Neuroprotection in Transient Focal Brain Ischemia After Delayed Intravenous Administration of Brain-Derived Neurotrophic Factor Conjugated to a Blood-Brain Barrier Drug Targeting System

Yun Zhang, PhD; William M. Pardridge, MD

Background and Purpose—Neuroprotection with brain-derived neurotrophic factor (BDNF) requires direct injection into the brain owing to poor transport of the neurotrophin through the blood-brain barrier (BBB) in vivo. The present studies investigate whether BDNF alone or conjugated to a BBB drug targeting system is neuroprotective in focal, reversible brain ischemia after delayed intravenous administration at 60 or 120 minutes after middle cerebral arterial occlusion.

Methods—BDNF was conjugated to the OX26 murine monoclonal antibody to the rat transferrin receptor, which undergoes transport into brain from blood via the BBB transferrin receptor transcytosis system. After a 1-hour occlusion of the middle cerebral artery in nitrous oxide–ventilated animals with normal blood sugar, the brain was reperfused, and either BDNF or the BDNF/OX26 conjugate was administered as a single intravenous injection at a dose of 50 μg per rat.

Results—After the intravenous administration of unconjugated BDNF, there was no neuroprotection on the basis of analysis of brain at either 24 hours or 7 days after a 1-hour middle cerebral arterial occlusion. In contrast, there was a 68% and 70% reduction in cortical stroke volume at 24 hours and 7 days, respectively, after intravenous administration of 50 μg per rat of the BDNF conjugate (P<0.01). No effects on subcortical stroke volume were observed.

Conclusions—These studies demonstrate marked neuroprotection in focal, transient brain ischemia with a single, delayed intravenous injection of BDNF if the neurotrophin is conjugated to a BBB drug targeting system. The neuroprotection is long lasting and persists for at least 7 days after a 1-hour middle cerebral artery occlusion. (Stroke. 2001;32:1378-1384.)

Key Words: antibodies, monoclonal ■ avidin ■ biotin ■ endothelium ■ receptors, transferrin

Brain-derived neurotrophic factor (BDNF) is neuroprotective in either global1 or regional2,3 brain ischemia after direct injection of the neurotrophic factor into the infarcted region of either brain2 or cerebrospinal fluid.1,3 The neurotrophic factor must be injected directly into the brain because the transport of BDNF across the brain capillary endothelial wall, which forms the blood-brain barrier (BBB) in vivo, is negligible.4 Although the BBB becomes disrupted in the later phases of regional brain ischemia, the BBB is intact for the first 3 to 4 hours after focal ischemia in the brain,5-7 which is the time that pharmacological intervention in brain ischemia and neuroprotection are possible.8

Although BDNF, per se, does not cross the BBB in pharmacologically significant amounts,4 neuroprotection with this neurotrophic factor is possible after intravenous administration in either transient forebrain9 or permanent focal brain ischemia10 if the BDNF is conjugated to a BBB drug targeting system. BDNF transport through the BBB is enabled after conjugation to a monoclonal antibody (MAb) to the rat transferrin receptor (TIR),11 which undergoes receptor-mediated transcytosis through the BBB via the brain capillary endothelial TIR.12,13 The BDNF was modified by attachment of 2000 Dalton strands of polyethylene glycol (PEG2000) to surface carboxyl groups.11 This modification is termed pegylation and results in prolonged plasma circulation of the neurotrophic factor. The pegylated BDNF was conjugated to the OX26 MAb with the use of avidin-biotin technology.11 In this approach, a single biotin moiety is attached to the tip of one of the PEG strands conjugated to the BDNF. This form of BDNF is designated BDNF-PEG2000-biotin and is immediately captured by a conjugate of streptavidin (SA) and the OX26 MAb. The conjugate of the OX26 MAb and SA is alternatively designated OX26/SA or SA-OX26, and the combined conjugate, wherein the BDNF-PEG2000-biotin is captured by the SA-OX26, is designated BDNF-PEG2000.
and SA was prepared via a stable thiol-ether linkage with the use of OX26 thiolated with Traut’s reagent and SA activated with m-maleimidobenzoyl N-hydroxysuccinimide ester.11 The PEG2000-hydrazide and the hydrazide-PEG2000-biotin were custom synthesized by Shearwater Polymers.11 The BDNF-PEG2000-biotin was prepared as described previously.11 The BDNF-PEG2000-biotin/SA-OX26 conjugate was formed by mixing 2 mg of BDNF-PEG2000-biotin and 8 mg of OX26/SA followed by purification of the conjugate from aggregates or unconjugated BDNF with a 2.6×4-cm bed of Sephacryl S300 HR.11 The final yield of conjugate was 4.5 mg; 14% of this was BDNF and 86% was OX26/SA, which reflects the 7:1 ratio of molecular weights of BDNF (28 000 Da) and OX26/SA (200 000 Da). The formulation has been characterized by SDS-PAGE, Western blotting, film autoradiography, gel filtration chromatography, and [3H]biotin binding assays.11 The biological activity of the BDNF-PEG2000-biotin/SA-OX26 conjugate is identical to unconjugated BDNF on a 1:1 molar basis, as demonstrated by cell survival studies and trkB autophosphorylation assays.11 The transport of the BDNF-PEG2000-biotin/SA-OX26 conjugate through the BBB in vivo has been demonstrated previously,11 whereas there is no transport of unconjugated BDNF through the BBB in vivo. The structure of the BDNF chimeric peptide is shown in Figure 1A. This bifunctional conjugate binds both the trkB receptor on neurons, to mediate BDNF neurotrophic action, and TIR on the BBB, to mediate uptake into the brain from blood.

