, so they are attempted to use in the field of drug delivery. For example, tetanus toxin C-fragment (TTC) derived from tetanus has been exploited as a molecular tool to specifically target motor neurons and has significantly alleviated symptoms in animal models of neurological diseases upon delivery of therapeutic molecules (18).

Rabies virus is highly neurotropic, and its glycoprotein is known to be the only protein component of the viral envelope that mediates viral entry into host cells (19). The sequence analysis reveals that the 330–357 amino acid sequence of glycoprotein is the key nerve binding region of the virus (20,21). Thus, we assumed the peptides derived from the rabies virus glycoprotein (RDPs) of sequence 330–357 had the similar cell specificity. In our previous study, we have reported that one of the RDPs can deliver the macromolecular protein into the hippocampus of brain, while the other RDP with different amino acid sequence was totally lack of delivery function (22). Here we showed that another RDP (39 amino acid residue, KSVRTWNEIIPSKGCLR VGGRCHPH VNGGGRRRRRRRRR) had the ability of targeted delivery proteins with different molecular weight and *p*I into the CNS. Furthermore, a potential therapeutic protein, brain-derived neurotrophic factor (BDNF), fused to RDP could be used for treatment of mouse experimental stroke.

MATERIALS AND METHODS

Construction of Recombinant Plasmid and Expression of Fusion Protein

The plasmid pET28b (+) (invitrogen) was used as the expression vector. The nucleotide acids of RDP and linker (GGGSGGGS) were inserted into the pET28b to construct the plasmid pET/RDP. Subsequently, Lac Z gene, Luciferase (Luc) gene or human BDNF gene was cloned into the pET/RDP to construct the recombinant plasmid pET/RDP-β-Gal, pET/RDP-Luc or pET/RDP-BDNF (Fig. 1). As controls, we also constructed the pET/β-Gal, pET/Luc and pET/BDNF plasmid. The nucleotide acid sequences of the plasmids were analyzed for their accuracy in Invitrogen Biotechnology Co. LTD. (Shanghai, China). The plasmids were transformed into the *E. Coli.* BL21 strain respectively, induced by 1 mmol/L IPTG to express the fusion proteins. The cells were harvested and sonicated, and the proteins were purified by Ni-NTA resin column (Amersham Pharmacia Co.) and were analyzed by SDS-PAGE.

Animals

Healthy male mice, CD-1 species, weighing 25~30 g, provided by the Beijing Vital River Lab Animal Technology Co. Ltd. (China), were used throughout this study. Animals were housed in environmentally controlled conditions (22°C, 12 h light-dark cycle with light cycle from 7:00 am to 19:00 pm and dark cycle from 19:00 pm to 7:00 am) with *ad libitum* access to standard laboratory mouse chow and water. Animal experiments were performed in accordance with the Chinese Guides for the Care and Use of Laboratory Animals.

RDP-β-Gal Administration and X-gal Staining

The RDP-β-Gal fusion protein in saline was intravenously (iv) injected into the mice (0.4 pmol). Subsequently, the mice were euthanized with overdose pentobarbital (45 mg/kg), and the cerebrum, spinal cord, liver, spleen, lung, kidney and heart were dissected out at 15 min, 1 hr and 8 hr after administration. Three mice were used in each time point. The tissues were fixed in 4% paraformaldehyde for 48 hr, and then the frozen sections (30 μm) were cut with a

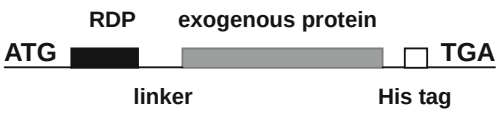

Fig. 1 The scheme for construction of the the recombinant plasmid containing RDP, linker and exogenous protein.

cryomicrotome. The sections from different tissues were put in a 24-well plate (Gibco, USA) and incubated with the freshly prepared X-gal solutions (1 g/L X-gal, 5 mmol/L $K_3[Fe(CN)_6]$, 5 mmol/L $K_4[Fe(CN)_6]$, 2 mmol/L $MgCl_2$) at 37°C until a detectable blue color was formed. The reaction was terminated by the addition of 1 mol/L Na_2CO_3. The sections were transferred to the slides, covered with coverslips and examined under a light microscope.

