An Antibody-Avidin Fusion Protein Specific for the Transferrin Receptor Serves as a Delivery Vehicle for Effective Brain Targeting: Initial Applications in Anti-HIV Antisense Drug Delivery to the Brain

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An Antibody-Avidin Fusion Protein Specific for the Transferrin Receptor Serves as a Delivery Vehicle for Effective Brain Targeting: Initial Applications in Anti-HIV Antisense Drug Delivery to the Brain

Manuel L. Penichet,* Young-Sook Kang,† William M. Pardridge,‡ Sherie L. Morrison,* and Seung-Uon Shin2§*

In the present study a novel Ab-avidin fusion protein has been constructed to deliver biotinylated compounds across the blood brain barrier. This fusion molecule consists of an Ab specific for the transferrin receptor genetically fused to avidin. The Ab-avidin fusion protein (anti-TfR IgG3-CH3-Av) expressed in murine myeloma cells was correctly assembled and secreted and showed both Ab- and avidin-related activities. In animal models, it showed much longer serum half-life than the chemical conjugate between OX-26 and avidin. Most importantly, this fusion protein demonstrated superior [3H]biotin uptake into brain parenchyma in comparison with the chemical conjugate. We also delivered a biotinylated 18-mer antisense peptide-nucleic acid specific for the rev gene of HIV-1 to the brain. Brain uptake of the HIV antisense drug was increased at least 15-fold when it was bound to the anti-TfR IgG3-CH3-Av, suggesting its potential use in neurologic AIDS. This novel Ab fusion protein should have general utility as a universal vehicle to effectively deliver biotinylated compounds across the blood-brain barrier for diagnosis and/or therapy of a broad range of CNS disorders such as infectious diseases, brain tumors as well as Parkinson’s and Huntington’s diseases. The Journal of Immunology, 1999, 163: 4421–4426.
and biotin. Indeed Ab-Av chemical conjugates have been used to deliver a mono-biotinylated drug (22). However, an important drawback of the chemical coupling procedure is the difficulty in producing a reproducible and homogeneous product. Genetic engineering provides an alternative approach for large scale production of homogeneous Ab-Av fusion proteins. The present work describes the brain delivery characteristics of a TfR-specific Ab containing chicken Av and its initial application in delivery to the brain of anti-HIV peptide nucleic acid, an 18-mer antisense to the rev gene of HIV-1 with lysine and tyrosine at the 5' end and biotin at the 3' (biotin-PNA) (23). The fusion protein demonstrated superior \(^{3}H\)biotin uptake into brain parenchyma in comparison with the chemical conjugate. In addition, the brain uptake of anti-HIV PNA was increased at least 15-fold when it was bound to the anti-TfR IgG3-CH\(_{3}\)-Av. Since the brain is a shelter for HIV, the successful brain delivery of anti-HIV peptide nucleic acid (PNA) with the anti-TfR IgG3-CH\(_{3}\)-Av may provide an effective treatment for cerebral AIDS.

Materials and Methods

Vector construction

The anti-TfR IgG3-C\(_{H}\)-Av H chain vector was constructed by the substitution of the variable region of anti-dansyl (5-dimethylaminonaphthalene 1-sulfonyl chloride) IgG3-C\(_{H}\)-Av fusion H chain (24) with the variable region of the H chain of anti-rat TfR mAb OX-26 (25) (Fig. 1). The anti-TfR \(\kappa\) L chain expression vector containing the Escherichia coli gpt gene for eukaryotic selection and the anti-TfR IgG3-hinge-transferrin fusion H chain expression vector containing the hisD gene for eukaryotic selective marker were constructed (S.-U. Shin, manuscript in preparation). The IgG3-CH\(_{3}\)-Av H chain specific for dansyl was available in the laboratory (24). The IgG3-hinge-transferrin DNA fragment (between Age I and BamHI) of the anti-TfR IgG3-CH\(_{3}\)-Av fusion protein producer was replaced with the Age I-BamHI DNA fragment (IgG3-C\(_{H}\)-Av H chain gene) of the anti-dansyl IgG3-C\(_{H}\)-Av H chain expression vector.

