Parkinson’s Disease: New Concepts and Future Treatments

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In addition, Dr. Hauser has consulted in litigation with lawyers representing various current and former manufacturers of welding consumables.
**BRAIN REGIONS** affected physically or functionally by Parkinson's disease are highlighted. The pars compacta region of the substantia nigra (dark area in detail) loses neurons that normally issue motion-controlling signals (arrows) to the striatum in the form of the naturally occurring chemical dopamine. Striatal neurons relay the messages to higher motor centers (gray).

Death of the nigral neurons lowers dopamine levels and thereby disrupts the circuit and, in turn, a patient's motor control. Dopamine-producing neurons outside the substantia nigra are not harmed much, but areas that lose other kinds of neurons, such as the raphe nuclei and locus ceruleus, contribute to depression and to additional nonmotor manifestations of the disorder.
Normal: section through cerebral peduncles and substantia nigra.

Parkinson's disease: substantia nigra depigmented.

Lewy inclusion bodies in cell of substantia nigra in Parkinson's disease; may also appear in locus ceruleus and tegmentum, cranial motor nerve nuclei, and peripheral autonomic ganglia.

Neurofibrillary tangle in nerve cell of substantia nigra as seen in postencephalitic parkinsonism, progressive supranuclear palsy, and parkinsonism-dementia complex.

Section of substantia nigra of normal animal; treatment of section with formaldehyde vapor causes formation of polymers with monamines (dopa and norepinephrine) which fluoresce to bright green under ultraviolet light.

PARKINSONISM: PATHOLOGIC CHANGES
PD Diagnosis

• 2 of 3 cardinal features
  – Bradykinesia
  – Rigidity
  – Rest tremor

• In addition:
  – Asymmetry
  – Substantial and sustained response to dopaminergic medications (under the right circumstances)
Imaging:
DatScan
 Imaging in the brain: Molecular targets of radioligands.\(^7\)

- **DOPA** → **dopamine**
- Neuronal dopamine metabolism (fluorodopa)
- Dopamine transporter (\(\beta\)-CIT, others)
- Presynaptic
- Postsynaptic

Adapted from Science 2000; 289: 409-411.
The new view of PD
Mapping of a Gene for Parkinson's Disease to Chromosome 4q21-q23.
Polymeropoulos, Mihael; Higgins, Joseph; Golbe, Lawrence; Johnson, William; Ide, Susan; Di Iorio, Giuseppe; Sanges, Giuseppe; Stenroos, Edward; Pho, Lana; Schaffer, Alejandro; Lazzarini, Alice; Nussbaum, Robert; Duvoisin, Roger


Figure 1. A large family with PD.
Lewy body–like pathology in long-term embryonic nigral transplants in Parkinson’s disease

Jeffrey H Kordower¹, Yaping Chu¹, Robert A Hauser², Thomas B Freeman³ & C Warren Olanow⁴

Fourteen years after transplantation into the striatum of an individual with Parkinson’s disease, grafted nigral neurons were found to have Lewy body–like inclusions that stained positively for α-synuclein and ubiquitin and to have reduced immunostaining for dopamine transporter. These pathological changes suggest that Parkinson’s disease is an ongoing process that can affect grafted cells in the striatum in a manner similar to host dopamine neurons in the substantia nigra. These findings have implications for cell-based therapies and for understanding the cause of Parkinson’s disease.
Figure 2 Parkinson’s disease–like pathology in long-term nigral grafts.
In the nongrafted host’s nigra, typical α-synuclein (a) and ubiquitin (b) neuropathology was observed. (c) Extensive α-synuclein pathology was seen in grafted neurons, including cytoplasmic and aggregated α-synuclein (arrows) as well as α-synuclein neurites (arrowheads). (d,e) Pathological aggregates of ubiquitin were also seen in grafted neurons (arrows). Scale bar, 40 μm. All people studied in this manuscript gave their informed consent to participate in transplant studies approved by the Institutional Review Board at the University of South Florida at Tampa. Proper informed consent was subsequently obtained for brain donation.
Schematic of environmentally dependent secondary structural changes for α-synuclein. Unstructured in buffer (left), α-helical in the presence of membranes (middle), and β-sheet in amyloid fibrils (right).
Figure 1  $\alpha$-Synuclein aggregation process. (a) In certain conditions, the natively unfolded $\alpha$-syn monomers are able to self-aggregate in soluble oligomers and then to form typical amyloid fibrils with $\beta$-sheet structure. These different species of $\alpha$-syn assemblies coexist in a highly dynamic equilibrium. (b) $\alpha$-Syn fibrillization is a nucleation-dependent process. Addition of exogenous preformed fibrils (red) speeds up the conversion of monomers to fibrils. The color reproduction of this figure is available on the html full text version of the manuscript.
What’s happening in PD?

