Neuroprotection in stroke: is it time to consider large-molecule drugs?

'More than a dozen neurotrophin drugs could enter clinical trials for stroke if these large-molecule pharmaceuticals can be made transportable through the blood–brain barrier.'

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The American Heart Association (Dallas, TX, USA) estimates that there are >500,000 strokes per year in the USA alone, and that 30% of these events result in death. Of the survivors in the USA, there are now approximately three million people in stroke rehabilitation, costing in excess of US$40 billion per year in medical costs. In 1996, thrombolytic therapy for stroke was introduced as the first advance in stroke therapy for decades. However, thrombolytic therapy only dissolves the thrombus that forms at brain arteries, and does not result in neuronal protection.

There have been extensive efforts worldwide to develop small-molecule neuroprotectants for the treatment of acute stroke. Clinical trials have failed with such uniformity that there is the risk of a developed perception that neuroprotection is not possible in stroke. There are several reasons why small-molecule neuroprotective agents have failed in human trials, but a principal cause is the poor transport of these agents across the blood–brain barrier (BBB).

The latest failure in stroke therapy is the Glycine Antagonism in Neuroprotection (GAIN) trial using gavestinel[1]. The failure of the GAIN trial is consistent with the poor BBB transport of gavestinel[2], particularly because this drug is >99% plasma-protein bound in the systemic circulation[3]. YM872 is an AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor antagonist that is considered a possible small-molecule neuroprotective agent[4]. However, YM872 is a highly polar molecule of reduced lipid solubility, and these properties eliminate significant free diffusion through the BBB *in vivo*. The BBB is intact in the first three hours after a stroke[5], when there is an opportunity for neuroprotection. Therefore, a drug that does not cross the BBB would not be expected to be an effective neuroprotective agent in clinical trials. Drugs that do not cross the BBB could still be neuroprotective in preclinical studies if the BBB is disrupted in animals subjected to experimental stroke. BBB disruption can be caused by either excessive electro-coagulation of the middle cerebral artery[2] or hyperglycemia, which causes vasculopathy and breakdown of the BBB in ischemic stroke[6].

Safety of small-molecule drugs

Apart from the problem of poor BBB transport, many small-molecule neuroprotective agents have poor safety profiles[2] because of the lack of receptor specificity of small molecules. Protein-based therapeutics, such as the neurotrophins, are receptor-specific and would be expected to have improved safety profiles in the brain. There are >30 neurotrophins produced in the brain[7] and, therefore, the number of neurotrophin drug candidates is expected to increase in the near future with the application of genomics-based gene discovery. Many of the neurotrophins are highly neuroprotective in stroke models when the protein is injected directly into the brain. Intracerebral administration is required because the neurotrophins do not cross the BBB and are generally not neuroprotective following intravenous administration. Because of this, current neurotrophin drug development is aimed at the discovery of small molecules that increase production of neurotrophins in the brain[8]. However, such molecules must be administered before the ischemic insult, and this is not practical in the treatment of human stroke.

Neurotrophin drug development

The most direct approach to neurotrophin drug development is to use the naturally occurring neurotrophins as large-molecule neuropharmaceuticals. However, because the neurotrophins do not cross the BBB, there must be a merger of neurotrophin drug discovery with BBB drug-targeting technology; this is illustrated in the case of brain-derived neurotrophic factor (BDNF). This neurotrophin was cloned in the 1980s[9] and failed in the 1990s as a

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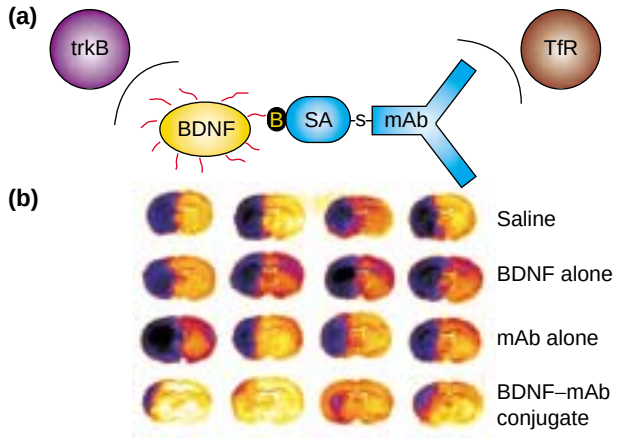