Transfection and initial characterization of anti-rat TfR IgG3-C\(_{H}\)-Av

All cells were cultured in DMEM (Life Technologies, Grand Island, NY) with 5% calf serum (HyClone, Logan, UT). A cell line that produces high levels of the anti-TfR \(\kappa\) L chain, TAUD3.1, was obtained by transfecting P3 \(\times\) 63Ag8.653 by electroporation with a chimeric mouse/human \(\kappa\) L chain gene with the variable region of OX-26 (Fig. 1), selecting with 0.33 \(3HXM\) (30\(3HXM\) contains 3.3 mM hypoxanthine, 49.3 mM xanthine, 0.52 mM mycophenolic acid, and 0.1 N NaOH) and detecting stable transfec-
tants secreting L chain by ELISA (26). One L chain-expressing transfectant, TAUD3.1, was electroporated (26) with the gene encoding anti-rat TfR IgG3-C\(_{H}\)-Av H chain; stable transfec-
tants were selected with 5 mM histidinol (Sigma, St. Louis, MO) and screened by an ELISA for the secretion of H chain (26). The fusion protein biosynthetically labeled with \(^{35}\text{S}\)methionine (ICN, Irvine, CA) was immunoprecipitated using rabbit anti-human IgG and a 10% suspension of staphylococcal protein A (IgGSorb; The Enzyme Center, Malden, MA) and then analyzed by SDS-PAGE with/without 2-ME. The fusion protein was purified from culture supernatants using protein G immobilized on Sepharose 4B fast flow (Sigma). Purity was assessed by Coomassie blue staining of SDS-PAGE gels. Protein concentrations were determined by bicinchoninic acid-based protein assay (BCA Protein Assay; Pierce, Rockford, IL) and ELISA.

Ag binding study

The binding of anti-TfR IgG3-C\(_{H}\)-Av to the TfR was studied by flow cytometry using the rat myeloma cell line Y3-Ag1.2.3. Cells (1 \(\times\) 10\(^{5}\)) were incubated with 1 \(\mu\)g of anti-TfR IgG3-C\(_{H}\)-Av, anti-DNS IgG3-C\(_{H}\)-Av (negative control), or anti-rat TfR IgG3 (positive control) (M. J. Coloma et al., manuscript in preparation), in a volume of 100 \(\mu\)l for 2 h at 4°C, washed, incubated 2h at 4°C with FITC-labeled goat anti-human IgG (PharMingen, San Diego, CA) and analyzed by flow cytometry (Becton Dickinson, Mountain View, CA).
Biotinylated human serum albumin binding assays

All steps were conducted in PBS, and plates were washed six times between each step with the same buffer. Ninety-six-well plates were coated with 50 ml/well biotinylated-BSA (Sigma; biotin:BSA ratio = 11:1, 5 ) overnight at 4°C, then blocked with 100 ml/well 3% BSA (overnight at 4°C) (24). All fusion proteins (by duplicate) were diluted and approx 50 ml/supernatant volume of 50 ml/well, and after overnight incubation at 4°C, goat anti-human k alkaline phosphatase conjugate (Sigma) was added, followed by 50 ml of the substrate p-nitrophenyl phosphate at 0.5 mg/ml in diethanolamine buffer (pH 9.6) (Sigma). The OD was read at 410 nm. To determine whether anti-TfR IgG3-C-HAv could be removed with biotin acrylic beads, varying concentrations of the fusion protein (0.5–250 nM) were preincubated with biotin acrylic beads (Sigma) (5 ml) at room temperature for 30 min. After brief centrifugation, the presence of the fusion protein in the supernatants was quantified by ELISA as described above.

For a competition ELISA, anti-rat TfR IgG3-C-HAv (2.5 nM) was preincubated with various concentrations of biotin-BSA (35.4 PM–36.3 nM) at 37°C for 2 h, and then ELISA was performed as described.

Pharmacokinetics and brain delivery of [3H]biotin bound to anti-TfR IgG3-C-HAv

Male Sprague Dawley rats (three rats per group) weighing 220 to 230 g purchased from Samyook Experimental Animals (Buann, Korea) were anesthetized with ketamine (100 mg/kg) and xylazine (2 mg/kg) by i.m. injection. The left femoral vein was cannulated with PE50 tubing and injected with 0.2 ml HEPES (pH 7.4) containing 0.1% native rat serum albumin and 5 ml of [3H]biotin (DuPont NEN Research Products, Bukyungsa, Korea) mixed with 20 ml of Ab-fusion proteins (0.1 nmol) or chemical conjugate (OX-26/Av). Five microcuries of [125I]biotin-PNA bound to anti-TfR IgG3-C-HAv was administered into the plasma compartment, while the rate of removal of [3H]biotin was measured by [125I]anti-TfR IgG3-CHAv labeled by incubation with [3H]biotin, and the radioactivity of each fraction was counted on a Packard Liquid Scintillation Analyzer (Model A2100 TR).