RDP-Luc Administration and Luc Activity Measurement

The RDP-Luc in saline was iv injected into the mouse (0.4 pmol). At 15 min, 30 min, 1 hr, 5 hr and 8 hr after administration, the mice were euthanized by decapitation, and the tissues of cerebrum and spinal cord were removed in ice bath and frozen in liquid nitrogen for use. Ten percent (1:10, w/v) homogenenate in ice-cold lysis buffer containing 5 mM EDTA, 1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 10 mM CHAPS, 1% Triton X-100 0.25 mM phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin and 50 mM Tris-HCl (pH 8.2) was prepared (5,000 rpm, 5 sec for twice with 30 sec interval) in ice bath. The Crude homogenates were incubated on ice for 10 min, then centrifuged at 5,000 r.p.m for 10 min at 4°C. The protein concentration of the supernatant was taken for BCA assay (Pierce), while the remainder was stored at −70°C until later use. Luc activity was measured by using the assay kit (Promega). The Luc activity was expressed as light units/mg Pr. The half-lives of Luc activity in mouse tissues were calculated by the log-linear elimination phase.

Immunofluorescence Staining of BDNF

The mice were iv injected RDP (synthesized by Chinapeptides Co. Ltd.,purity >99%), BDNF and RDP-BDNF (0.4 pmol), respectively. At 15 min after injection, the mice were euthanized and fixed by intracardical perfusion of 4% paraformaldehyde in PBS (0.01 M, pH 7.4). After the brains were cryoprotected in sucrose at 4°C, the coronal sections were cut on a cryostat microtome. The slices were incubated in 10% normal goat serum diluted in PBS at 4°C overnight. For the BDNF recognition, a human BDNF monoclonal antibody (Santa Cruz; 1:1000) was used as primary antibody, and FITC-labeled goat anti-human IgG (Beijing Boaoshen Biotech. Co., China) was used as secondary antibody. The PBS was used to wash the slices before each addition. The slices were air dried and placed on coverslips using a fluorescent mounting medium. All immunostaining sections were observed with a fluorescence microscope (Olympus Optical Co., Ltd., Japan), and were photographed using the same magnification and identical color scale setting as a correction for background staining.

Permanent Middle Cerebral Artery Occlusion (MCAO) and RDP-BDNF Treatment

The preparation method of mouse permanent MCAO model was described previously (23). Briefly, the mice were anesthetized with 10% chloral hydrate (0.1 ml/10 g), then a low neck incision was done, and the right common carotid artery (CCA), internal carotid artery (ICA) and external carotid artery (ECA) were exposed and isolated. The ECA was distally ligated, and the CCA and the ICA was clamped with a curved microvascular clamp. A small incision into the wall of the ECA was done and the 6-0 monofilament nylon suture was slowly inserted retrogradely *via* arteriectomy in the direction of the ICA until resistance was felt. The silk suture around the ECA stump was lightly tightened around the nylon suture to prevent bleeding, and to secure the nylon suture in a permanent position. The microvascular clamp at the ICA and CCA was removed, and the skin was sutured. Subsequently, the anesthetized mouse was iv injected RDP-BDNF (0.4 pmol). A total of 15 mice were used for this study with 5 mice in each of the 3 treatment groups control (saline), BDNF (0.4 pmol), RDP-BDNF. The suture was left in place for the duration of the experiment.

The mice were euthanized 24 hr later, and brains cut into 2 mm coronal sections with a mouse brain matrix (Harvard Appamouseus, Holliston, MA). The brains were immediately stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC) and fixed in formalin. The sections were scanned with a Scanwizard scanner, and the images processed in Adobe Photoshop. The border between infarction and non-infarction region was outlined using an image analysis system, and the area of infarction was measured by subtracting the area of the non-lesioned ipsilateral hemisphere from that of the contralateral side. The volume of the infarct (mm^3) was computed from the average infarct area for all 4 sections from the same brain multiplied by the total section length. A total of 20 sections (5 mice/group) were used for every average infarct volume.

The neurologic deficit at 24 hr was scored as follows: 0, no deficit; 1, failure to extend the contralateral forepaw fully; 2, intermittent circling; 3, sustained circling without moving forward; 4, walks only when stimulated with decreased level of consciousness (23).

