Glutamate Biomarkers: ALS, Astroglia and Glutamate transporters

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Depts of Neurology and Neuroscience
Targets for monitor Glutamate Neurotransmission
Astrocyte: Human vs rodent - complexity of processes

Single rat astrocyte envelopes 100,000 synapses
Single Human astrocyte envelopes ~2,000,000 synapses!!

(Oberhelm et al, J. Neurosci, 2009)
ALS – An Overview

Classification

- Sporadic ALS
  - Cause unknown
  - 90% to 95% of cases
- Familial ALS (FALS)
  - Genetically linked
  - 5% to 10% of cases
- Environmental ALS (e.g., Western Pacific ALS)
  - Possible dietary cause
  - Sometimes accompanied by symptoms of Parkinson’s disease and dementia
  - 2 fold incr risk Gulf War vets
Altered glutamate regulation in ALS:
Increased cerebrospinal fluid [glutamate] and decreased glutamate transport

CSF levels of glutamate elevated 3-10 fold in up to 40% of all ALS patients (n>300 worldwide)

Glutamate transport decreased by up to 95% in motor cortex and spinal cord

Focal, peri-neuronal loss of EAAT2 expression in sporadic, Mutant SOD1 and TDP43- based ALS: Leads to toxic extracellular glutamate

Human

<table>
<thead>
<tr>
<th></th>
<th>ALS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAAT2</td>
<td><img src="https://example.com/image1" alt="Image" /></td>
<td><img src="https://example.com/image2" alt="Image" /></td>
</tr>
<tr>
<td>EAAT1</td>
<td><img src="https://example.com/image3" alt="Image" /></td>
<td><img src="https://example.com/image4" alt="Image" /></td>
</tr>
<tr>
<td>EAAT3</td>
<td><img src="https://example.com/image5" alt="Image" /></td>
<td><img src="https://example.com/image6" alt="Image" /></td>
</tr>
</tbody>
</table>

EAAT2: Motor cortex

Rothstein et al, Ann Neurol, 1995

Rodent

Glutamate transport

Howland et al, PNAS 2001
ALS Therapeutics: Anti-glutamate repeatedly positive

- **Riluzole Phase 2/Phase 3**: up to 1 year increase survival (repeatedly positive in multiple followup trials)
- **Topiramate**: 25% longer median survival (those not losing wgt)
- **Ceftriaxone**: now under study
  - Nasal biopsy biomarker planned
Glial Cells contribute to pathogenesis in neurodegenerative diseases: Astroglia/Microglia and oligos are an important therapeutic target for ALS

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target Neurons</th>
<th>Involvement of Other Cell Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer's disease</td>
<td>cortical and hippocampal neurons</td>
<td>not directly tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>microglial dysfunction contributes to pathogenesis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not directly tested</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>dopaminergic neurons</td>
<td>express enzyme that induces toxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>their activation precedes neurodegeneration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>elevated expression in oligodendrocytes suffices for disease</td>
</tr>
<tr>
<td>Huntington's disease</td>
<td>striatal neurons</td>
<td>mutant expression renders neurons vulnerable in culture</td>
</tr>
<tr>
<td></td>
<td></td>
<td>their activation occurs early and progresses with disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not directly tested</td>
</tr>
<tr>
<td>Spinocerebellar ataxia</td>
<td>Purkinje cells</td>
<td>mutant expression in Bergmann glia suffices for disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not directly tested</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not directly tested</td>
</tr>
<tr>
<td>Prion disease</td>
<td>cortical neurons</td>
<td>PrP expression suffices for disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>microglial activation decreases prion infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>probably not important for pathogenesis</td>
</tr>
</tbody>
</table>
Astrocytic Glutamate Transporter Dysregulation in Disease

- Multiple acute and chronic neurologic injuries associated with altered glutamate transporter (GLT1/EAAT2) expression (e.g. ALS, HD, Alz, MS)
- Likely represents an altered astroglia and/or altered neuron-glial communication, astroglial-microglial interaction
- Understanding these pathways provides insight into
  - normal astroglial-neuron interactions
  - Pathways for drug discovery/defect correction
Altered expression/regulation of GLT1/EAA T2

- Due to loss of axonal/dendritic signaling
  - Altered astroglial promoter activation
  - KBBP binding protein; Neuron –stimulated astroglial transporter promoter regulation
    - Yang et al, Neuron, 2009
- Epigentent
  - Yang et al., 2009, Glia
- Micro RNA
  - Regan et al, unpublished
- Other pathways:
  - e.g. altered trafficking→degradation; sumylation (trotti)
- NOTE: In most cases- diseases that lead to reactive astrocytes/altered dendrites almost always associated with loss of GLT1
NINDS Neurodegeneration Drug Screening Consortium

- 26 Academic laboratories
- 29 Screening assays
- Blindly screened 1040 FDA approved drugs
  - ~750 FDA approved drugs, ~300 nutritionals/controlled substances
- Assays focused on various aspects of neurodegenerative disease pathways
  - Diseases: HD, ALS, PD, SMA, Kennedy’s
- 11 Assays ALS-Relevant:
  - SOD1 toxicity
  - Excitotoxicity
  - Mitochondrial function
  - SOD1 Protein aggregation

Screening Results: Best drug Cephalosporin antibiotics with 4 hits
Late ceftriaxone treatment increases survival of G93A SOD1 mice, delays loss of MN and increases EAAT2/GLT1.

