Modeling Parkinson’s disease: systems to test gene-environment interactions

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Outline

- Parkinson’s disease (PD)
- Prominent models of PD
- New rotenone model
- Designing systems for testing gene-environment interactions
Parkinson’s disease (PD)  
Signs and Symptoms

- Motor
  - Akinesia/bradykinesia
  - Resting tremor
  - Rigidity
  - Gait disturbances

- Non-motor
  - Sleep disorders
  - Depression
  - Dementia
  - Olfactory deficits
  - Autonomic dysfunction

Table 2  
Incidence of psychosis, dementia and depression in a German cohort of PD patients [13]

<table>
<thead>
<tr>
<th>No Symptom</th>
<th>Psychosis</th>
<th>Dementia</th>
<th>Depression</th>
<th>% PD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Symptom</td>
<td>36 %</td>
<td>2 %</td>
<td>15 %</td>
<td>18 %</td>
</tr>
<tr>
<td>No Symptom</td>
<td>9 %</td>
<td>3 %</td>
<td>11 %</td>
<td>6 %</td>
</tr>
</tbody>
</table>

Ziemssen and Reichmann, 2007
PD-Pathology

• Loss of dopaminergic neurons of the substantia nigra
• Loss of dopaminergic terminals in the striatum
• Lewy bodies in surviving neurons
  – Contain α-synuclein
  – Other disease states
  – Other neuron types
  – Pathological role?


PD-Pathogenesis

Braak et al., 2004

• Progressive from lower brain stem to Cortex at latest stages
PD Etiology-Sporadic

• Sporadic
  – ~90-95%
  – Average onset at 60 (1% over 50)
  – Environmental exposures
    • Pesticides
    • Illicit drug use
    • Rural living
    • Rural drinking water consumption
    • Smoking is protective
  – Polymorphisms
    • Dopaminergic transmission
    • Protein aggregation/degradation
    • Xenobiotic metabolism
PD Etiology-Genetic

- **Autosomal dominant**
  - α-synuclein
    - Point mutations
    - Duplications
    - Triplications
  - Leucine-rich repeat kinase 2 (LRRK2)
    - >40 missense variants, 7 of them pathogenic,
    - including the common G2019S

- **Autosomal recessive**
  - Parkin
    - >100 mutations (point mutations, exon rearrangements)
  - PINK1
    - >40 point mutations, rare large deletions
  - DJ-1
    - >10 point mutations and large deletions

- Penetrance
  - Variable

- Animal models
  - Minimal phenotype

- Important conclusions
  - Genetic influences continue to be identified
  - Most cases likely result from a combination of environmental and genetic influences
  - Understanding such contributions could delay or prevent many cases
PD-Pathogenesis

- Mitochondrial complex I dysfunction
- Oxidative stress
- Iron overload
- Proteosome activity
- α-synuclein aggregation

Greenamyre and Hastings 2004
Prominent neurotoxic models of PD

- Major Models
  - MPTP
  - 6-OHDA
  - Rotenone
  - Paraquat

Betarbet et al., 2002
The rotenone model

• Natural compound found in roots of several plants
• Used as insecticide and to kill fish
• Highly lipophillic
• Complex I inhibitor
• Administered via osmotic pump
  – Intravenous
  – Subcutaneous
• At 2-3.0 mg/kg/day
  – Selective lesion to the nigrostriatal dopamine system
  – Does not effect cellular respiration – ATP production

Betarbet et al., 2000
Mechanism of action

Ricci et al., 2003

home.ncifcrf.gov/mtdp/Catalog/2dgif/26258.gif
• Limitations of the rotenone model
  • % of animals that exhibit a lesion
  • Distribution of the lesion
  • Location of the lesion
  • Magnitude
Potential sources of variability

• Rotenone potency/purity
  – Mass spec
    • Variability between vials
    • Variability in unidentified peaks
  – Complex I inhibition assay
    • No differences observed between vials

• Stability
  – Sonication
  – 37° C for 28 days

• Delivery
  – Osmotic pump delivery
  – Brain rotenone levels correlate with neurotoxicity
Does the rotenone model need to be improved?

All models of PD need improvement &
We need new models of PD

• For study of the pathological mechanisms of PD
  – This model has already provided a large amount of data

• For study of neuroprotection/potentiation pathways
  – Yes
    • Consistency
    • Stereology
    • Behavior
    • Statistical analysis
Research goals

• Develop an environmental PD model that can be used to assess neuroprotection and potentiation
• Test gene-environmental interactions
  – Protective pathways
  – Potentiation
    • Low dose environmental toxicant administration
    • Genetic background
Ip. rotenone 1.5, 2.5 mg/kg/day for 60 days

Fig. 1. Levels of DA and its metabolites DOPAC and HVA in the CPU and PFC tissues. Control group N = 10, compared with low dose (1.5 mg/kg) N = 9, medium dose (2.5 mg/kg) N = 7. Mann–Whitney U-test —*significant at P < 0.05; and **significant at P < 0.01, compared to respective control.
• Appears to produce a consistent deficit to the nigrostriatal dopamine system
• Pathology to nigrostriatal system or other brain areas has not been characterized
• Avoids invasive surgical procedure
• Rotenone administered daily