Reversible MCAO

All animal protocols were approved by the UCLA Animal Research Committee. Adult male Sprague-Dawley rats (weight, 250 to 350 g) were purchased from Harlan Breeders (Indianapolis, Ind). Focal cerebral ischemia was produced by intraluminal MCAO following the method of Longa et al.15 After fasting overnight, the animal was lightly anesthetized with halothane by inhalation, and endotracheal intubation was performed by transillumination.16 The endotracheal catheter was polyethylene (PE-100) tubing (7 cm long) and was connected to a model 680 Harvard small animal ventilator. The animal was artificially ventilated with a mixture of 70% N2O/30% O2 at 50 cm H2O at a rate of 90 strokes per minute and a volume of 5 mL per stroke. The body temperature was maintained with a Harvard thermal blanket with a rectal probe. The systolic blood pressure was measured with a model 29 rat tail amplifier (IITC Inc/Life Science Instruments). The left femoral artery was cannulated with PE-50 tubing. Blood was collected via the femoral artery catheter, and arterial blood pH, PCO2, and PO2 were measured with a model 238 pH/blood gas analyzer (Ciba Corning Diagnostics). Blood glucose was monitored with an Accu-Chek III Monitor and Chemstrip bG test strips (Boehringer Mannheim). The right common carotid artery and the right external carotid artery were exposed, and the occipital artery and superior thyroid artery were electrocoagulated. The right pterygopalatine artery was ligated, the right common carotid artery was clamped, and a 4-0 nylon suture was inserted retrogradely via arteriectomy of the external carotid artery into the internal carotid artery. The tip of the suture was rounded near a flame before insertion. The suture was slowly advanced until resistance was felt. The external carotid artery was ligated, and the common carotid artery clamp was released. The skin incision was sutured, leaving 10 mm of nylon silk protruding. The animal was allowed to recover and was kept warm with a heating lamp. One hour after ischemia, the intraluminal nylon suture was withdrawn to allow for reperfusion. The rat was sedated with halothane and killed by decapitation at 23 hours or 7 days after the 1-hour period of ischemia. Neurological status before the animal was killed was measured as described by Longa et al.18 Some animals in each treatment group died during the night; these animals were replaced in the study and were not included in the calculation of infarct volumes. There were no significant differences between the mortality in the 4 treatment groups shown in Table 1 for the 24-hour study or in the 2 treatment groups shown in Figure 4 for the 7-day study (P=0.75, Fisher’s exact test). For the 24-hour study, the 4 treatment groups received saline, unconjugated OX26 MAb, unconjugated BDNF, or