Results

Construction, expression, and in vitro properties of mouse/human anti-TfR IgG3-C-HAv

The strategy for the expression of anti-TfR IgG3-C-HAv is illustrated in Fig. 1. Clones expressing anti-TfR IgG3-C-HAv fusion proteins were identified by an ELISA, and transfectants faithfully express up to 10^6 cells/well. Purified anti-TfR IgG3-C-HAv fusion proteins were stable at 4°C in PBS for 1 yr. Anti-TfR IgG3-C-HAv fusion proteins were biosynthetically labeled with [35S]methionine in immunoprecipitated using anti-human IgG and staphylococcal protein A and analyzed by SDS-PAGE under nonreducing (A) and reducing (B) conditions. Included for comparison are anti-TfR IgG3 without attached Av, OX-26 (the murine IgG2a anti-TfR that donated the variable regions), and a previously characterized anti-dansyl IgG3-C-HAv. The positions of the m.w. standards are indicated at the side.

Stability of [3H]biotin fusion protein complex in serum

The serum stability of the [3H]biotin anti-TfR IgG3-C-HAv complex was examined by fast protein liquid chromatography (FPLC) using a Suprose 6HR 10/30 column (Pharmacia Biotech, Uppsala, Sweden). A 50–ml aliquot of either 60-min serum samples, or of an in vitro preparation containing 7.5 ml of [3H]biotin and 30 mg of anti-TfR IgG3-C-HAv as a control (injected) was injected into the column. The samples were passed through the column in the presence of 0.01 M PBS (pH 7.4) containing 0.05% Tween 20 at a flow rate of 0.25 ml/min. Fractions (0.5 ml) were collected, and the radioactivity of each fraction was counted on a Packard Liquid Scintillation Analyzer (Model A2100 TR).

Pharmacokinetics, brain delivery, and serum stability of [3H]biotin bound to anti-TfR IgG3-C-HAv

Rats were injected i.v. with OX-26 (IgG2a anti-TfR) labeled with tritium, or with OX-26 chemically conjugated to Av or anti-TfR IgG3-C-HAv labeled by incubation with [3H]biotin, and the radioactivity was followed for 60 min. (Fig. 5). [3H]Biotin bound to the OX-26/Av chemical conjugate was removed rapidly from the plasma compartment, while the rate of removal of [3H]biotin bound to anti-TfR IgG3-C-HAv is similar to that of [3H]-labeled OX-26 (Fig. 5). The corresponding pharmacokinetic parameters
obtained by fitting the data to a mono- or bi-exponential equation are given in Table I. These data show that $[^{3}H]$biotin bound to anti-TfR IgG3-C\textsubscript{H3}-Av is cleared from the peripheral compartment 5.8-fold more slowly than $[^{3}H]$biotin bound to the OX-26/Av chemical conjugate. The plasma “area under the plasma concentration curve” (AUC) of $[^{3}H]$biotin bound to the anti-TfR IgG3-C\textsubscript{H3}-Av for the period of 0 to 60 min was increased by a factor of 2.8 compared with that of $[^{3}H]$biotin bound to the OX-26/Av conjugate, as a consequence of both a longer half-life of elimination (80.6 ± 6.4 min vs 20.5 ± 2.2 min) and an increased “mean residence time” (MRT) (114 ± 7 min vs 16.0 ± 1.3 min). Brain uptake of $[^{3}H]$biotin bound to anti-TfR IgG3-C\textsubscript{H3}-Av was increased by a factor of 6.1 compared with that of the OX-26/Av conjugate (Table I) reflecting both a 2.6-fold increase in the BBB PS product (2.25 ± 0.65 $\mu l \cdot min^{-1} \cdot g^{-1}$ vs 0.85 ± 0.02 $\mu l \cdot min^{-1} \cdot g^{-1}$) and the higher AUC. These results showed that the fusion protein has much longer serum half-life than the chemical conjugate between OX-26 and avidin, and most importantly this fusion protein demonstrated superior $[^{3}H]$biotin uptake into brain parenchyma in comparison with the chemical conjugate.