• Propogation of abnormal aggregation of alpha-synuclein
  – Synuclein mutations
  – Too much synuclein
  – Problems with clearing synuclein

• Decreased energy state in neurons

• Inflammation
Olfaction

• A high prevalence of decreased olfactory function is present in Parkinson’s disease patients

• Many patients report a loss or change in their sense of smell years before the onset of parkinsonism

• Corresponds with Braak Stage I

REM Sleep Behavior Disorder

- Schenck et al (1996) followed a group of men over age 50 with RBD and reported that almost 40% went on to develop Parkinson’s disease.

- The average interval between onset of RBD and the appearance of parkinsonism was 13 years.

Autonomic Nerve Involvement

• There are Lewy body-like aggregates of alpha-synuclein in neurons that go to the heart and gut as well.
FIG. 1. α-SYN immunohistochemistry of colon at 40x magnification (A, C, E) and 120x magnification (B, D, F). Nerve fiber staining was seen in PD subjects (A, B). Some subjects with inflammatory bowel disease showed a pattern of immunostaining in round cells of unknown origin (C, D). Controls and most subjects with inflammatory bowel disease showed no α-synuclein immunostaining (E, F). Scale bar in E and F represents 50 and 20 μm and applies to A, C, E and B, D, F, respectively.
Incidence of Parkinson’s Disease by Frequency of Bowel Movements in Midlife

Test for trend: $P=.005$.

Cardiac Sympathetic Denervation

• More than 20 studies have agreed that virtually all patients with PD have a loss of sympathetic innervation of the heart

• Documented by heart imaging studies
  – Sympatho-neuronal imaging agents $^{123}$I-MIBG and 6-$^{[18F]}$fluorodopamine

• Also demonstrated by neurochemical assessments during catheterization

• Indicates that peripheral sympathetic nervous system involvement is a universal, possibly early feature of the disease

“Pre-Motor PD”

• Early Features
  – Hyposmia
  – REM behavior disorder
  – Cardiac Sympathetic Denervation

• Midlife Risk Factors or Very Early Features?
  – Constipation
  – Sleepiness
The Future of PD: earlier diagnosis

Can we identify individuals who have pre-motor Parkinson’s disease?

– Smell Test
– Dopamine brain scan
– EKG
– Colon biopsy
The Future of PD: biomarkers
The Parkinson’s Progression Markers Initiative (PPMI)

Parkinson’s Disease and Movement Disorders Center
Biomarker

• Diagnose
• Evaluate progression of disease
PPMI

- **Subjects**
  - 400 *de novo* PD subjects (“possible PD” with positive DaTSCAN and unmedicated for at least 6 months)
  - 200 age- and gender-matched healthy controls
  - Followed for a minimum of 3 years and a maximum of 5 years

- **Assessments**
  - Motor assessments (new UPDRS)
  - Neuropsychiatric / cognitive testing
  - Olfaction
  - DaTSCAN imaging, MRI

- **Biologics**
  - DNA collected at screening
  - Serum and plasma collected at each visit
  - Urine collected annually
  - CSF collected at baseline, 6 months, 12 months and annually thereafter
Preliminary Discoveries of Promising PD Biomarkers

CSF Alpha-synuclein is reduced in PD subjects

(Mollenhauer et. al, 2008)

