Neuroprotection in Parkinson’s Disease

Graham A. Glass, MD

Assistant Professor of Neurology
University of California San Francisco

San Francisco VA Medical Center
Parkinson’s Disease Research, Education and Clinical Center (PADRECC)
Current Therapy in PD

- Parkinson’s Disease is one of the only “neurodegenerative” diseases in which medications alleviate symptoms
- The advent of carbidopa/levodopa significantly altered longevity in PD patients
- DBS therapy has allowed improved management in a number of patients
- Many years into PD, motor symptoms take a back seat to other problems, and we are more limited in therapies for these problems.
Levodopa Extends Lifespan

• Comparison of longevity in pre- and post-levodopa treatment patients revealed that patients treated with levodopa had less excess mortality than non-levodopa treated patients

Sweet et al., Ann Int Med 1975:83;456-463

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
<th>Number of Deaths</th>
<th>Age at Death, yrs.</th>
<th>Duration of Illness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Pre-levodopa (13)</td>
<td>802</td>
<td>340</td>
<td>65.9</td>
<td>10.8</td>
</tr>
<tr>
<td>Post-levodopa (present series)</td>
<td>100</td>
<td>32</td>
<td>73.1</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>38-91</td>
<td>1-41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63-90</td>
<td>3-28</td>
</tr>
</tbody>
</table>
Table 2 Progression of disability in IPD: latencies to reach successive H+Y stages

<table>
<thead>
<tr>
<th>Study</th>
<th>HY 1</th>
<th>HY 2</th>
<th>HY 3</th>
<th>HY 4</th>
<th>HY 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-levodopa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoehn + Yahr, 1967</td>
<td>3.0</td>
<td>6.0</td>
<td>7.0</td>
<td>9.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Marttila + Rinne, 1977</td>
<td>—</td>
<td>2.9</td>
<td>5.5</td>
<td>7.5</td>
<td>9.7</td>
</tr>
<tr>
<td>Post-levodopa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoehn, 1983</td>
<td>—</td>
<td>9.0</td>
<td>12.0</td>
<td>12.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Hely et al., 1999</td>
<td>—</td>
<td>—</td>
<td>4.0</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Muller et al., 2000</td>
<td>—</td>
<td>3.0</td>
<td>5.5</td>
<td>14.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Lucking et al., 2000</td>
<td>—</td>
<td>11.0</td>
<td>19.0</td>
<td>26.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Poewe, W. Neurology 2006;66:S2-S9
<table>
<thead>
<tr>
<th>Neuropsychiatric</th>
<th>Percent of subjects experiencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive decline</td>
<td>84</td>
</tr>
<tr>
<td>Dementia</td>
<td>48</td>
</tr>
<tr>
<td>Daytime sleepiness</td>
<td>79</td>
</tr>
<tr>
<td>Depression (mostly mild)</td>
<td>50</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>21</td>
</tr>
<tr>
<td>Axial motor</td>
<td></td>
</tr>
<tr>
<td>Falls</td>
<td>81</td>
</tr>
<tr>
<td>Fractures</td>
<td>23</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>50</td>
</tr>
<tr>
<td>Severe dysarthria</td>
<td>27</td>
</tr>
<tr>
<td>Autonomic</td>
<td></td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td>41</td>
</tr>
<tr>
<td>Symptomatic postural hypotension</td>
<td>35</td>
</tr>
</tbody>
</table>

As reported by Hely et al.\textsuperscript{29}
What are people doing to slow down PD?