Systemic clearance of $[^{3}H]$biotin bound to anti-TfR IgG3-C\textsubscript{H3}-Av is mainly by the liver, which cleared 5.6 ± 0.7% ID/g within 60 min following an i.v. injection, while its renal clearance is minor with 0.37 ± 0.18% ID/g (Table II). This means that the binding of $[^{3}H]$biotin to anti-TfR IgG3-C\textsubscript{H3}-Av is very stable in serum. The serum stability of the $[^{3}H]$biotin/anti-TfR IgG3-C\textsubscript{H3}-Av fusion protein complex was examined by FPLC (data not shown). Examination of the FPLC profile indicated that more than 90% of the plasma radioactivity ($[^{3}H]$biotin) eluted as the anti-TfR IgG3-C\textsubscript{H3}-Av complex 60 min after injection, with little free $[^{3}H]$biotin detected in the serum. These results suggest that it should be possible to use the Ab-Av fusion protein as a vehicle to deliver biotinylated compounds to the brain.

Brain uptake of $[^{125}I]$biotin-PNA bound to anti-TfR IgG3-C\textsubscript{H3}-Av

Experiments were then performed to determine whether the anti-TfR IgG3-C\textsubscript{H3}-Av fusion protein can be used to deliver a biotinylated 18-mer antisense specific for the rev gene of HIV-1 (biotin-PNA), a molecule with therapeutic potential against HIV, to the...
The PS product for the [125I]biotin-PNA was increased 5.6-fold, rat brains. [125I]Biotin-PNA was injected i.v. into rats with or without anti-TfR IgG3-C5-Av, and the brain uptake was analyzed as described above (Table III). The brain uptake of unconjugated [125I]biotin-PNA was negligible, with a PS product of 0.12 ± 0.01 μl · min⁻¹ · g⁻¹ and a brain uptake of 0.0083 ± 0.0009% ID/g. In contrast, the brain uptake of [125I]biotin-PNA bound to anti-TfR IgG3-C5-Av was 0.12 ± 0.01% ID/g at 60 min after an i.v. injection, and its BBB PS product was 0.67 ± 0.09 μl · min⁻¹ · g⁻¹. The PS product for the [125I]biotin-PNA was increased 5.6-fold, and brain uptake was increased 14.5-fold when the [125I]biotin-PNA was bound to anti-TfR IgG3-C5-Av. Thus, this novel Ab-Av fusion protein can deliver the biotinylated antisense drug anti-HIV PNA across the BBB, suggesting that brain delivery of anti-HIV PNA with the anti-TfR IgG3-C5-Av may provide an effective treatment for cerebral AIDS.

Discussion

Following i.v. injection, biotin bound to Av is rapidly removed from plasma with a half-life of 1.3 min (24). This rapid rate of plasma clearance has been attributed to the attached carbohydrate and the cationic charge of Av, which has 9 lysine and 8 arginine residues leading to an isoelectric point of 10. It is not surprising that chemical conjugation of Av to OX-26 leads to a reduced plasma AUC and a marked reduction of brain targeting compared with OX-26 (Table I) (29). It was therefore unexpected that genetic fusion of Av to human IgG3 would result in a protein with a half-life similar to that of unconjugated OX-26. In related studies, we have shown that the half-life of anti-TfR IgG3-C5-Av is similar to that of anti-TfR IgG3 (M. J. Coloma et al., manuscript in preparation).

It is difficult to explain why the Ab chemically conjugated to Av has such different pharmacokinetic properties compared with the Ab genetically fused to Av. Perhaps the chemical treatment per se partially denatures the conjugate, leading to its more rapid clearance. Alternatively, the site of Av addition may make important contributions to the pharmacokinetic properties. The fusion proteins are homogeneous with one Av attached at the end of the H chain. The conjugated proteins would be expected to be heterogeneous, varying both in the site and number of attached Av. The IgG-Av fusion protein behaves similarly to the IgG-C4D immunoadhesin, which is an IgG-C4D fusion protein (30). Free C4D, a cationic protein like Av, is rapidly removed from the bloodstream (30). However, the plasma clearance of C4D is greatly reduced when the protein is administered in the form of an IgG-C4D fusion protein (30).