(ceftriaxone, 200 mg/kg ip x 5-7 days; Start Rx: 90 days age)

(c) Rothstein et al., Nature 2005
Ceftriaxone/ß-Lactam: Subsequent published effects in other disease models

• Neural injury:
  – Radiation induced neural injury (invitro)
  – ALS model (invitro/in vivo)
  – Sindbis induced paralysis (in vivo)
  – GP120 toxicity (in vitro)
  – Huntington’s disease mouse (in vivo)
  – EAE induced damage (in vivo)
  – Stroke (in vivo)

• Synaptic activity/Behavior
  – Depression models (in vivo; = efficacy to Prozac)
  – Cocaine readministration
• Cell lines:
  • Rodent-astroglia (EAAT2 promoter fragment)
  • Human immortalized (EAAT2 promoter fragment)
  • Rodent EAAT2 BAC luciferase
How to effectively target drugs to monitor Glutamate Transporters? (e.g. EAAT2)

Development of an Astroglial Biomarker Program
Biomarkers: How to improve CNS drug discovery

Your Nose: A window to your brain

• How do we know if drugs really get to the brain and “work”?

• Need to **sample** brain tissue

• Instead:
  • Nasal olfactory mucosa
  • This tissue is easily accessible “real” nervous system tissue that can be biopsied, repeatedly in patients
Human and Rodent Expression of Brain Astroglial proteins EAAT2/GFAP in Olfactory tissue: Induction by an Astroglial Activator

A. Mouse and Human Biopsy: Detectable Astroglial EAAT2/GLT1 Protein

B. Experimental Astroglial EAAT2 Activator TAP: Induction of Rodent Nasal EAAT2

(Sattler et al, SFN 2007)
Human Nasal Olfactory Biopsy: EAAT2 Biomarker
Reliable Bioassay for Human EAAT2

- Experience in local biopsy protocol
- >80 controls:
  - 40 Psychiatry (A. Sawa)
  - 24 controls (age: 20-50; JHU/UMD)
  - 30 European
- Reliable, rapid clinic procedure
- Stable, low variability assays
- Ratio EAAT2/GFAP mRNA (qPCR) and EAAT2/OMP (qPCR)

(Sattler et al, SFN 2007)
Pathfinding Drug: RUX122 Validates Astroglial Biomarker

RUX122 Has No Effect on Neuronal and non-CNS Genes
Imaging Astroglial Biology

- Diagnostic Tool
- Biomarker for
  - patient selection
  - monitoring therapeutic effect
- Same biomarker in transgenic mouse model
Dynamic PET Imaging of Glutamate Transporters

• Phenotype ALS patients
  ▫ Loss of EAAT2 and increased CSF-Glutamate occurs in 40% of sporadic ALS patients
  ▫ In rodents, transporter changes occur at disease onset

• Measure Drug Efficacy
  ▫ Image before and during drug treatments targeted to glutamate transporters to monitor effects on transporter levels

• Broader Applications
  ▫ Altered excitatory transmission in multiple disorders (HD, AD, Epilepsy, Depression, Glioblastoma)
Ligand Development

1. Protein & Ligand Models
   R. Bridges
   Penetration: Potency & Selectivity

2. Ligand Synthesis
   J. Gerdes
   Syntheses

3. In vitro Pharmacology

4. CNS Distribution
   PK/PD

R. Sattler (Hopkins)
Synthetic Ligand Panel

1. $R = CH_3$
2. $R = H$
3. $R = CH_3$
4. $R = H$
5. $R = CH_3$
6. $R = H$
7. $R = CH_3$
8. $R = H$
9. $R_1 = R_2 = H$
10. $R_1 = R_2 = CH_3$
11. $R_1 = CH_3, R_2 = H$
12. $R_1 = H, R_2 = CH_3$

John Gerdes, University of Montana
Pharmacological Profile of EAAT ligands

IC₅₀ ([³H]-D-Aspartic Acid uptake measurements in overexpressed C17 cells) EAAT2 vs EAAT3

(Previously reported literature values: Dunlop, 2006; Greenfield, 2005)
Dose-Dependent Brain Penetration of RM005

**Brain**

<table>
<thead>
<tr>
<th>Time after injection [min]</th>
<th>RM005 (acid) [ng/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Graph of RM005 (acid) in Brain at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>3</td>
<td>![Graph of RM005 (acid) in Brain at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>10</td>
<td>![Graph of RM005 (acid) in Brain at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>30</td>
<td>![Graph of RM005 (acid) in Brain at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>60</td>
<td>![Graph of RM005 (acid) in Brain at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>90</td>
<td>![Graph of RM005 (acid) in Brain at 3mg/kg and 10mg/kg]</td>
</tr>
</tbody>
</table>