Figure 1. (a) Brain-derived neurotrophic factor (BDNF) is conjugated with 2000 Dalton strands of polyethylene glycol (PEG) and the pegylated BDNF homodimer contains a single biotin residue (denoted as 'B' in the figure) at the tip of one of the PEG strands. This BDNF–PEG^{2000}–biotin is then mixed with a thio-ether (-S-) conjugate of streptavidin (SA) and an anti-transferrin receptor (TfR) monoclonal antibody (mAb) to form the BDNF–mAb conjugate shown in the figure. The conjugate forms instantaneously and is injected intravenously into rats subjected to the permanent middle-cerebral-artery occlusion (MCAO) stroke model. (trkB is the BDNF receptor.) **(b)** The middle cerebral artery was occluded in adult rats, and 5–50 μg per rat doses of BDNF chimeric peptide were administered intravenously for up to 2 h after arterial occlusion. Controls included the intravenous administration of BDNF alone or mAb alone. The BDNF–mAb conjugate reduced the stroke volume by 65%, from 350 ± 21 mm^3 to 121 ± 23 mm^3 [mean ± SD, $p<0.01$ by analysis of variance with Bonferroni correction (ANOVA)][12]. There was no reduction in stroke volume following the intravenous administration of either the unconjugated BDNF, unconjugated mAb, or saline. The brain was removed 24 h after MCAO, and brain sections were treated with 2,3,5-triphenyltetrazolium chloride (TTC). TTC stains viable brain tissue, which appears yellow after visualization of the image. Infarcted brain is unstained by TTC, which appears blue. A coronal section from the brain of four different rats in each of the four treatment groups is shown.

treatment of amyotrophic lateral sclerosis (ALS) in Phase III trials[10]. BDNF does not cross the BBB, either in the brain or spinal cord, and the peripheral administration of native BDNF would not be expected to cause neuroprotection in the brain, because this large molecule does not cross the BBB. However, recent work has shown that if BDNF is conjugated to a BBB-drug targeting system, then this neurotrophin is neuroprotective in either global[11] or regional[12] brain ischemia following the delayed intravenous administration of low doses of the drug. The BDNF is conjugated to a BBB drug-targeting vector, which is a ligand transported by a receptor-mediated transcytosis (RMT) system localized within the BBB. This BDNF–vector conjugate is called a chimeric peptide. One suitable transport vector is a peptidomimetic monoclonal antibody (mAb) that is transported by an endogenous BBB-RMT system, such as the transferrin receptor (TfR). The mAb undergoes receptor-mediated transcytosis across the BBB via the endogenous transferrin-receptor transport system, and carries any drug attached to the mAb across the BBB. The BDNF is conjugated to the mAb with avidin–biotin technology, as shown in Fig. 1a. Alternatively, a BDNF–mAb fusion gene and fusion protein could be produced. In either case, it is necessary that the bifunctionality of the chimeric peptide be maintained so that the drug binds both to the TfR to enable transport across the BBB, and to the neuronal trkB BDNF receptor to cause neuroprotection subsequent to transport (Fig. 1a). The delayed intravenous administration of BDNF chimeric peptide at doses as low as 5 μg per rat, results in 45–65% reduction in stroke volume in the middle-cerebral-artery occlusion model (Fig. 1b). By contrast, the intravenous administration of the unconjugated BDNF has no neuroprotective effect (Fig. 1b), because the neurotrophin does not cross the BBB.

There is an inherent amplification in the overall drug-development process once a BBB drug-targeting technology is developed. A single brain drug-targeting technology can be applied to the drug development of many neurotrophins that can be individually developed as neuroprotective agents for the treatment of acute stroke. In addition, such drugs could also prove to be beneficial in the long-term treatment of various brain disorders, including Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases. However, large-molecule drugs cannot be put to work in human disorders without the application of a BBB drug-targeting technology. Given the inability to develop small-molecule drugs as neuroprotective agents, now might be the time to direct efforts towards large-molecule neurotrophin drug development.

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