The amount of a drug delivered to the brain is typically expressed as the % ID/g, which is a function of the BBB permeability-surface area (PS) product and the plasma AUC (28). The more efficient brain uptake of [3H]biotin bound to anti-TfR IgG3-C5-Av (compared with the chemical conjugate) with an accumulation of 0.25% ID/g at 60 min after the i.v. bolus reflects both its improved PS and AUC. This brain concentration is 3-fold higher than the brain uptake after 60 min of the classical neuroactive alkaloid morphine (0.081% ID/g) (28) and is comparable to that of OX-26.

Antisense oligodeoxynucleotides such as anti-HIV PNA may provide an effective therapy for HIV type 1 present in cerebral AIDS. Indeed, antisense oligonucleotides administered by intracerebroventricular injection or infusion have actually demonstrated brain delivery of antisense oligonucleotides such as anti-HIV PNA across the BBB, suggesting that brain delivery of anti-HIV PNA with the anti-TfR IgG3-C5-Av may provide an effective therapy for cerebral AIDS.

Table I. Pharmacokinetic parameters* for [3H]OX-26 and [3H]biotin bound to the OX-26/Av conjugate, or anti-TfR IgG3-C5-Av 60 min after i.v. injection in the rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[3H]OX-26</th>
<th>OX-26/Av conjugate</th>
<th>Anti-TfR IgG3-C5-Av</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (μl/min)</td>
<td>2.99 ± 0.38</td>
<td>6.75 ± 0.43</td>
<td>2.91 ± 0.32</td>
</tr>
<tr>
<td>A2 (μl/min)</td>
<td>0.62 ± 0.20</td>
<td>2.75 ± 0.58</td>
<td>2.75 ± 0.58</td>
</tr>
<tr>
<td>K1 (min⁻¹)</td>
<td>0.25 ± 0.02</td>
<td>0.58 ± 0.07</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>K2 (min⁻¹)</td>
<td>0.035 ± 0.003</td>
<td>0.0087 ± 0.0005</td>
<td>0.0087 ± 0.0005</td>
</tr>
<tr>
<td>t1/2a (min): distribution</td>
<td>65 ± 5</td>
<td>2.82 ± 0.22</td>
<td>1.24 ± 0.15</td>
</tr>
<tr>
<td>t1/2b (min): elimination</td>
<td>81 ± 5</td>
<td>20.5 ± 2.2</td>
<td>80.6 ± 4.8</td>
</tr>
<tr>
<td>AUC0–60 min (% IDmin/ml)</td>
<td>132 ± 19</td>
<td>48.5 ± 4.0</td>
<td>134 ± 29</td>
</tr>
<tr>
<td>AUCss (% IDmin/ml)</td>
<td>282 ± 52</td>
<td>50.4 ± 5.0</td>
<td>332 ± 89</td>
</tr>
<tr>
<td>Vm (ml/kg)</td>
<td>133 ± 15</td>
<td>143 ± 17</td>
<td>172 ± 23</td>
</tr>
<tr>
<td>Vss (ml/kg)</td>
<td>1.45 ± 0.23</td>
<td>8.94 ± 0.61</td>
<td>1.54 ± 0.29</td>
</tr>
<tr>
<td>VDbrain (mg/min/g)</td>
<td>0.65 ± 1.0</td>
<td>0.25 ± 0.09</td>
<td>0.25 ± 0.09</td>
</tr>
</tbody>
</table>

* For the pharmacokinetic parameters, the subscripts 1 represents the distribution phase and the subscript 2 the elimination phase. A indicates the intercept value on the y-axis in Fig. 5. K is the transfer rate, and CL the clearance rate. AUC0–60 min and AUCss are the first 60 min and steady-state area under the drug concentration curve, respectively. Vm is the systemic volume of distribution, MRT the mean residence time, and VDbrain the brain volume of distribution.