• Other therapies further treat symptoms.
• Some are thought to be neuroprotective and efforts are being made to prove this (selegiline, rasagiline, Mirapex and Mirapex LA)
• Tons of studies as seen below.....but.
<table>
<thead>
<tr>
<th>Trials</th>
<th>Active agents</th>
<th>Putative mechanisms</th>
<th>N</th>
<th>Primary outcomes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed, Published Trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Tetrad and Langston$$^{15}$$</td>
<td>selegiline</td>
<td>antioxidant/anti-apoptotic</td>
<td>54</td>
<td>Time to levodopa Rx</td>
</tr>
<tr>
<td>2. DATATOP$$^4$$</td>
<td>selegiline and tocopherol</td>
<td>antioxidant/anti-apoptotic</td>
<td>800</td>
<td>Time to levodopa Rx</td>
</tr>
<tr>
<td>3. SINDEPAR$$^{16}$$</td>
<td>selegiline$$^{b}$$</td>
<td>antioxidant/anti-apoptotic</td>
<td>101</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>4. ROADS$$^{17}$$</td>
<td>lazabemide (4 dosages)</td>
<td>antioxidant/anti-apoptotic</td>
<td>321</td>
<td>Time to levodopa Rx</td>
</tr>
<tr>
<td>5. Swedish Selegiline$$^{18}$$</td>
<td>selegiline</td>
<td>antioxidant/anti-apoptotic</td>
<td>157</td>
<td>Time to levodopa Rx</td>
</tr>
<tr>
<td>6. Norwegian-Danish$$^{19}$$</td>
<td>selegiline</td>
<td>antioxidant/anti-apoptotic</td>
<td>163</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>7. QE2$$^{20}$$</td>
<td>coenzyme Q10 (3 dosages)</td>
<td>antioxidant/mitochondrial stabilizer</td>
<td>80</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>8. Jankovic and Hunter$$^{21}$$</td>
<td>riluzole</td>
<td>NMDA antagonist</td>
<td>20</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>9. TEMPO$$^{22}$$</td>
<td>rasagiline (2 dosages)</td>
<td>antioxidant/anti-apoptotic</td>
<td>404</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>10. ELLDOPA$$^{5}$$</td>
<td>levodopa (3 dosages)</td>
<td>dopaminergic</td>
<td>361</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>11. U.K. Low-dose Pergolide$$^{23}$$</td>
<td>pergolide</td>
<td>antioxidant</td>
<td>106</td>
<td>Time to levodopa Rx</td>
</tr>
<tr>
<td>12. NET-PD futility #1$$^{24}$$</td>
<td>minocycline, creatine</td>
<td>anti-inflammatory, mitochondrial</td>
<td>200</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>13. TCH346$$^{25}$$</td>
<td>TCH346 (3 dosages)</td>
<td>anti-apoptotic</td>
<td>301</td>
<td>Time to dopaminergic Rx</td>
</tr>
<tr>
<td>14. NET-PD futility #2$$^{26}$$</td>
<td>GPI-1485, coenzyme Q10</td>
<td>trophic factor antioxidant, mitochondrial stabilizer</td>
<td>213</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>15. PRECEPT$$^{27}$$</td>
<td>CEP-1347 (3 dosages)</td>
<td>anti-apoptotic</td>
<td>806</td>
<td>Time to dopaminergic Rx</td>
</tr>
<tr>
<td>Ongoing or Unpublished Trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. NIL-A$$^c$$ (completed 2002)</td>
<td>GPI-1485 (2 dosages)</td>
<td>trophic factor</td>
<td>300</td>
<td>Change in UPDRS motor Rx</td>
</tr>
<tr>
<td>17. Riluzole$$^e$$ (completed 2002)</td>
<td>riluzole (2 dosages)</td>
<td>NMDA antagonist</td>
<td>1084</td>
<td>Time to dopaminergic Rx</td>
</tr>
<tr>
<td>18. Guilford GPI-1485$$^d$$ (completed 2006)</td>
<td>GPI-1485</td>
<td>trophic factor</td>
<td>~200</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>19. MitoQ trial$$^e$$ (completed 2007)</td>
<td>mitoquinone (2 dosages)</td>
<td>mitochondrial antioxidant</td>
<td>120</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>20. QE3$$^f$$</td>
<td>coenzyme Q10 (2 dosages)</td>
<td>antioxidant/mitochondrial stabilizer</td>
<td>600</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>21. ADAGIO$$^g$$ (completed 2008)</td>
<td>rasagiline (2 dosages)</td>
<td>antioxidant/anti-apoptotic</td>
<td>1176</td>
<td>Change in UPDRS</td>
</tr>
<tr>
<td>22. NET-PD LS Creatine$$^h$$</td>
<td>creatine</td>
<td>mitochondrial stabilizer</td>
<td>1720</td>
<td>Global statistic</td>
</tr>
<tr>
<td>23. PROUD$$^j$$</td>
<td>pramipexole</td>
<td>dopaminergic</td>
<td>535</td>
<td>Not reported</td>
</tr>
</tbody>
</table>
ADAGIO Trial