Plasma DJ-1 is elevated in PD subjects and increases with the progression

(Waragai et. al, 2007)
P-PPMI
P-PPMI

• Hyposmics
• RBD
• LRRK2
The Future of PD: improving motor symptom treatment
Levodopa

- Levodopa remains the most effective treatment for motor features of PD
- BUT, its use is associated with the development of
  - Fluctuations
  - Dyskinesias
Levodopa/Carbidopa

- $T_{1/2}$ of levodopa alone is 60 minutes
- $T_{1/2}$ of levodopa/carbidopa is 90 minutes
Synthesis of Dopamine From Levodopa in Presynaptic Neuron
Change in Clinical Response Over Time

**EARLY PARKINSONS DISEASE**

- Clinical response
- Serum levodopa (up arrows)

**MODERATE PARKINSONS DISEASE**

- Clinical response
- Serum levodopa (up arrows)

**ADVANCED PARKINSONS DISEASE**

- Clinical response
- Serum levodopa (up arrows)
- Dyskinesia

With permission from R. Hauser
The Future of PD: Levodopa

- Infusion Pump
- Long-acting oral preparations
- Levodopa prodrugs
- Inhaled levodopa
Duodopa

- Levodopa-carbidopa intestinal gel
- Gel is infused via a PEG tube in the abdomen and an external pump.
- Approved for use in Europe and has been shown to decrease motor fluctuations and dyskinesias.
- Phase 3 studies are now completed
Impax IPX066 (Rytary)

• A new extended release oral formulation of carbidopa/levodopa
• In an open label study, diary data indicated that IPX066 provided 2.00 hours greater reduction in OFF time (P<0.0001) compared with CD-LD IR.
• Phase 3 studies now completed.

Hsu et al AAN 2010
Xenoport XP21279

- Levodopa prodrug
- XP21279 is designed to target natural nutrient transporter mechanisms expressed throughout the length of the GI tract, including the colon.
- Once absorbed, XP21279 is rapidly converted to L-Dopa by the body's endogenous enzymes.
Inhaled levodopa

• Dry powder L-dopa aerosol that utilizes a breath actuated device (ARCUS™ respiratory delivery platform) to deliver a precise dose to the deep lung for rapid, predictable and consistent therapeutic onset

• Being developed as an adjunct PRN therapy to standard oral L-dopa therapy to address the OFF episodes as they emerge and enable patients to reliably control their symptoms

• In normal healthy volunteers, therapeutic plasma levels of LD were achieved within 5 minutes of inhalation

• Now in clinical trial for PD patients with fluctuations on oral levodopa
The Future of PD:
Other medications to improve symptoms

• A2a antagonists
  – Kyowa: istradefylline
  – Biotie: tozadenant
A2a antagonists

- Non-dopamine medications
- May reduce OFF time in patients with fluctuations on levodopa
- Could possibly prevent or reduce development of dyskinesia (unknown)
The Proposed Mechanism of Antiparkinsonian Activity of KW-6002 Restoration of Balance in the GABAergic Output Pathways from the Striatum

**Normal**

- CORTEX → STRIATUM (GABAergic)
- STRIATUM → GP (A2A)
- GP → SNc (dopamine)
- SNc → SNr/GPi (GABAergic)
- SNr/GPi → STN (GABAergic)
- STN → THALAMUS

**PD**

- CORTEX → STRIATUM (GABAergic)
- STRIATUM → GP (A2A)
- GP → SNc (dopamine)
- SNc → SNr/GPi (GABAergic)
- SNr/GPi → STN (GABAergic)
- STN → THALAMUS

**PD + KW-6002**

- CORTEX → STRIATUM (GABAergic)
- STRIATUM → GP (A2A)
- GP → SNc (dopamine)
- SNc → SNr/GPi (GABAergic)
- SNr/GPi → STN (GABAergic)
- KW-6002 (A2A)
- STN → THALAMUS

Key:
- Red arrows: excitatory
- Blue arrows: inhibitory
Role of mGluR5 receptors in dyskinesia

mGluR5 antagonism might exert anti-dyskinetic actions through reduction in levodopa-induced phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1/2), and mitogen-and-stress activated kinase 1 (MSK-1).