**Plasma**

<table>
<thead>
<tr>
<th>Time after injection [min]</th>
<th>RM005 (acid) [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Graph of RM005 (acid) in Plasma at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>3</td>
<td>![Graph of RM005 (acid) in Plasma at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>10</td>
<td>![Graph of RM005 (acid) in Plasma at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>30</td>
<td>![Graph of RM005 (acid) in Plasma at 3mg/kg and 10mg/kg]</td>
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<tr>
<td>60</td>
<td>![Graph of RM005 (acid) in Plasma at 3mg/kg and 10mg/kg]</td>
</tr>
<tr>
<td>90</td>
<td>![Graph of RM005 (acid) in Plasma at 3mg/kg and 10mg/kg]</td>
</tr>
</tbody>
</table>

**Iv Injection of acid**

n.a.: not applicable
Brain Penetration of RM005 After ProDrug Injection

Iv injection of ester
Increased Brain Penetration of RM005 After ProDrug Injection

![Graph showing increased brain penetration of RM005 after prodrug injection compared to acid injection over time.](image-url)
Generation of labelled transporter PET candidate

Prodrug tracer $[^{18}F]1$

Key Intermediate

Path C

Previously prepared ligand for pharmacology
RM005 AND RM006 Cross the BBB in WT Mice

Results thus far:

• iv injections of the methyl ester prodrug of aspartyl amide (Fluoro) leads to higher drug levels in brain than acid injection

• Labeling now underway- small animal testing soon

• Primate planned (?)2010

• Early human trial (Control/ALS) 2011?
Tools to Study Astroglia/Glutamate Transporters: normal & disease genetic regulation

- **BAC (full length gene) transgenic reporter mice:**
  - GLT1-eGFP/GLAST-DsRed
  - ALDHL1H1-eGFP
  - GFAP-eGFP
  - MCT1-TdTm

- **BAC-TRAP**
  - GLT1, ALDHL1H1, GFAP bac TRAP
    - For translated astroglial RNA capture

- **TG**
  - GLT1-8.3kb promoter/TdTm

(Regan et al, J. Neurosci, 2006)
iPS astroglia: Characterization

- GFAP+ after 4 mo (2ES, 4 iPS)
- Abundance 10%
- Differentiation: serum/BMP4

**TABLE 2. Antibodies used to assess Glial Differentiation**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAP</td>
<td>Glial fibrillary acidic protein</td>
<td>Astrocyte</td>
</tr>
<tr>
<td>GLT1</td>
<td>Glutamate transporter</td>
<td>Mature Astrocyte</td>
</tr>
<tr>
<td>AQ4</td>
<td>Aquaporin 4</td>
<td>Astrocyte</td>
</tr>
<tr>
<td>ALDH1L1</td>
<td>Aldehyde dehydrogenase 1.1</td>
<td>Mature astrocyte</td>
</tr>
<tr>
<td>GLAST</td>
<td>Glutamate transporter</td>
<td>Astrocyte/oligo</td>
</tr>
<tr>
<td>CC1</td>
<td>adenomas polyposis coli</td>
<td>Oligodendroglia</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
<td>Oligodendroglia</td>
</tr>
<tr>
<td>MAG</td>
<td>Myelin associated protein</td>
<td>Oligodendroglia</td>
</tr>
<tr>
<td>CNPase</td>
<td>Cyclic nucleotide phosphatase</td>
<td>Oligodendroglia</td>
</tr>
<tr>
<td>O4</td>
<td>Pro-oligodendroglia antigen</td>
<td>Oligo progenitor</td>
</tr>
<tr>
<td>PDGFRα</td>
<td>Platelet derived growth factor α</td>
<td>NG2/glial progenitor</td>
</tr>
<tr>
<td>NG2</td>
<td>Chondroitin sulfate proteoglycan</td>
<td>NG2/glial progenitor</td>
</tr>
</tbody>
</table>
Generation of EAAT2-BAC firefly luciferase reporter mice: utility for In vivo drug screening

EAAT2 Luciferase Promoter Reporter Mice
Tissue Activity

Body Region

100,000 fold incr in brain

(image adapted from GFAP luciferase mouse study; Xenogen)
Acknowledgements

Credit in my lab: Astroglial Biology Group

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Melissa Regan PhD

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D. Kieran

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J. Gerdes

Harvard Univ
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Merit Cudkowicz

Columbia
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Chris Henderson

Support: NIH, MDA, P²ALS/Packard Center
Penetration Profile in WT Mice: RM005 and RM006

Study Design:
- iv injections of acid and methyl ester
- 3mg/kg; 10mg/kg
- Tissue collection (brain and plasma) at 1; 3; 10; 30; 60; 90 min
Penetration Profile in WT Mice

Method development with Ligand Standards #9 (acid) and #10 (dimethyl ester)

#9 = RM001

L-β-threo-benzyl-aspartic Acid

#10 = RM002

L-β-threo-benzyl-aspartate dimethyl ester

Initial Method Development Test Agents

Tissue LC/MS Measure

In vivo metabolism possible

Penetration