Statistical Analysis

All data were given as mean ± S.E.M. The half-lives of Luc activity in cerebra and spinal cords were estimated during the log-linear elimination phase after i.v administration of RDP-Luc. For comparison between three groups a one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test was performed. A p value of <0.05 was considered to be statistically significant.

RESULTS

RDP-Mediated Tissue Transduction

To evaluate the beneficial effects of this RDP transduction system, β-Gal was firstly cloned into the pET expression vector to produce RDP-β-Gal protein *in vitro*. SDS-PAGE analysis showed that the expression band of RDP-β-Gal was about 120 kDa (Fig. 2a), which was consistent with its molecular weight (122 kDa).

The RDP-β-Gal fusion protein was iv injected into the mice to examine whether RDP could mediate the protein into the CNS, thus the distribution of β-Gal in the mouse cerebrum, spinal cord, kidney; liver, lung, heart and spleen was tested at 15 min, 1 hr and 8 hr after administration. The results showed that a strong β-Gal staining was detected in all brain regions and the spinal cord at 15 min, the staining became light at 1 hr, and gradually disappeared at 8 hr after RDP-β-Gal injection (Fig. 2b). However, there was no significant staining in β-Gal group and control (saline) group in the cerebrum and spinal cord.

Among peripheral tissues, the strong colors were observed in kidney at 15 min and 1 hr after RDP-β-Gal and β-Gal injection, which implied that the proteins