- Delayed-start design with four arms, either early or delayed administration of two doses (1mg, 2mg) of rasagiline
- 1,146 subjects vs. 404 in TEMPO trial
- 9 months for both arms of study vs. 6 months in TEMPO
- 3 Endpoints vs. 1 in TEMPO

Olanow et al., NEJM 2009;361:1268-78
ADAGIO Endpoints

Olanow et al., NEJM 2009;361:1268-78
ADAGIO Trial Results

• 1mg dose met all 3 endpoints
  – Baseline to end change in UPDRS
  – Slope of curves in weeks 12-36
  – Non-inferiority in slope of weeks 48 to 72

• 2mg dose met none
Problems with Interpretation of ADAGIO

• Possibly biased sample due to selection of patients likely to ‘survive’ placebo phase of study

• Using UPDRS, especially ‘old’ UPDRS has problems
  – Not very sensitive to early changes in symptoms
  – Subjective
  – Likely not linear progression

• Difference between early and delayed start groups (about 2 UPDRS total points) was only about 1% of total

• Failure or 2mg dose to meet any endpoints

Adapted from Ahlskog and Uitti Neurology 2010;74;1143-1148
Why didn’t 2mg work?

– Possible Explanations
  • Symptomatic benefit masked disease-modifying effect
    – But, symptomatic effect was equal between doses in first phase
    – MAO-B nearly completely inhibited at both doses
  • Disease modifying effect may be independent of MAO-B inhibition and more potent at lower doses
    – But propargylamine compound TCH346 failed in large Trial
Other Problems

- Variability in response to rasagiline was twice the magnitude of the positive finding of the study:
  - Delayed-start, active phase 2mg: +1.16 pts
  - Delayed-start, active phase 1mg: -0.23
  - Early-start, first phase 2mg: -1.11
  - Early-start, first phase 1mg: -1.26
  - Early-start, second phase 1mg: -1.56
  - Early-start, second phase 2mg: -2.36
  Range: 3.52 pts

- Design assumed that symptomatic effect would plateau by 12 weeks, but this does not seem to be the case

- Does rasagiline even do better than levodopa?
Adagio vs. Elldopa

Fahn et al. NEJM 2004;351:2498-508
I can’t get no satisfaction
Still no neuroprotection for Parkinson disease

• Editorial written following PSG Trial based on CEP-1347, an anti-apoptotic therapy in PD

• 2 points made in this editorial
  – Animal Models for PD
  – simplification of apoptosis vs. necrosis of cells

J. Eric Ahlskog
Neurology 2007;69:1476-1477
Back to the Drawing Board!

• What are we protecting from?
• Retrospective thinking
• Faulty logic based on grossly incomplete models of PD
• Measurements of PD progression very limited
• Inability to “screen” good compounds based on poor animal models
• Mouse substantia not nigra – not pigmented
• Equal over-expression of α-synuclein does not lead to aggregation/neuronal demise in mice
• Mouse lifespan much shorter – most studies ignore effects of aging
• A53T α-synuclein mutation in humans is normal sequence in mouse
Does everyone with “pre-clinical” PD end up getting PD?

