Growth Factor Transplantation for the treatment of Parkinson’s disease

Barry Snow
Levodopa

Levodopa → Dopamine
Marked long-duration improvement and mild short-duration improvement in patient with moderate disease. Short-duration improvement occurs after peak plasma concentration of levodopa.
Levodopa

Dopamine
Deep Brain Stimulation
What we need:

- A way of encouraging the nigrostriatal neurones to re-grow
- At least restoration of dopamine storage and release
- Preferably reinstatement of neuronal connections.
BILATERAL FETAL MESENCEPHALIC GRAFTING IN TWO PATIENTS WITH PARKINSONISM INDUCED BY 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

Håkan Widner, M.D., Ph.D., James Tetrud, M.D., Stig Rehncrona, M.D., Ph.D., Barry Snow, M.D., Patrik Brundin, M.D., Ph.D., Björn Gustavii, M.D., Ph.D., Anders Björklund, Ph.D., Olle Lindvall, M.D., Ph.D., and J. William Langston, M.D.

Abstract. Background. Intracerebral transplantation of fetal dopaminergic neurons is a promising new approach for the treatment of Parkinson’s disease. Patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have a relatively stable lesion limited to the nigrostriatal system, rendering them ideal candidates for transplantation. Improvement of motor function after neural grafting has previously been observed in nonhuman primates with MPTP-induced parkinsonism.

Methods. We grafted human fetal tissue from the ventral mesencephalon (obtained six to eight weeks after conception) bilaterally to the caudate and putamen in two immunosuppressed patients with severe MPTP-induced parkinsonism, using a stereotaxic technique. The patients were assessed regularly with clinical rating scales, timed tests of motor performance, and $[^{18}F]$fluorodopa positron-emission tomography during the 18 months before the operation and the 22 to 24 months after the operation.

Results. Both patients had substantial, sustained improvement in motor function and became much more independent. Postoperatively, the second patient’s maintenance dose of levodopa was decreased to 150 mg daily, which was 30 percent of the original dose. Striatal uptake of fluorodopa was unchanged 5 to 6 months postoperatively but was markedly and bilaterally increased at 12 to 13 and 22 to 24 months in both patients, closely paralleling the patients’ clinical improvement. There were no serious complications.

Conclusions. Bilateral implantation of fetal mesencephalic tissue can induce substantial long-term functional improvement in patients with parkinsonism and severe dopamine depletion and is accompanied by increased uptake of fluorodopa by the striatum. The results in these patients resemble those obtained in MPTP-treated primates and suggest that this will be a useful model for the assessment of transplantation therapies in Parkinson’s disease. (N Engl J Med 1992;327:1556-63.)
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Results. Although the patients continued to improve, the postoperative clinical and functional improvements did not exceed those observed in the preoperative period. Postoperative [18F]fluorodopa positron emission tomography revealed no significant increase in striatal fluorodopa uptake.
NEUROPATHOLOGICAL EVIDENCE OF GRAFT SURVIVAL AND STRIATAL REINNERRVATION AFTER THE TRANSPANTATION OF FETAL MESENCEPHALIC TISSUE IN A PATIENT WITH PARKINSON’S DISEASE

JEFFREY H. KORDOWER, Ph.D., THOMAS B. FREEMAN, M.D., BARRY J. SNOW, M.D., FRANÇOIS J.G. VINGERHOETS, M.D., ELLIOTT J. MUFSON, Ph.D., PAUL R. SANBERG, Ph.D., ROBERT A. HAUSER, M.D., DONALD A. SMITH, M.D., G. MICHAEL NAUERT, M.D., DANIEL P. PERL, M.D., AND C. WARREN OLANOW, M.D.
Fetal ventral mesencephalon → Blastocyst → Fibroblasts → Neural stem/progenitor cells → Pluripotent cells (ES and iPS) → Tissue rich in dopaminergic neuroblasts → Dopaminergic neuron precursors/neuroblasts

Simon, Stott, Barker 2013
Central & Peripheral Nervous Systems

Neurotrophic factors as a therapeutic target for Parkinson’s disease

Jonathan R Evans† & Roger A Barker
†University of Cambridge, Cambridge Centre for Brain Repair, Department of Clinical Neurosciences, UK