Rylander et al. J Pharmacol Exp Ther 2009; 330: 227-235
The Future of PD: slowing disease progression
Medications that are being investigated as to whether they slow progression of the disease

- Isradipine
- Pioglitazone
- Nicotine Patch
‘Rejuvenation’ protects neurons in mouse models of Parkinson’s disease

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Why dopamine-containing neurons of the brain’s substantia nigra pars compacta die in Parkinson’s disease has been an enduring mystery. Our studies suggest that the unusual reliance of these neurons on L-type Caᵥ1.3 Ca²⁺ channels to drive their maintained, rhythmic pacemaking renders them vulnerable to stressors thought to contribute to disease progression. The reliance on these channels increases with age, as juvenile dopamine-containing neurons in the substantia nigra pars compacta use pacemaking mechanisms common to neurons not affected in Parkinson’s disease. These mechanisms remain latent in adulthood, and blocking Caᵥ1.3 Ca²⁺ channels in adult neurons induces a reversion to the juvenile form of pacemaking. Such blocking (‘rejuvenation’) protects these neurons in both in vitro and in vivo models of Parkinson’s disease, pointing to a new strategy that could slow or stop the progression of the disease.
Juvenile Dopamine Neuron

Na channels used for pacemaking
With aging, Ca channels are expressed and take over pacemaking function. Ca may enter mitochondria and decrease energy state making them vulnerable.
Adding a Calcium Channel Blocker

When a calcium channel blocker is added, Na channels regain pacemaking function, the energy status improves, and dopamine neurons become more resistant to toxins.

Isradipine, a calcium channel blocker that enters the brain is now being evaluated as a possible neuroprotective agent.
Pioglitazone
The PPAR-γ agonist pioglitazone modulates inflammation and induces neuroprotection in parkinsonian monkeys

Christine R Swanson¹², Valerie Joers¹², Viktorya Bondarenko¹, Kevin Brunner¹, Heather A Simmons¹, Toni E Ziegler¹, Joseph W Kemnitz¹²³, Jeffrey A Johnson¹²⁴ and Marina E Emborg¹²⁵*

Abstract

Background: Activation of the peroxisome proliferator-activated receptor gamma (PPAR-γ) has been proposed as a possible neuroprotective strategy to slow down the progression of early Parkinson’s disease (PD). Here we report preclinical data on the use of the PPAR-γ agonist pioglitazone (Actos®, Takeda Pharmaceuticals Ltd.) in a paradigm resembling early PD in nonhuman primates.

Methods: Rhesus monkeys that were trained to perform a battery of behavioral tests received a single intracarotid arterial injection of 20 ml of saline containing 3 mg of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Twenty-four hours later the monkeys were assessed using a clinical rating scale, matched accordingly to disability, randomly assigned to one of three groups [placebo (n = 5), 2.5 (n = 6) or 5 (n = 5) mg/kg of pioglitazone] and their treatments started. Three months after daily oral dosing, the animals were necropsied.

Results: We observed significant improvements in clinical rating score (P = 0.02) in the animals treated with 5 mg/kg compared to placebo. Behavioral recovery was associated with preservation of nigrostriatal dopaminergic markers, observed as higher tyrosine hydroxylase (TH) putaminal optical density (P = 0.011), higher stereological cell counts of TH-ir (P = 0.02) and vesicular monoamine transporter-2 (VMAT-2)-ir nigral neurons (P = 0.006). Stereological cell counts of Nissl stained nigral neurons confirmed neuroprotection (P = 0.017). Pioglitazone-treated monkeys also showed a dose-dependent modulation of CD68-ir inflammatory cells, that was significantly decreased for 5 mg/kg treated animals compared to placebo (P = 0.018). A separate experiment to assess CSF penetration of pioglitazone revealed that 5 mg/kg p.o. induced consistently higher levels than 2.5 mg/kg and 7.5 mg/kg. p.o.