**Materials and Methods**

**Production of BDNF Chimeric Peptide**

The human recombinant BDNF was obtained from Amgen under a Material Transfer Agreement. The OX26 MAb was generated from OX26 hybridoma serum-free conditioned media and purified by protein G affinity chromatography.14 Recombinant SA was purchased from Sigma Chemical Co. A 1:1 conjugate of the OX26 MAb and SA was prepared via a stable thiol-ether linkage with the use of OX26 thiolated with Traut’s reagent and SA activated with m-maleimidobenzoyl N-hydroxysuccinimide ester.11 The PEG2000-hydrazide and the hydrazide-PEG2000-biotin were custom synthesized by Shearwater Polymers.11 The BDNF-PEG2000-biotin was prepared as described previously.11 The BDNF-PEG2000-biotin/SA-OX26 conjugate was formed by mixing 2 mg of BDNF-PEG2000-biotin and 8 mg of OX26/SA followed by purification of the conjugate from aggregates or unconjugated BDNF with a 2.6×4-cm bed of Sephacryl S300 HR.11 The final yield of conjugate was 4.5 mg; 14% of this was BDNF and 86% was OX26/SA, which reflects the 7:1 ratio of molecular weights of BDNF (28 000 Da) and OX26/SA (200 000 Da). The formulation has been characterized by SDS-PAGE, Western blotting, film autoradiography, gel filtration chromatography, and [3H]biotin binding assays.11 The biological activity of the BDNF-PEG2000-biotin/SA-OX26 conjugate is identical to unconjugated BDNF on a 1:1 molar basis, as demonstrated by cell survival studies and trkB autophosphorylation assays.11 The transport of the BDNF-PEG2000-biotin/SA-OX26 conjugate through the BBB in vivo has been demonstrated previously,11 whereas there is no transport of unconjugated BDNF through the BBB in vivo. The structure of the BDNF chimeric peptide is shown in Figure 1A. This bifunctional conjugate binds both the trkB receptor on neurons, to mediate BDNF neurotrophic action, and TIR on the BBB, to mediate uptake into the brain from blood.

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the MAb-BDNF conjugate; there were 10, 10, 10, and 6 rats per group, respectively. The number of rats that died in each of the 4 respective groups was 6, 6, 6, and 2, respectively, and 4 animals in each group survived the length of the study. For the 7-day study, the 2 treatment groups received unconjugated BDNF or MAb-BDNF conjugate; there were 8 rats in each of these 2 groups. The number of rats that died before the end of the study was 4 in each group. The cause of death was hemorrhage secondary to perforation of either the internal carotid artery or MCA; 1 rat died of respiratory failure.

**Drug Treatment Schedules**

BDNF-PEG\(^{2000}\)-biotin/SA-OX26 was dissolved in PBHST (0.01 mol/L Na\(_2\)HPO\(_4\), 0.5 mol/L NaCl, 0.05% Tween-20, pH 7.4). The conjugate was 14% BDNF by weight, 10,11 these rats were administered 50 mg of OX26 MAb. In the control; the second group received 50 mg per rat of unconjugated OX26 MAb. The fourth group received BDNF-PEG 2000-biotin/SA-OX26 conjugate equivalent to 50 mg per rat of BDNF. Since the conjugate was 14% BDNF by weight,10,11 these rats were administered 50 mg of BDNF that was attached to 225 mg of OX26 MAb. In these 4 groups, drugs were administered into the femoral vein via a 30-gauge needle at 1 hour after MCA occlusion. The fifth group received the conjugate at a dose equivalent to 50 mg per rat of BDNF; administered 1 hour after reperfusion, which is 2 hours after MCAO. The animals were briefly anesthetized in a halothane box before intravenous injection.

**Measurement of Infarct Volumes**

After decapitation, the rat brain was quickly removed and chilled in a freezer, and 6×2-mm coronal slices were prepared with a brain matrix (ASI Instruments, Inc). The brain sections were stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC) at 37°C for 15 minutes. The TTC stained viable brain tissue dark red, whereas infarcted tissue was unstained. After staining, the sections were fixed in phosphate-buffered formalin (10%) at 4°C followed by scanning on a 1200-dpi UMAX scanner. The images were transferred to Adobe Photoshop 5.5 on a G4 Power Macintosh and then quantified with the use of NIH Image software. The border between infarcted and noninfarcted tissue was outlined with the image analysis system, and the area of infarction was measured by subtracting the area of the nonlesioned ipsilateral hemisphere from that of the contralateral hemisphere.17 In addition, contralateral and ipsilateral hemisphere areas were measured, and the difference between ipsilateral and contralateral areas in each section was used to calculate the edema volume.19 The infarct areas on each slice were summed and multiplied by slice thickness to give the infarct volumes.10

**Statistical Analysis**

Data are presented as the mean±SD of each group. A 1-way ANOVA followed by the Bonferroni correction was used to assess statistical differences for the physiological variables or infarct volumes, and P<0.05 was considered statistically significant. All ANOVA analyses were performed with Program 7D of the BMDP Statistical Software programs developed by the UCLA BMDP Computing Facility. The nonparametric Mann-Whitney test was used to determine significant differences in neurological scores, and differences in mortality in the treatment groups were evaluated with Fisher’s exact test.