Table II. Organ clearance and delivery of [3H]biotin bound to the anti-IgG3-C5-Av fusion protein

<table>
<thead>
<tr>
<th>Organ</th>
<th>Organ Clearance (μl/min/g)</th>
<th>Uptake (% ID/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>2.25 ± 0.65</td>
<td>0.25 ± 0.09</td>
</tr>
<tr>
<td>Lung</td>
<td>2.54 ± 0.78</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>Heart</td>
<td>1.18 ± 0.49</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.44 ± 0.69</td>
<td>0.37 ± 0.18</td>
</tr>
<tr>
<td>Liver</td>
<td>46.4 ± 12.8</td>
<td>5.60 ± 0.69</td>
</tr>
</tbody>
</table>

Table III. Brain uptake of biotin-PNA with or without anti-TfR IgG3-C5-Av

<table>
<thead>
<tr>
<th>Injectate</th>
<th>PS Product (μl/mg brain)</th>
<th>Brain Uptake (% ID/g brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[125I]-Biotin-PNA</td>
<td>0.12 ± 0.01</td>
<td>0.0083 ± 0.0009</td>
</tr>
<tr>
<td>Anti-TfR IgG3-C5-Av[125I]-biotin-PNA</td>
<td>0.67 ± 0.09</td>
<td>0.12 ± 0.03</td>
</tr>
</tbody>
</table>

* Measurements were made 60 min after i.v. injection. Data are mean ± SE (n = 3, rats).
selective inhibition of in vivo gene expression in the brain (31, 32). However, it would be desirable to have a noninvasive method of administering the oligonucleotides, but unfortunately they show negligible transcellular transport (33). In the present study, the brain uptake of free biotin-PNA (biotinylated anti-HIV PNA) injected i.v. was negligible (0.0083% ID/g). When biotinylated PNA was bound to the OX-26/streptavidin (SA) chemical conjugate, the brain uptake of systemically administered biotin-PNA was enhanced to about 0.075% ID/g (23). However, when anti-TfR IgG3-C3-Av was used as the delivery vehicle, the brain uptake of biotinylated PNA increased to 0.12% ID/g, a 15-fold increase compared with free biotin-PNA. Thus, the brain uptake of biotin-PNA with the genetically engineered anti-TfR IgG3-C3-Av is higher than that of biotin-PNA with the OX-26/SA chemical conjugate. Nevertheless, the brain uptake of biotin-PNA bound to anti-TfR IgG3-C3-Av was half of that bound to anti-TfR IgG3-C3-Av. The PS product (0.67 µl/min/g brain) of anti-TfR IgG3-C3-Av/biotin-PNA decreased to 30% of the PS product (2.25 µl/min/g brain) of anti-TfR IgG3-C3-Av/biotin. The decreased brain uptake may reflect the poor intrinsic intracellular permeability of the PNA moiety in the complex.

A major concern is whether sufficient quantities of drugs can be delivered using anti-TfR IgG3-C3-Av to have a therapeutic effect. Recent studies have demonstrated that the brain uptake of BDNF-polyethylene glycol (PEG)-biotin conjugated to OX26/SA was 0.144 ± 0.004% ID/g (34). Thus, the brain uptake of BDNF is ~2-fold greater than that of morphine. When BDNF-PEG-biotin bound to OX26/SA was administered i.v. daily for 1 wk after a 12-min period of transient forebrain ischemia, the hippocampal CA1 neuronal density was normalized; unconjugated BDNF or OX26/SA had no effect (35). Our studies suggest that anti-TfR IgG3-C3-Av is even more effectively taken up into the brain than OX26/SA conjugates. Therefore, it would be expected to be an even more effective drug delivery vehicle capable of delivering therapeutic levels of drugs to the brain.

Our studies have indicated that anti-TfR IgG3-C3-Av may be able to serve as a universal vehicle for targeting the brain with a vast array of different compounds, including chemicals, proteins, and DNA. In particular we have demonstrated that anti-TfR IgG3-C3-Av can enhance the brain uptake of anti-HIV PNA and may provide a treatment for cerebral AIDS. Although we have focused our discussion on targeting to the cerebral hemisphere, the anti-TfR IgG3-C3-Av can also be useful for targeting other structures of the CNS such as the cerebellum and spinal cord, which are also limited by the BBB. Therefore, the results presented here suggest that our universal vehicle will have a large number of potential applications in the diagnosis and/or therapy of various CNS disorders.

Acknowledgments

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References