Incidental Lewy Body Disease and Preclinical Parkinson Disease

Anthony DelleDonne, PhD; Kevin J. Klos, MD; Hiroshige Fujishiro, MD, PhD; Zeshan Ahmed, BSc; Joseph E. Parisi, MD; Keith A. Josephs, MD, MST; Roberta Frigerio, MD; Melinda Burnett, MD; Zbigniew K. Wszolek, MD; Ryan J. Uitti, MD; J. Eric Ahlskog, PhD, MD; Dennis W. Dickson, MD

- 8-17% of patients who pass away without ever having a PD symptom are discovered to have “pre-clinical PD
- iLB’s are found in various tissues in the nervous system.
Parkinson’s as we think about it now

Parkinson’s as a Multi-system Disease

The Parkinson’s Complex

- Substantia Nigra
- Pons
- Basal Forebrain
- Medulla
- Amygdala
- Hypothalamus
- Olfactory Bulb
- Spinal Cord (intermediolateral column)
- Peripheral Autonomic Nervous System (heart, intestinal track, bladder)
- Olfactory Cortex
- Temporal Cortex
- Neocortex

Langston, 2006
What triggers PD Pathology?

ETIOLOGY

Oxidative Stress

Inflammation

Protein Aggregation

Mitochondrial Dysfunction

Excitotoxicity

APOPTOSIS

Glutathione in PD

- GSH is the most abundant antioxidant in the brain and is selectively reduced in PD
- The magnitude of glutathione depletion correlates with severity of PD
- Earliest indicator of nigral degeneration
- Not decreased in other atypical parkinsonian syndromes
Glutathione Role in PD

• Removes reactive oxygen and nitrogen species
• Depletion results in reduced DA content, increased lipofuscin deposition and increased numbers of dystrophic axons in dopaminergic fibers, mitochondrial damage
• Glutathione levels cannot be restored by direct supplementation because glutathione crosses the blood brain barrier via a saturatable mechanism and is not taken up by neurons
Neuronal GSH synthesis:

- cysteine availability is rate-limiting for GSH synthesis
- most cell types obtain Cys-Cys (cystine) from the extracellular space, rather than free cysteine.
- but mature CNS neurons are different; neurons take up free cysteine itself, indirectly provided by astrocytes

GSH = glu-cys-gly
NAC

- Cell permeable precursor of cysteine that crosses the BBB, enters neurons and is capable of restoring GSH in a concentration dependent fashion
- Oral bioavailability is 9.1%
- NAC crosses mice BBB at 2.4L/g-min which is comparable to many centrally active peptides
- Already in clinical use
NAC is a membrane-permeable cysteine precursor.
Animal Data

- Two studies have shown that NAC is protective from MPTP-ism.
- Mice deficient in EAAC1 were shown to have decreased neuronal GSH content, increased neuronal oxidative stress, and widespread age-dependent neuronal loss. These mice showed a 42% loss of SN dopaminergic neurons over one year of life.
EAAC1 was originally classified as a glutamate transporter

$\text{Na}^+ \text{- dependent, concentrative Excitatory Amino Acid Transporters:}$

EAAT1 = GLAST - astrocyte specific
EAAT2 = GLT1 - astrocyte specific
EAAT3 = EAAC1 - neuron specific
EAAT4
EAAT5

The large majority of glutamate uptake in brain is performed by astrocytes.

- Unlike the other EAATs, EAAC1 is not clustered around synapses, and it has a 10-fold greater affinity for cysteine than for glutamate, suggesting that cysteine uptake is its primary role.
NAC protective

- When these mice were given oral NAC starting at age 3 weeks there was no loss of dopaminergic SNc neurons at age 12 months, reduced nitrotyrosine immunoreactivity in dopaminergic SNc neurons, and improved motor performance.
EAAC1−/− mouse brain slices:
Neurons show reduced capacity to scavenge ROS (DCF fluorescence)
Age-dependent loss of SNc dopaminergic neurons in EAAC1−/− mice
Reduced GSH content in EAAC1−/− brain neurons (C5-maleimide fluorescence)
NAC restores GSH content in EAAC1−/− brain neurons

wild type

EAAC1−/−

EAAC1−/− +NAC

EAAC1−/− +NAC+BSO

NAC+BSO

BSO

NAC

* *

GSH (μmol per mg protein)

0
0.5
1.0
1.5
2.0

Time (h)