**Results**

Rats subjected to 1 hour of MCAO were divided into 4 groups and treated with either saline, BDNF, unconjugated OX26 MAb, or the BDNF conjugate. The physiological parameters for the rats in all 4 groups were within normal limits, as shown in Table 1. The differences in blood pressure between the treatment groups were not significantly different (Table 1).

The TTC stains for 4 different rats in the 4 different groups are shown in Figure 1B and demonstrate a visible reduction in stroke volume in the animals treated with the BDNF conjugate. The total hemispheric infarct volumes and hemispheric edema volumes for the 4 groups of rats are shown in Table 2. There was no significant difference in either the edema volume or the infarct volume in rats treated with either unconjugated OX26 MAb or 50 μg per rat of unconjugated BDNF administered 1 hour after the insertion of the MCA suture (Table 2). However, there was a 54% reduction (P<0.01) in the total hemispheric infarct volume and a 60% reduction (P<0.01) in the hemispheric edema volume after intravenous administration of 50 μg per rat of BDNF conjugate. The neurological score at 24 hours after 1 hour of MCAO was 2.5±0.6 (mean±SD) in the saline-treated ani-

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**TABLE 1. Physiological Variables in Animals Treated With Saline, BDNF, OX26, and the BDNF Conjugate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>BDNF</th>
<th>OX26 Conjugate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure, mm Hg</td>
<td>110±22</td>
<td>104±18</td>
<td>116±20</td>
</tr>
<tr>
<td>PCO(_2), mm Hg</td>
<td>135±30</td>
<td>134±10</td>
<td>140±10</td>
</tr>
<tr>
<td>PO(_2), mm Hg</td>
<td>44±4</td>
<td>40±6</td>
<td>44±5</td>
</tr>
<tr>
<td>pH</td>
<td>7.40±0.04</td>
<td>7.42±0.04</td>
<td>7.41±0.04</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.9±0.4</td>
<td>36.9±0.4</td>
<td>36.7±0.6</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>102±8</td>
<td>101±6</td>
<td>102±8</td>
</tr>
</tbody>
</table>

Data are mean±SD (n=4 rats per group). No significant differences in physiological variables between groups were found.
mals and 1.5±0.6 (mean±SD) in the animals treated with 50 μg per rat of BDNF conjugate; this difference was statistically significant at the 0.05 level.

The hemispheric infarct zones were subdivided into cortical and subcortical infarct areas; these data are shown in Table 2. None of the treatments resulted in a decrease in the subcortical infarct volume. Although unconjugated BDNF had no effect on the cortical infarction volume, the single intravenous injection of 50 μg per rat of the BDNF conjugate administered at 60 minutes after MCAO resulted in a 68% reduction (P<0.01) in cortical stroke volume (Table 2). If the intravenous administration of the conjugate was delayed 2 hours after insertion of the catheter (ie, 1 hour after removal of the suture), there was a 31% reduction (P<0.05) in the cortical infarct volume (Table 2). The mean±SD values of the infarct areas for each of the 6 coronal sections of brain are shown in Figure 2 for the animals treated with saline, BDNF, or the conjugate at 60 minutes after arterial occlusion.

In the 7-day study, additional groups of rats were subjected to 1 hour of MCAO. After removal of the MCAO, the animals were treated with a single intravenous dose of either unconjugated BDNF or BDNF conjugate at a dose of 50 μg per rat. The animals were killed 7 days later, and the total hemispheric infarct volume, the cortical infarct volume, and the subcortical infarct volume were measured (Figure 3). Neither the BDNF nor the BDNF conjugate caused a measurable reduction in the subcortical infarct volume (Figure 3), and these volumes were no different from those of the saline-treated animals at 24 hours (Table 2). Intravenous administration of the unconjugated BDNF resulted in no decrease in either the total infarct volume or the cortical infarct volume, compared with the saline-treated animals at 24 hours (Figure 3, Table 2). Conversely, the total hemispheric infarct volume at 7 days was reduced by 53% (P<0.01) with the BDNF conjugate compared with the total hemispheric infarct volume after treatment with the unconjugated BDNF, and the cortical infarct volume was reduced 70% (P<0.01) with the BDNF conjugate compared with the unconjugated BDNF (Figure 3). The TTC stains of the 6 different coronal sections obtained 7 days after treatment with either the BDNF or the BDNF conjugate are shown in Figure 4A, and the mean±SD values of the infarct areas for each section are shown in Figure 4B.