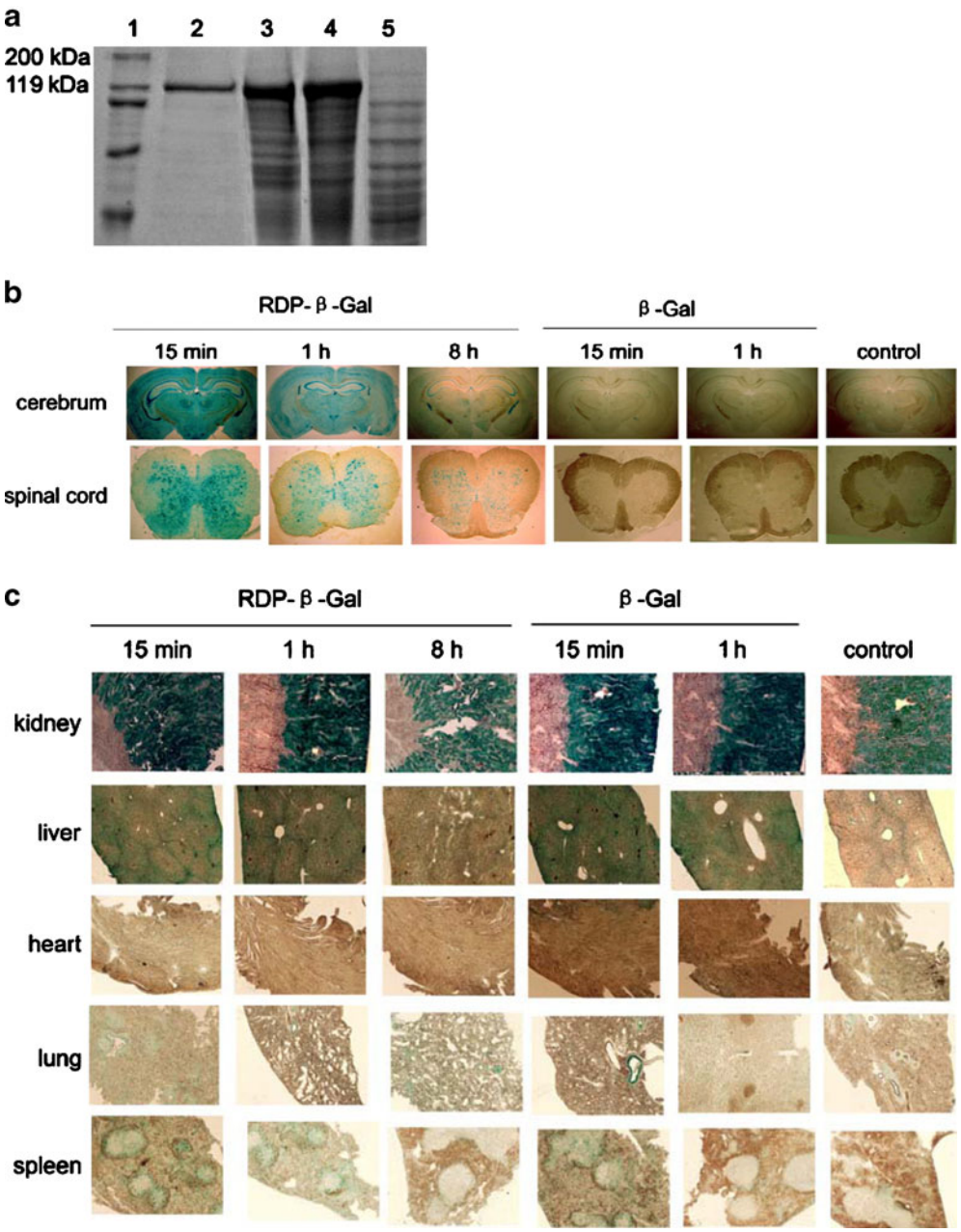

Fig. 2 Targeted delivery of RDP-β-Gal into the mouse CNS after iv injection. (**a**) SDS-PAGE of the expressed and purified fusion proteins in BL21 (DE3). Lane 1, protein molecular weight markers; lanes 2, purified RDP-β-Gal; lane 3, cell lysate of induced *E. coli* harboring pET/RDP-β-Gal; lane 4, supernatant of cell lysate of induced *E. coli* harboring pET/RDP-β-Gal; lane 5, uninduced *E. coli*. (**b**) Analysis of β-Gal enzymatic activity assessed by X-gal staining in the cerebra and spinal cords. (**c**) X-gal staining in the peripheral tissues, including kidney, liver, heart, lung and spleen. Samples were analyzed at time points after protein administration. There were 3 mice per group. The results clearly showed that RDP mediated the β-Gal selectively into the CNS, while the control protein β-Gal did not cross the BBB when delivered in the same manner.

might be cleared by the kidney (Fig. 2c). No significant difference was observed among the groups of other tissues. These results indicated that RDP could transport β-Gal across the BBB and selectively enter the nerve cells.

Time-Course of RDP-Luc in the Cerebrum and Spinal Cord

To explore the efficiency of RDP in the CNS drug delivery, the time–course relationship of RDP-Luc was studied in the cerebra and the spinal cords of the mice. RDP-Luc fusion protein was expressed and purified *in vitro*, and the molecular weight was 68 kDa (Fig. 3a). The time-course relationship showed that after iv injection of RDP-Luc, the Luc activity in cerebra of the mice rapidly increased within 15 min, and then declined to the background levels in 8 hr (Fig. 3b). The half-life ($t_{1/2}$) was 60.7 min. The results in spinal cords were similar with that in cerebra (Fig. 3c), the $t_{1/2}$ was 62.6 min. These results suggested that the RDP could rapidly mediate the protein into the CNS, and the $t_{1/2}$ in the CNS was about 1 hr. Also, the transport was specific to the protein with the RDP, because the control protein Luc did not cross the BBB when delivered in the same manner.

Neuroprotection in Experimental Stroke in the Mouse with RDP-BDNF Fusion Protein

To determine whether RDP could cargo therapeutic macromolecules into brain, we examined the immunofluorescence staining of BDNF in the mouse brains after RDP-BDNF administration. The results showed that strong fluorescence positive stained cells were detected in the mouse brains of RDP-BDNF group, and the immunopositive staining appeared mainly in the cytoplasm of the BDNF-positive cells, whereas there was few fluorescence positive staining in the mouse brains of BDNF groups (Fig. 4b). These results demonstrated that RDP-BDNF could enter into neuronal cells from iv administration and was located in the cytoplasm.