0 4 6

0
500
1000
1500
2000
0h 4h 6h

NAC

BSO

NAC+BSO

0 4 6
Pole test
CSF (data expressed as mean±SE)  
* different from control

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NAC</th>
<th>NACA</th>
<th>GSH</th>
<th>One way Anova P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystine (CySS: μM)</td>
<td>0.67±0.13</td>
<td>1.01±0.06</td>
<td>1.34±0.15</td>
<td>2.41±0.48*</td>
<td>0.003</td>
</tr>
<tr>
<td>Cysteine (Cys: μM)</td>
<td>6.51±0.75</td>
<td>7.62±0.44</td>
<td>7.95±0.72</td>
<td>10.92±0.64*</td>
<td>0.003</td>
</tr>
<tr>
<td>CyS-GSH (μM)</td>
<td>1.30±0.31</td>
<td>1.05±0.26</td>
<td>1.18±0.26</td>
<td>2.15±0.51</td>
<td>0.160</td>
</tr>
<tr>
<td>GSH (μM)</td>
<td>6.15±0.31</td>
<td>5.95±0.24</td>
<td>4.77±0.18</td>
<td>9.81±1.33*</td>
<td>0.001</td>
</tr>
<tr>
<td>GSSG (μM)</td>
<td>1.71±0.56</td>
<td>1.26±0.19</td>
<td>1.16±0.13</td>
<td>2.75±0.53</td>
<td>0.059</td>
</tr>
<tr>
<td>Eh (GSSG/GSH)</td>
<td>-126.47±4.19</td>
<td>-127.93±2.74</td>
<td>-123.07±2.39</td>
<td>-130.38±3.06</td>
<td>0.455</td>
</tr>
<tr>
<td>Eh (CySS/Cys)</td>
<td>-123.92±0.56</td>
<td>-122.73±1.03</td>
<td>-120.13±1.19</td>
<td>-121.49±2.15</td>
<td>0.283</td>
</tr>
<tr>
<td>Total GSH</td>
<td>10.86±1.50</td>
<td>9.53±0.33</td>
<td>8.27±0.37</td>
<td>17.47±2.44*</td>
<td>0.003</td>
</tr>
<tr>
<td>Total Cys (μM)</td>
<td>9.19±1.28</td>
<td>10.69±0.73</td>
<td>11.82±1.15</td>
<td>17.89±1.37*</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Why do we need to rethink design

• Traditional Double-Blind Placebo trials are too large and costly for questionable agents (Creatine/CoQ10).
• Delayed Start Trials are large, costly, and exclude the use of patients on therapy with other agents (ADAGIO n=1176)
• Traditional Futility designs often rely on historic control, still require large numbers of patients and result in needless replication
Futility designs have relied on historic progression rates to determine modulation.

Problematic if your group progresses more rapidly or slowly than “historic controls.”

Calibration of appropriate rate of progression to compare active agent to occurs such that if the estimated increase in UPDRS scores from baseline to 24 weeks in the calibration group falls outside the 95% CI for the projected rate of historic controls (CoQ10 vs Creatine).
Which Historic Controls

- ELLDOPA trial database is open via PSG
- Rate of Progression for 24 weeks in the 300mg and 600mg treated groups is 5.12 UPDRS points
- Re-Calibration occurs if the estimated increase in UPDRS scores from baseline to 24 weeks in the calibration group falls outside the 95% CI for the projected rate of ELLDOPA progression
Non-Superiority??

- Based on ELLDOPA data and recommendations by the NET-PD investigators a 30% reduction in progression (5.12 vs 3.58).
- Something of an arbitrary cut-off
- Historically, futility (non-superiority is needed to keep “n” low)—CoQ10, GPI-1485, placebo (71, 71,71)
Linear Mixed Models and Power??

• For repeated continuous outcomes
• Makes use of interim UPDRS measures at weeks 2, 4, 8, and 16
• Better for patients who do not complete the study (vs. “last observation carried fwd)
• Significantly improves power and deals more accurately with disease progression (9 mos Ahlskog argument)
Conclusions

• Identification of appropriate agents requires advances in animal models
• Assessment of promising agents requires advances in trial design
• NAC may represent an agent worthy of further evaluation
thank you