Discussion

The results of these studies are consistent with the following conclusions. First, BDNF chimeric peptides are neuroprotective in focal transient brain ischemia after delayed intravenous administration of a single dose of 50 μg per rat. Second, BDNF chimeric peptides reduce cortical stroke volume with no effect on subcortical stroke volume. Third, the neuroprotection after intravenous BDNF chimeric peptide is observed despite delayed administration of the conjugate 1 or 2 hours after MCAO. Fourth, the neuroprotective effect of the BDNF chimeric peptide is long lasting, with a 70% reduction in cortical infarct volume at 7 days after 1-hour MCAO.
Prior work with the permanent MCAO model demonstrated that 50 μg per rat of BDNF chimeric peptide reduced the total hemispheric infarct volume 65% after intravenous administration of the conjugate in 2 sequential intravenous doses at 0 and 3 hours after MCAO. A dose response was observed in the permanent MCAO model. A 43% reduction in hemispheric infarct volume was observed after the intravenous administration of a 10-fold lower dose of BDNF conjugate (5 μg per rat), and no significant effect on infarct volume was observed after the intravenous administration of 1 μg per rat of BDNF conjugate. The present studies using the reversible MCAO model involved only a single intravenous administration of the BDNF chimeric peptide. Since degrees of neuroprotection were observed in the present study that are comparable to prior work using 2 sequential doses, the present studies indicate that there is little beneficial effect from a second dose of conjugate administered 3 hours after the initial dose. Prior studies with the permanent MCAO model indicated there was a therapeutic time window and the reduction in total hemispheric stroke volume was 65%, 55%, and 19% when the BDNF chimeric peptide was administered at 0, 1, and 2 hours after permanent MCAO, respectively. A similar therapeutic window exists in the reversible MCAO model since the reduction in the cortical infarct volume is 68% and 31% after the intravenous administration of the BDNF chimeric peptide at 1 and 2 hours, respectively, after MCAO (Table 2).

This study shows that the BDNF chimeric peptide administered intravenously causes no change in the subcortical stroke volume (Table 2, Figure 3). This observation is consistent with prior studies wherein the BDNF (34 μg) was administered directly to the brain by intracerebral infusion of the unconjugated neurotrophin over a 24-hour period, and the infusion was started at 30 minutes after permanent MCAO. BDNF was administered by intracerebral infusion because of the limited transport of this neurotrophin across the BBB and limited access of BDNF to neuronal sites after intravenous administration. The molecular basis of the preferential action of BDNF in cortex versus subcortical regions is not known but may be due to regional differences in expression of the BDNF receptor, trkB, in brain ischemia. Alternatively, BDNF neuroprotection may be mediated via N-methyl-D-aspartate (NMDA) receptors, and there may be regional differences in the expression of the NMDA receptor.

Neuroprotection with unconjugated BDNF after intravenous administration has been recently reported for a 2-hour reversible MCAO model. In this study BDNF was infused over a 3-hour period, and the infusion was started at 30 minutes after MCAO. No neuroprotective effect of intravenous unconjugated BDNF was observed for the subcortical region of brain. However, a 55% reduction in cortical stroke volume was observed after the intravenous infusion of 300 μg per rat of unconjugated BDNF. This dose is 6-fold greater than the dose of BDNF chimeric peptide administered in the present studies, 50 μg per rat. Despite the large dose of unconjugated BDNF administered intravenously, it is unexpected that neuroprotection is achieved with intravenous BDNF because the BBB transport of BDNF is negligible when there is no BBB disruption. Intravenous administration of unconjugated BDNF was neuroprotective in a reversible MCAO model that was performed with chloral hydrate anesthesia and under conditions that caused a significant hyperglycemia, with a plasma glucose level of 220±47 mg%. This level of hyperglycemia, in conjunction with a 2-hour reversible MCAO, causes vasculopathy and premature disruption of the BBB. Therefore, modest hyperglycemia may accelerate opening of the BBB in focal brain ischemia and enable high doses of unconjugated neurotrophic factor to enter the brain after intravenous administration.