The therapeutic effect of RDP-BDNF was examined in experimental stroke in mice. The results were shown in Fig. 4c–e. In saline control group, reproducible brain infarct was obtained with TTC, which stains healthy brain red, and leaves infarcted brain unstained. The TTC staining of coronal brain sections from a representative mouse was shown in Fig. 4c. The volume of the hemispheric infarct was given in Fig. 4c for the mice. The iv injection of the RDP and BDNF had no significant effect on the stroke volume in the MCAO mice as shown in Fig. 4c and d. The injection of a same dose RDP-BDNF fusion protein reduced the hemispheric infarct volume by 85% (Fig. 4c and d)

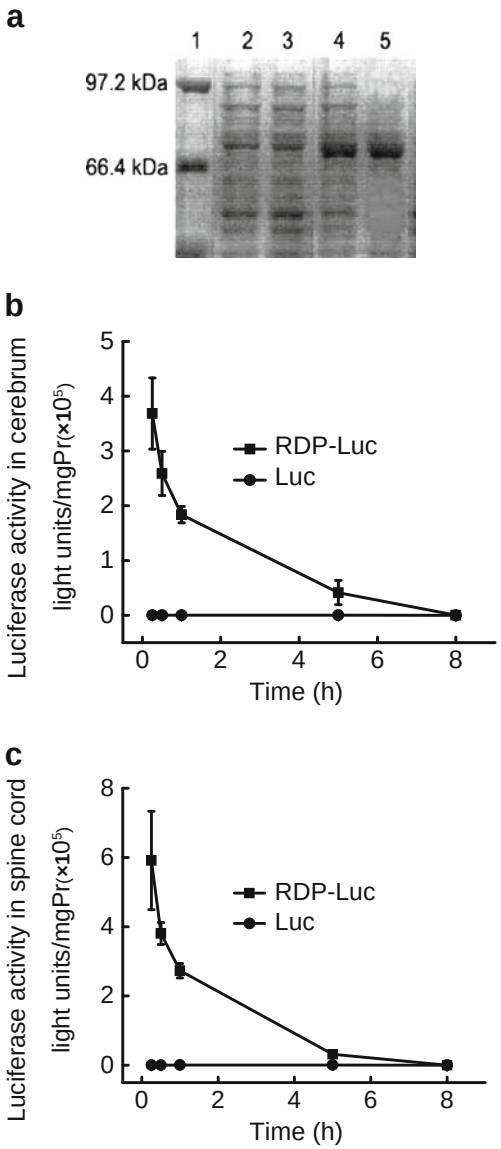

Fig. 3 Time-course relationship of RDP-Luc delivery in the cerebrum and spinal cord. (**a**) Expression and purification of RDP-Luc by SDS-PAGE. Lane 1, protein molecular weight markers; lanes 2, uninduced *E. coli*.; lane 3, uninduced *E. coli* harboring pET/RDP-Luc; lane 4, supernatant of cell lysate of induced *E. coli* harboring pET/RDP-Luc; lane 5, purified RDP-Luc. (**b**) Time-course curve of RDP-Luc delivery in the cerebrum. (**c**) Time-course curve of RDP-Luc delivery in the spinal cord. The results suggested that the RDP-Luc rapidly entered the nerve cells, and the $t_{1/2}$ in the CNS was about 1 hr according to the log-linear elimination phase. Data were expressed as mean ± S.E.M (n=4 mice/time point).

The neural deficit was scored at 24 hr after the MCAO, and the results showed that the neural deficit in the mice treated with RDP and BDNF was not significantly different from the neural deficit in the control rats (Fig. 4e). Treatment with the RDP-BDNF fusion protein reduced the neural deficit by 69% ($p<0.01$).