Conclusions: Our results indicate that oral administration of pioglitazone is neuroprotective when administered early after inducing a parkinsonian syndrome in rhesus monkeys and supports the concept that PPAR-γ is a viable target against neurodegeneration.
Pioglitazone

- Clinical trial currently enrolling
Nicotine Patch

• Protects dopamine neurons in animal studies
• Currently in clinical trial
Other Types of Symptomatic Medications
Droxidopa

- Under investigation to treat low/falling blood pressure
- Some data indicates that it may reduce falls
Other Trials

- rotigotine patch – apathy, motivation, mood, fatigue?
- rasagiline – thinking and memory
A Novel “Molecular Tweezer” Inhibitor of α-Synuclein Neurotoxicity in Vitro and in Vivo

Shubhangi Prabhudesai · Sharmistha Sinha · Aida Attar · Aswani Kotagiri · Arthur G. Fitzmaurice · Ravi Lakshmanan · Magdalena I. Ivanova · Joseph A. Loo · Frank-Gerrit Klärner · Thomas Schrader · Mark Stahl · Gal Bitan · Jeff M. Bronstein

Summary  Aggregation of α-synuclein (α-syn) is implicated as being causative in the pathogenesis of Parkinson’s disease, multiple system atrophy, and dementia with Lewy bodies. Despite several therapies that improve symptoms in these disorders, none slow disease progression. Recently, a novel “molecular tweezer” (MT) termed CLR01 has been described as a potent inhibitor of assembly and toxicity of multiple amyloidogenic proteins. Here we investigated the ability of CLR01 to inhibit assembly and toxicity of α-syn. In vitro, CLR01 inhibited the assembly of α-syn into β-sheet-rich fibrils and caused disaggregation of pre-formed fibrils, as determined by thioflavin T fluorescence and electron microscopy. α-Syn toxicity was studied in cell cultures and was completely mitigated by CLR01 when α-syn was expressed endogenously or added exogenously. To determine if CLR01 was also protective in vivo, we used a novel zebrafish model of α-syn toxicity (α-syn-ZF), which expresses human, wild-type α-syn in neurons. α-Syn-ZF embryos developed severe deformities due to neuronal apoptosis and most of them died within 48 to 72 h. CLR01 added to the water significantly improved zebrafish phenotype and survival, suppressed α-syn aggregation in neurons, and reduced α-syn-induced apoptosis. α-Syn expression was found to inhibit the ubiquitin proteasome system in α-syn-ZF neurons, resulting in further accumulation of α-syn. Treatment with CLR01 almost completely
Fig. 1  Structure of the molecular tweezers CLR01 and CLR03.
Fig. 3 CLR01 inhibits α-synuclein (α-syn) toxicity in cell culture. (a) CLR01 inhibits endogenously expressed α-syn toxicity in HEK293 cells. α-Syn expression was induced by adding doxycycline (Dox) in the absence or presence of CLR01. Cell numbers and cell death measured using propidium iodide were determined by flow cytometry (N=12 per condition; *p < 0.0003; **p < 0.007). (b) Differentiated PC-12 cells were treated with 20 μM α-syn incubated in the absence or presence of increasing concentrations of CLR01 for 48 h and cell viability was measured using the MTT assay. Inset: Viability of PC-12 cells treated similarly with α-syn in the presence or absence of 10-fold molar excess of CLR01 or CLR03. The data are an average of at least 3 independent experiments with 6 wells per condition. Not significant (NS) = xxx; PI = xxxx.
Fig. 5 CLR01 ameliorates α-synuclein (α-syn) neurotoxicity in zebrafish (ZF).
(a) ZF embryos were treated with CLR01 at 8 hpf and were monitored for abnormal appearance and survival. Bright-field and fluorescent overlay images were taken at 72 hpf (top). Green bars represent normal-appearing embryos and red bars represent abnormal embryos (N=132/condition). (b) CLR01 prevents α-syn-induced apoptosis. ZF embryos expressing DsRed or α-syn were incubated in acridine orange 24 hpf and apoptotic cells were counted (N=6 per condition). Ten μM CLR01 reduced α-syn-induced apoptosis to control levels (*p<0.007 Syn-DsRed vs Syn-DsRed/CLR01 and DsRed control). Representative images are shown on the right.
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