Background: The search for therapeutic agents that might alter the disease course in Parkinson’s disease (PD) is ongoing. One area of particular interest involves neurotrophic factors (NTFs), with those of the glial cell line-derived neurotrophic factor (GDNF) family showing greatest promise. The safety and efficacy of these therapies has recently come into question. Furthermore, many of the key questions pertaining to such therapies, such as the optimal method of delivery, timing of treatment and selection of patients most likely to benefit, remain unanswered. Objective: In this review we sought to evaluate the therapeutic potential of NTFs in the treatment of PD. We
Expert Opinion

Central & Peripheral Nervous Systems

Neurotrophic factors as a therapeutic target for Parkinson’s disease

Jonathan R Evans† & Roger A Barker
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Objective: In this review we sought to evaluate the potential of NTFs in the treatment of PD. We...
GDNF

- Complex delivery system
- Single growth factor
- Rapid transition to pivotal study
  - Different protocol to unblinded studies
  - ?under powered
## Microarray Analysis of Choroid Plexus Neurotrophic Genes Expression

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<thead>
<tr>
<th>Name</th>
<th>Expression Index</th>
<th>Rank/24000</th>
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<tr>
<td><strong>TGFbeta 1</strong></td>
<td>12.6</td>
<td>240</td>
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<tr>
<td><strong>IGF-2</strong></td>
<td>11.8</td>
<td>528</td>
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<tr>
<td><strong>VEGF</strong> (confirmed by ELISA)</td>
<td>10.29</td>
<td>2097</td>
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<tr>
<td><strong>EGF</strong></td>
<td>9.04</td>
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<td><strong>IGF-1</strong></td>
<td>7.92</td>
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<tr>
<td><strong>FGF-2</strong></td>
<td>6.93</td>
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<tr>
<td><strong>Growth hormone</strong></td>
<td>6.39</td>
<td>13317</td>
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<tr>
<td><strong>BMPs</strong></td>
<td>5.24</td>
<td>16628</td>
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<tr>
<td><strong>Nerve growth factor</strong></td>
<td>4.79</td>
<td>18152</td>
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<tr>
<td><strong>BDNF</strong> (confirmed by ELISA)</td>
<td>4.31</td>
<td>19716</td>
</tr>
<tr>
<td><strong>FGF-2</strong></td>
<td>6.93</td>
<td>11758</td>
</tr>
<tr>
<td><strong>TGFbeta 3</strong></td>
<td>6.76</td>
<td>12272</td>
</tr>
<tr>
<td><strong>FGF</strong></td>
<td>5.267</td>
<td>16726</td>
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<tr>
<td><strong>Nerve growth factor</strong></td>
<td>4.79</td>
<td>18152</td>
</tr>
<tr>
<td><strong>NT-3</strong> (LOWEST)</td>
<td>3.98</td>
<td>20869</td>
</tr>
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</table>
Rat brain at 6 Months

(calcein)

Skinner 2009
porcine endogenous retrovirus (PERV)

- highly prone to recombination events
- capable of intracellularly moving within the pig genome as retrotransposons
- remote possibility of recombination with human retroviruses, including human endogenous retroviruses

- Cytomegalovirus
- lymphotrophic herpes virus
- encephalomyocarditis virus
- hepatitis E
- circo-viruses
Released 1807 by Captain Abraham Bristow of the *Sarah*
High Health Status Pigs:
• US FDA Guidelines 2003
• Low copy numbers for PERV
• No gene marker for locus required for recombination PERV-C with PERV-A
• No demonstrated transmission of PERV

Molecular Diagnostic Laboratory
• International Accreditation New Zealand certified

Manufacturing Plant:
• GMP certification annual recertification and audit
• Cell Processing
• Encapsulation Technology
• Toxicity data
• Quality Certified Encapsulated Product

Preclinical Data
• Safety and efficacy studies in animals:

Monitoring
• Safety monitoring for donors and recipients
## Acceptance Criteria

<table>
<thead>
<tr>
<th>Free Clusters of Choroid Plexus Cells</th>
<th>Acceptance Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yield Clusters/CP</strong></td>
<td>≥ 40,000</td>
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<tr>
<td><strong>Cluster Size (new)</strong></td>
<td>≥ 65 µm</td>
</tr>
<tr>
<td><strong>Viability</strong></td>
<td>≥ 95%</td>
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<tr>
<td><strong>DNA pg/cluster</strong></td>
<td>≥ 500</td>
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<tr>
<td><strong>Functional Assay pg/µg DNA/day</strong></td>
<td>≥ 100</td>
</tr>
<tr>
<td><strong>Purity (new)</strong></td>
<td>Preliminary &gt; 90%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean Capsule Diameter (µm)</th>
<th>600-700</th>
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<tbody>
<tr>
<td><strong>Capsule Size Uniformity</strong></td>
<td>≥ 90%</td>
</tr>
<tr>
<td><strong>Capsule Integrity</strong></td>
<td>≥ 90%</td>
</tr>
<tr>
<td><strong>CPE Viability</strong></td>
<td>≥ 90%</td>
</tr>
<tr>
<td><strong>% Capsules with clusters</strong></td>
<td>≥ 80%</td>
</tr>
<tr>
<td><strong>Functional Assay pg/µg DNA/day</strong></td>
<td>≥ 100</td>
</tr>
<tr>
<td><strong>Bacteriology and Mycology</strong></td>
<td>Interim</td>
</tr>
<tr>
<td></td>
<td>No growth after 7 days</td>
</tr>
<tr>
<td></td>
<td>Final</td>
</tr>
<tr>
<td></td>
<td>No growth after 14 days</td>
</tr>
<tr>
<td><strong>Mycoplasma</strong></td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Endotoxin</strong></td>
<td>&lt; 1 EU/mL</td>
</tr>
<tr>
<td><strong>Release for Dispensing</strong></td>
<td>d20</td>
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<tr>
<td><strong>Capsule Integrity</strong></td>
<td>≥ 90%</td>
</tr>
<tr>
<td><strong>CPE Viability</strong></td>
<td>≥ 90%</td>
</tr>
<tr>
<td><strong>In-process sterility compliance</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Final Product Sterility</strong></td>
<td></td>
</tr>
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</tbody>
</table>
Porcine pancreatic islet cells for diabetes

• 35 subjects
• No persisting adverse events
• No infectious complications
• Modest clinical improvement at best
• Large amounts of tissue to produce the expected insulin output
Animal neurological studies

- Rat stroke
- Rat QA HD
- Monkey QA HD
- Rat 6OHD PD
- Monkey MPTP PD
6OHDA Rats

Circling

Skinner 2011
% Change of turns vs. Weeks post implant

- CP cell capsules
- Empty capsules
- Sham

Pig cells for PD

Based on a solid background:

- Many transplant studies (well developed assessment protocols)
  - Adrenal
  - Foetal
  - Gene therapy
- Pig cells used before in PD
- Previous Growth factor studies
  - GDNF
- Patients already accept burr holes and brain probes for DBS
- Sham surgery for PD established
- LCT encapsulated cells well tolerated in diabetes
Auckland Phase I safety Study with efficacy observations

Patients screened and selected for DBS

Offered recruitment to transplant study (n=4)

Clinical assessment
Vancouver PET

Unilateral putaminal transplant NT encapsulated cells

Observe 6 months

Clinical assessment
Vancouver PET

Excellent response
Keep observing

Partial response
Contralateral transplant

No response
DBS
Oversight

Medsafe approval
  Clinical studies
  GMP
Minister of health
Ethics
ADHB RRC
MAB
  Haaken Widner, Roger Barker, Tom Foltyn, David Eidleberg
DSMB
  Rod Ellis-Pegler, Tim Anderson, Andrew Hughes
Right to publish
Nil COI
Enhanced consent process
Where we will go with the data:

• Retain the option to do exploratory studies
  – Dose ranging
  – Bilateral
  – Earlier disease