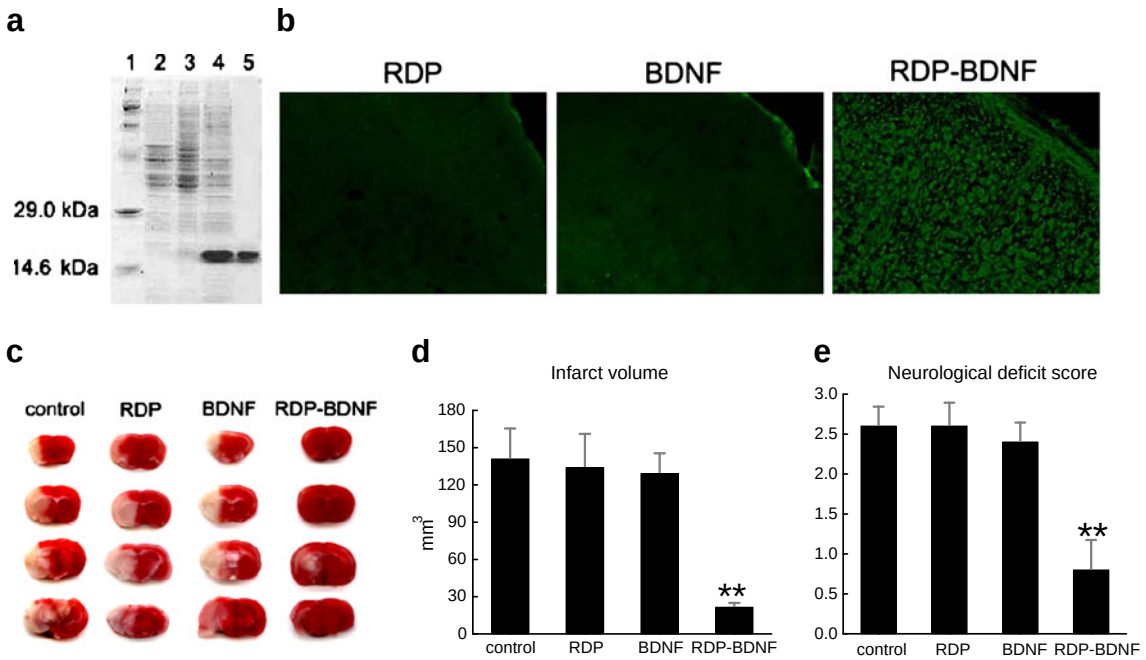

Fig. 4 Neuroprotection in experimental stroke with RDP-BDNF. (**a**) Expression and purification of RDP-BDNF in BL21. Lane 1, protein molecular weight markers; lanes 2, uninduced *E. coli*.; lane 3, uninduced *E. coli* harboring pET/BDNF; lane 4, supernatant of cell lysate of induced *E. coli* harboring pET/RDP-BDNF; lane 5, purified RDP-BDNF. (**b**) Representative photographs of BDNF immunostraining in cerebral cortex. Magnification 100. The mice were respectively injected RDP, BDNF and RDP-BDNF (three mice in per group) and were euthanized at 15 min after injection. (**c**) TTC stains of coronal sections from a representative mouse from each of 3 treatment groups. (**d**) Hemispheric infarct volume in the 3 treatment groups. (**e**) Neurologic deficit in the 3 treatment groups at 24 hours after MCAO. Data are mean ± S.E.M (n=5 mice/group). *$p < 0.01$ difference from control.

DISCUSSION

The brain-targeting protein delivery holds great promise in curing nervous system degenerative diseases like Alzheimer's disease, Parkinson's disease, stroke and brain tumors in clinical applications. However, it has not been achieved until now. The results of this study demonstrated that three biologically active proteins with different molecular weight and *p*I, β-Gal (115.5 kDa, *p*I 5.3), Luc (60.7 kDa, *p*I 6.4) and BDNF (13.6 kDa, *p*I 9.6) (Table I) mediated by RDP could be efficiently delivered into the mouse CNS after iv injection. The results also showed that neuroprotective properties of BDNF in stroke were retained after fusion to RDP. This study suggested a simple and effective approach for targeted delivery of exogenous proteins into the CNS through systemic administration.

The exogenous proteins often form insoluble inclusion bodies when expressed in *E. coli*, while our results showed that proteins linked with RDP were expressed in soluble condition. One of the possible reason is that RDP is a basic peptide, including 12 Arginine, 2 Lysine and 2 Histidine (41% basic amino acids in RDP sequence) with a pI of 12.3 (Table I), then its highly cationic nature increases the production of the soluble proteins.

β-Gal and Luc are currently used as reporters for many biological functions. They can be useful tools to evaluation the distribution of fusion proteins *in vivo* (10,24). β-Gal is stable and resistant to proteolytic degradation and easily assayed. It can catalyze the hydrolysis of X-gal, which produces a blue or green color in cells and tissues containing β-Gal (25,26). Besides tracing of *in vivo* distribution, Luc seems useful to further analyze the quantification of protein delivery in organs for kinetic studies in a simple and reliable way (27). In this study, we used the β-Gal and Luc as reporter enzymes to examine the delivery efficiency of RDP, and the result showed that RDP could mediate exogenous proteins across the BBB and selectively enter the nerve cells in 15 min, and the $t_{1/2}$ was about 1 hr.

