Biologic Drug Delivery Across the Blood-Brain Barrier with IgG Fusion Proteins

William M. Pardridge, M.D.
University of California
ArmaGen, Inc.
In 2017, there are no biologics that are FDA approved for CNS disease, where the drug crosses the blood-brain barrier (BBB)
Brain Uptake of Therapeutic Antibodies: The myth of a brain uptake of 0.1-0.2%

A frequently cited claim is that the brain uptake of therapeutic antibodies is low but significant, and the brain antibody concentration is 0.1-0.2% of the serum antibody concentration.

The evidence for this claim is the observation that the CSF concentration is 0.1-0.2% of the serum concentration of the antibody.

This assumes that drug entry into CSF, across the blood-CSF barrier at the choroid plexus, is equal to drug entry into brain parenchyma, across the blood-brain barrier, at the brain capillary endothelium.
### Blood to CSF vs Blood to Brain

<table>
<thead>
<tr>
<th>Blood-CF CSF Barrier @ Choroid Plexus</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG CSF to serum ratio</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>0.1-0.2%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood-Brain Barrier @ Capillary Endothelium</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG brain to serum ratio</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>&lt;0.01%</td>
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</table>

The blood-CSF barrier is 100-fold leakier than the BBB.
The Blood-Brain Barrier

~0% of all large molecule drugs cross the BBB

~2% of all small molecule drugs cross the BBB
Intravenous HRP is trapped in the lumen of brain capillaries (Brightman)
blood

red cell

GLUT1
abluminal membrane

GLUT1
luminal membrane

Intra-endothelial volume
0.8 uL/gram

Extra-vascular volume
700 uL/gram

brain
Endogenous BBB Transporters

Nutrient Transport: Carrier-Mediated Transport

Peptide Transport: Receptor-Mediated Transport
Immune-staining of capillaries in brain with an antibody to a BBB peptide receptor
Human and primate BBB insulin receptor: Mediates transport of insulin and an insulin receptor antibody

Human brain capillaries

Emulsion auto-radiography of rabbit brain after carotid artery infusion of $^{125}\text{I}$-insulin

Immunocytochemistry of Rhesus monkey brain with MAb against human insulin receptor (HIR)

Brain scanning in Rhesus monkeys

Peptide alone

Peptide-HIRMAb
IgG Fusion Proteins for Delivery of Protein Therapeutics to the Human Brain

Chimeric or humanized HIRMAb + Recombinant protein = IgG fusion protein: a New Biological Entity
AGT-181: HIRMAb-IDUA fusion protein
A BBB-penetrating form of iduronidase (IDUA)

HIRMAb-IDUA fusion protein

HIRMAb domain

IDUA domain

AGT-181

Brain uptake in the Rhesus monkey

Intracellular delivery of AGT-181 to lysosomes of Hurler fibroblasts

HIRMAb=monoclonal antibody (MAb) to human insulin receptor (HIR)

IDUA alone AGT-181

Brain uptake in the Rhesus monkey

Intracellular delivery of AGT-181 to lysosomes of Hurler fibroblasts

HIRMAb=monoclonal antibody (MAb) to human insulin receptor (HIR)
Reduction of glycosaminoglycans (GAGs) in brain of MPS mice with systemic administration of IgG-lysosomal enzyme fusion proteins

**Hurler mouse (MPSI)**

- 6 month old MPSI mice
- null for iduronidase (IDUA)
- treat twice weekly (1 mg/kg) IV for 6 weeks
- measure brain GAGs by light and electron microscopy

**Sanfilippo A mouse (MPSIIIA)**

- 2 week old MPSIIIA mice
- null for sulfamidase (SGSH)
- treat thrice weekly (5 mg/kg) IP for 6 weeks
- measure brain and liver Heparan Sulfate (HS) by LC-MS

**Treatment causes a 73% reduction in brain lysososomal inclusion bodies**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brain HS (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1200</td>
</tr>
<tr>
<td>cTfRMAb-IDUA</td>
<td>800</td>
</tr>
</tbody>
</table>