• Then move to pivotal sham-controlled trial
Issues to address

• Surgical complications
  – Less than DBS
• Inflammatory reaction
• Infection
• Runaway dyskinesias
  – no serotonergic activity
• The generalised degeneration of PD
• Oncogenicity
Tumourgenicity – Human PD Trials


- 40 patients 34-75 years of age with severe PD
- Bilateral transplantations of cultured mesencephalic tissue from 4 embryos into the putamen
- In 15% of patients that received transplants, dystonia and dyskinesia's reoccurred even after reduction/discontinuation of L-DOPA
- 2 patients underwent post-mortems
  - #1 – Traffic accident 7 mths into the trial,
  - #2 – Myocardial infarction 3 years after transplantation
  - No evidence of tumour growth
  - Evidence of fiber outgrowth from the transplant—excess dopamine
- No reported tumours during 1 year follow up
Tumourgenicity – Human PD Trials


- Unilateral implantation of porcine embryonic neural cells into the caudate and putamen in combination with immunosuppressants (cyclosporine)

- Implantations well tolerated for advanced PD at 1 year

- No evidence of transmission of porcine pathogens or PERV

- Clinical benefit variable
  - Small amount of tissue
  - Immunosuppressants may be useful for porcine xenograft survival

- No reported evidence of tumour growth in 12 patients assessed over 1 year
Tumourgenicity – LCT Rodent Studies

• Historical longevity rat study (2007)
  – Implantations of NT CELL capsules for 2-6mths into Sprague Dawley Rats
  – Histological section analysed and showed “absence of any neoplastic proliferative changes”
    – Alistair Johnson (Gribbles Veterinary)

• Longevity study currently in progress (2011-13)
  – 1-18 month duration
  – Implantation of 10 NT CELL capsules into each hemisphere
  – Subjects: Normal Male Sprague Dawley Rats
  – Currently up to 10 mth time point
  – One hemisphere fixed in 4% PFA for histopathology
  – Histopathology report being obtained
    • Gross pathology
    • Signs of inflammation
    • Any signs of tumour / neoplasia – present or absent.
    • Vessel growth – normal / abnormal
  – Specific markers are useful if any abnormal structures are observed and we wish to identify
    specific tumour types
What is the potential future for this treatment?

• Potentially of use for all people with PD
  – Incidence 10/100,000/year
• Early treatment
• Treatment centres
Correlation of Striatal Fluorodopa Uptake in the MPTP Monkey with Dopaminergic Indices

Brian D. Pate, PhD,* T. Kawamata, MD,† T. Yamada, MD,† E. G. McGeer, PhD,*† K. A. Hewitt,* B. J. Snow, MD,* T. J. Ruth, PhD,* and D. B. Calne, MD*

Striatal $^{18}$F-6-fluorodopa (FD) uptake constants were measured by positron emission tomography in (1) normal cynomolgus monkeys and (2) a series of cynomolgus and rhesus monkeys that had received intracarotid infusions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). After the animals were killed, the number and average size of dopaminergic neurons in the substantia nigra pars compacta were measured. Striatal levels of dopamine and its metabolites, and the striatal activities of the dopaminergic synthetic enzymes, were also determined. The striatal FD uptake constants showed highly significant positive correlations with both number and size of dopaminergic neurons, indicating atrophy of surviving neurons in MPTP-treated animals. The uptake constants also showed significant positive correlations with striatal levels of dopamine, total catecholamines, and the activities of the synthetic enzymes. Both histochemical and biochemical data on tyrosine hydroxylase suggested some contralateral enzyme loss in these MPTP-treated monkeys, as well as decreased enzyme activity in surviving neurons on the lesioned side. However, residual enzyme activities were apparently not rate limiting to striatal FD uptake. It is concluded that PET-FD measurements by positron emission tomography provide a good index of the integrity of the nigrostriatal pathway.

Effects of MPTP and CP treatment on TH density in the putamen

Figure 4
Group
R/L ratio of TH density

The MPTP lesion and CP implantation on dopamine neurons in the SN

The number of TH-positive neurons

Substantia nigra

Left
Right

Sham
Control
Treated

Effects of MPTP and CP treatment on TH density in the putamen

Sham
Control
Treated

R/L ratio of TH density