Mortality: ~33% at 2.5 mg, 50% at 3.0 mg

Alam and Schmidt. 2002. Behav Brain Res. 136: 317-324
Daily ip. rotenone

- 50x Stock made up at 150 mg/mL in 100% DMSO
- Diluted in a natural medium-chain triglyceride
- Final solution at 3.0 mg/mL in 98% miglyol 812 N, 2% DMSO; given at 1 mL/kg
- Regimen validation
- Peripheral complex I inhibition

Cannon et al., 2009
• Bilateral dopamine denervation results in severe motor deficits

• Temporal development of severe bradykinesia, postural instability, and rigidity

Cannon et al., 2009
Quantitative assessment of behavioral deficits
Rotenone-induced behavioral deficits

Woodlee et al., 2008

Cannon et al., 2009
Histological characterization of the lesion
Rotenone-induced dopamine terminal loss

<table>
<thead>
<tr>
<th>3.0 mg/kg/day</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>young-adult</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>D</td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>adult</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>F</td>
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<tr>
<td>F</td>
<td>G</td>
</tr>
<tr>
<td>G</td>
<td>H</td>
</tr>
<tr>
<td>middle-aged</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>J</td>
</tr>
<tr>
<td>J</td>
<td>K</td>
</tr>
<tr>
<td>K</td>
<td>L</td>
</tr>
</tbody>
</table>

Cannon et al., 2009
High magnification view of dystrophic neurites

Cannon et al., 2009
Specificity to dopaminergic terminals

Cannon et al., 2009
Specificity to dopaminergic terminals

Cannon et al., 2009
Striatal catecholamine alterations

Cannon et al., 2009
Unbiased stereological assessment of cell loss

Cannon et al., 2009
α-synuclein expression

Cannon et al., 2009
Aggregate formation

Cannon et al., 2009
Model Conclusions

• Dopamine-dependent behavioral deficits
• Consistent and reproducible lesions
• Quantifiable dopamine depletion and cell loss
• Other lines of investigation
  – GI dysfunction
  – Iron accumulation
  – Microglial activation
• Well-suited for use in neuroprotective and potentiation experiments
Gene-environment interactions

- Identification of new therapeutic approaches
  - PD gene modulation in the rotenone model
- Assessing increased toxicant sensitivity in transgenic animals
  - Rotenone exposure in transgenic rats
Transgene delivery

- PD related genes and goals
  - α-synuclein
    - shRNA knockdown
  - DJ-1
    - Express human in the rat brain
  - Parkin
    - Express human in the rat brain
  - Can we use what we know from genetic cases to develop treatments for sporadic cases?

- Viral delivery
  - System
    - Adeno-associated virus (AAV)
    - Lenti
    - Herpes

- Ultimate goal
  - Are these regimens protective in the rotenone model?
Viral vector reporter screen

- System
- Pseudotype
- Infusion parameters
  - Volume
  - Location
  - Rate
- Time-course
Transgene expression
Transgene expression in dorsolateral striatum

Unpublished results removed
Transgene expression: colocalization with dopamine neuron cell bodies and terminals

Unpublished results removed
α-synuclein shRNA
α-synuclein shRNA design and optimization

Unpublished results removed
α-synuclein shRNA plasmid

Unpublished results removed
Validation

• Restriction analysis
• Sequencing
  – Promoter, transgene, poly-a tail
• In vitro expression
α-Synuclein knockdown in vitro: CHO cells transfected with α-Synuclein shRNA plasmid

Unpublished results removed
Loss-of-function PD genes

Human transgene expression
DJ-1 plasmid

Unpublished results removed
DJ-1 expression after transfection

Unpublished results removed
Parkin plasmid

Unpublished results removed
Unpublished results removed
Current status

• For all three plasmids
  – AAV expression vectors in hand
  – Testing in vivo and in vitro

• Behavioral, neurochemical and histological effects to be tested
  – Alone
  – Under rotenone treatment

• Offers many advantages to transgenic animals
  – Regional
  – Temporal
Testing the role of genetic susceptibility to toxicants

• Incomplete penetrance of PD genes
• Could PD mutations render increased sensitivity to environmental toxicants?
• Transgenic rats
  – α-synuclein
    • A53T
    • E46K
Histological and neurochemical characterization of PD- transgenic rats
Human synuclein expression in transgenic rats

Unpublished results removed
Striatal neurochemistry: A53T and E46K vs. wild-type

Unpublished results removed
Conclusions

• Environmentally relevant model of PD in place
• Viral delivery optimized
• Examining the therapeutic potential of PD gene modulation in a ‘sporadic’ model
• Potential to examine relevant genetic susceptibility to environmental toxicants
• Lots of work to do!
Acknowledgements

• University of Pittsburgh
  – J. Timothy Greenamyre, M.D., Ph.D.
  – Ed Burton, M.D., Ph.D.
  – Laura Montero, B.S.

• Cornell
  – C.J. Li. Ph.D.
  – Kindiya Geghman, B.S.
References