**70% reduction in brain HS**
First BBB Trojan Horse Clinical Trials
(INDs approved 2015)

Mucopolysaccharidosis Type I
Hurler Syndrome
HIRMAb-IDUA fusion protein
Valanafusp alpha (AGT-181)
IDUA = iduronidase

Mucopolysaccharidosis Type II
Hunter Syndrome
HIRMAb-IDS fusion protein (AGT-182)
IDS = iduronate 2-sulfatase

The Mucopolysaccharidoses (MPS),
Lysosomal storage disorders
BBB-penetrating decoy receptors
Re-engineering the TNFR2 extracellular domain as a BBB-penetrating IgG fusion protein

etanercept

BBB-penetrating etanercept:

HIRMAb-TNFR2
Brain uptake in the Rhesus monkey:
Etanercept (Fc:TNFR) vs HIRMAb-TNFR fusion protein

(1) Brain uptake of entanercept (Fc:TNFR) is confined to brain plasma volume

(2) Brain uptake of HIRMAb-TNFR, 3% ID/brain, compares to small molecules
Therapeutic effects in mouse models of neural disease: Etanercept vs BBB-penetrating-TNFR2 fusion protein

**Parkinson’s disease**

Striatal tyrosine hydroxylase (TH) after 6-hydroxydopamine toxin exposure

- IV TfRMAb-TNFR2 reverses motor deficit by 80% and increases striatal TH enzyme activity by 130%
- **IV Enbrel has no effect**

**stroke**

Dye staining of mouse brain shows infarct after 60 min reversible occlusion MCA

- IV TfRMAb-TNFR2 reduces stroke volume by 50% and improves neural deficit by 50%
- **IV Enbrel has no effect**

**Alzheimer’s disease**

Immunoreactive Abeta amyloid plaque in PSAPP double transgenic AD mouse

- IP TfRMAb-TNFR2 reduces total plaque and brain Aβ\textsuperscript{1-42}, reduces ICAM-1 and improves recognition memory
- **IP Enbrel has no effect**
BBB-Penetrating Bi-Specific Antibodies Targeting Abeta Amyloid in Alzheimer’s Disease
Re-engineering an anti-Aβ antibody as a BBB-penetrating tetravalent bi-specific antibody (BSA)

- Amyloid
  - Brain uptake $< 0.01\%$ ID/brain

- Anti-Aβ ScFv

- BBB receptor
  - Brain uptake $= 2\%$ ID/brain

- Anti-BBB MAb

- Anti-BBB/anti-Aβ BSA
  - Brain uptake $= 2\%$ ID/brain
Chronic treatment of AD transgenic mouse with BBB-penetrating bi-specific antibody (BSA)

APPswe, PSEN1dE9 double transgenic Alzheimer’s disease (AD) mouse

**Methods**

1. Treat AD mice with either saline or 5 mg/kg BBB-penetrating anti-Aβ BSA administered subcutaneously (SQ)
2. Treat daily for 12 weeks (mice 12-15 months old)
3. Measure brain amyloid plaque with 6E10 immune labeling and Thioflavin-S in cortex and hippocampus
4. Quantify immune response with anti-drug antibody (ADA) ELISA
5. Measure cerebral micro-hemorrhage with Prussian blue
Amyloid plaque reduction in AD mouse brain with a BBB-penetrating anti-Aβ BSA

60% reduction in brain amyloid plaque with daily SQ administration of BBB-penetrating anti-Aβ BSA (5 mg/kg) for 12 weeks
Human BBB Trojan horse fusion proteins:
Re-engineering protein therapeutics for delivery to human brain

Lysosomal enzymes:
- IDUA
- IDS
- ASA
- SGSH
- NAGLU

Decoy Receptors:
- TNFR-II

Neurotrophins:
- EPO
- GDNF

Therapeutic antibodies:
- Anti-Aβ MAb
BLOOD BRAIN BARRIER