For neurotrophic factors such as BDNF to be neuroprotective in brain after intravenous administration of the unconjugated neurotrophin, there must be significant transport of these proteins across the BBB during the therapeutic window when neuroprotection is still possible. BDNF is a strongly cationic protein, and this peptide is absorbed to brain capillaries via electrostatic interactions that are inhibited by other polycationic proteins such as protamine. However, BDNF is not significantly transported across the BBB. The hypothesis that BDNF is, in fact, transported through the BBB is derived from the observation that there is brain uptake of radioactivity after the intravenous injection of [125I]BDNF. However, owing to its cationic nature, BDNF is rapidly removed from blood by peripheral tissues, particularly the liver, with a plasma half-life <10 minutes. The neurotrophic factor is rapidly metabolized in peripheral tissues, and this degradation is followed by the release of radiolabeled low-molecular-weight metabolites such as iodotyrosine back to the bloodstream. The [125I]tyrosine may cross the BBB and account for the radioactivity in brain after intravenous injection of [125I]BDNF. However, owing to its cationic nature, BDNF is rapidly removed from blood by peripheral tissues, particularly the liver, with a plasma half-life <10 minutes. The neurotrophic factor is rapidly metabolized in peripheral tissues, and this degradation is followed by the release of radiolabeled low-molecular-weight metabolites such as iodotyrosine back to the bloodstream. The [125I]tyrosine may cross the BBB and account for the radioactivity in brain after intravenous injection of [125I]BDNF. This interpretation is supported by prior work using 2 different approaches. First, there is no measurable uptake of radioactivity in the brain after injection of [125I]BDNF intravenously in rats when the peripheral metabolism of the neurotrophic factor is completely blocked by pegylation of the peptide. Second, the brain uptake of radioactivity is suppressed 10-fold after the intravenous administration of the BDNF chimeric peptide.
injection of a neuropeptide labeled with $^{111}$In, relative to the same peptide labeled with $^{125}$I. Peptide degradation products labeled with $^{111}$In, which are formed by metabolism in peripheral tissues, are not re-exported back to blood. There is no measurable uptake of brain radioactivity after the systemic administration of a neuropeptide labeled with $^{111}$In, unless the BBB is disrupted.

In summary, these studies demonstrate that BDNF chimeric peptides have neuroprotective effects in focal irreversible brain ischemia after delayed intravenous administration. Doses of BDNF chimeric peptides as low as 5 µg per rat result in significant neuroprotection after intravenous administration. The use of a BBB drug targeting system enables neuroprotection with neurotrophic factors at low doses and without the need for BBB disruption. The need for administration of low systemic doses of neurotrophins is underscored by the peripheral toxicity observed in humans after the intravenous administration of large doses of neurotrophic factors, since neuroprotection is possible only during the initial hours after focal ischemia, when the BBB is not usually disrupted.

**References**

the therapeutic time window for most neuroprotective agents is less than 6 hours. Thus, to be effective, such neuroprotective agents must cross an “intact” BBB.

Lipophilic compounds may cross the BBB by diffusion, but polar compounds are excluded unless they are substrates for transporters present at the BBB. Thus, brain-derived neurotrophic factor (BDNF), a potential neuroprotective agent, does not cross the intact BBB in pharmacologically significant amounts, but there is a specific transporter for the much-larger transferrin molecule. To circumvent the BBB, there has been much effort in the last decade to either modify drugs so that they become substrates for naturally occurring BBB transporters or to conjugate drugs to substrates of such transporters. Thus, in pioneering work, Pardridge and colleagues have shown that the entry of a number of drugs into brain can be enhanced by conjugation to OX26, a monoclonal antibody to the transferrin receptor.

In the current study, Zhang and Pardridge demonstrate that while intravenous injection of BDNF does not reduce the infarct volume that results from 1 hour of transient middle cerebral artery occlusion in the rat, injection of BDNF conjugated to OX26 causes a 70% reduction in cortical infarct size. The experiments involved giving the conjugate at 1 hour after the onset of occlusion. Delaying the drug treatment another hour decreased the protective effect, so there was only a 31% reduction in cortical infarct size. Whether this decrease in efficacy is purely a reflection of the progression of parenchymal cell damage or whether the delivery of the BDNF conjugate to the injured parenchymal cells changes with time is still unclear. Thus, the endothelial endocytosis of the transferrin receptor could be altered by the ischemia, or the migration of the drug within the brain extracellular space may also be limited in damaged brain (for example, because of swelling of the astrocytic foot processes surrounding the cerebral capillaries).

Targeting drugs to a BBB transporter may not only increase drug BBB permeability but may also increase the percentage of injected dose that enters the brain; ie, it may result in preferential targeting to the brain. As suggested by Zhang and Pardridge, this may be important in limiting the therapeutic dose and potential systemic toxicity. Thus, this study serves as a reminder that development of effective treatments for stroke requires not only the discovery of protective agents but also insight in how to deliver those agents to the ischemic brain.

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