BDNF is a member of the neurotrophin family, and involved in nerve growth and survival (28). Zhou *et al.*

Table I The Molecular Weight and Theoretical *p*I of RDP and Relative Proteins

	RDP	β-Gal	RDP-β-Gal	Luc	RDP-Luc	BDNF	RDP-BDNF
Molecular weight (kDa)	4.8	115.5	122.0	60.7	68.0	13.6	19.5
Theoretical *p*I	12.3	5.3	5.9	6.4	8.8	9.6	10.5

reported that iv administration of recombinant human BDNF (rhBDNF) conjugated with protein transduction domain (PTD) is able to promote the survival and growth of neurons impaired by Aβ 25-35 *in vitro*, and PTD-rhBDNF 5 mg/kg is used for treatment of Alzheimer's disease model mice due to its ability of transport through the BBB *in vivo* (29). PTD is a kind of CPPs that lack of cell specificity. The PTD fusion proteins distribute in every organ because PTD can cross various plasma membranes and physiological barriers. In the kinetics study, the concentration of PTD fusion protein in brain is the lowest of all the organs *in vivo* (24). The present study demonstrated that the RDP could selectively deliver proteins into the CNS, and RDP-BDNF fusion protein was biologically active in the brain and produced neuroprotection in the infarction regions of the brain. Moreover, since we had examined that repeated administration of RDP-BDNF fusion protein for 7 days did not show any toxic reactions in mice, this approach might be a potential new treatment for human CNS diseases.

The internalization mechanism of RDP is still unclear. Rabies virus can be transported to the CNS after binding to specific receptors at nerve endings, with subsequent internalization and retrogradely axoplasmic transport in several weeks (30), therefore the possibility of axonal transport of RDP delivery is excluded. It is known that RVG plays an essential role in the pathogenicity of the rabies virus (19,31), and it interacts specifically with the nicotinic acetylcholine receptor (nAchR) on neuronal cells to enable viral entry into neuronal cells. There are many nAchR subtypes, and they all form from combinations of five subunits (α, β, γ, δ/ε) (32). Different compositions of nAchR subunits exhibit different specificities for various ligands and are thereby pharmacologically distinguishable (33). Molecular biological studies have identified nine α subunits (α2-α10) and three β subunits (β2-β4) in the CNS. At least two distinct functional nAchR subtypes (α7 and α4β2) are widely distributed in the mammalian CNS (34). Some evidences demonstrate that multiple nAchR subtypes (α3, α5, α7, and α10) are present in brain capillary endothelial cells (BCECs) (35,36). Since α7 subtype of nAchR is expressed in both BCECs and brain parenchyma,it is assumed that α7 subtype mediated transport might be involved in RDP delivery. In the previous study, a 29 amino-acid peptide derived from the RVG (YTIWMPENPRPGTPCDIFTNSRGKRASNG) was exploited as an agent for brain-targeting siRNA or microRNA delivery through the α7 subtype of nAchR mediated transcytosis (37,38). Additionally, RVG29 can bind with another receptor — γ aminobutyric acid type B receptors (GABABR), which is expressed in all major brain structures and BCECs, and block of GABABR with α-bungarotoxin can decrease the cellular uptake of RVG29 (39). GABABR has been demonstrated a heterodimeric G-protein coupled receptor which involves clathrin mediated endocytosis (40). Thus, GABABR of BCECs seems partly involved in the uptake of RVG29 compounds through a clathrin mediated energy-depending endocytosis (39). In this study, different types of proteins fusing with RDP rapidly entered the neuronal cells in 15 min, which was assumed that the internalization route might also be receptor-mediated endocytosis, the important mechanism by which various agents cross the BBB.

In summary, the present study demonstrated the targeted delivery of different types of biologically active proteins to the CNS by addition of the RDP. Although the RDP sequence is 39 amino acids, the length did not appear to affect the function of the recombinant proteins, because RDP fusion proteins had been shown to not only rapidly cross the mouse BBB *in vivo*, but to retain the protein activities in the CNS. The present report also showed that iv injection of an RDP-BDNF fusion protein significantly reduced the stroke volume and neural deficit induced by permanent MCA thread occlusion in mice. Thus, this study suggested a simple and effective approach for targeted delivery of exogenous proteins into the CNS through iv injection.

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