PD Update
(Parkinson’s is a synucleinopathy)

Bill Stamey, M.D.
Mid Coast Medical Group Neurology
Brunswick, Maine
April, 2016
Outline

• This slide show is from the talk given in Portland, Maine, April, 2016 and is an attempt to address part of:
  – What causes PD
  – New ways to diagnose PD
  – New treatment trials
The brain is made up of cells called neurons

Neurons are very specialized and groups of neurons form different functional areas.
Neurons communicate along the axon

Neurons communicate via a long branch called an axon.
And meet at the synapse

The axon ends at the synapse.
Signals go to the next neuron via the dendrite

The synapse passes a signal to the next neuron via the dendrite.
Neurons with Lewy bodies (LB)

We have known since 1912 that there is an abnormal inclusion in the neurons of PD patients called a Lewy body (LB). The LB is the pathologic hallmark of PD, and is present in neurons only in certain locations. This discovery opened the door to understanding PD. A huge amount of work has been done on the formation, the contents, the meaning of LBs.

Lewy FH. Zur pathologischen anatomie der Paralysis Agitans. Dtsh Z Nervenheilk. 1913;50:50-55
In 2003 Heiko Braak, a German pathologist, made a very important observation that changed the way we think about disease. He showed that in PD LBs are first seen in lower parts of the brain and spread upwards to higher regions. He described this upward spread in stages, 1 through 6.

Braak stage I

Also, LB are in the medulla, or bottom of the brainstem at the dorsal motor nucleus (DMN) of vagus.

The vagus is a very long nerve that connects with the gut. Involvement here is associated with constipation. In both cases the nerves are easily exposed to the outside environment whether through the nose or through the gut, and Dr. Braak questioned whether this exposure, coupled with the spread of LB from cell to cell meant PD patients were infected by some sort of virus.
Braak stage II

LB spread up to the pons which regulates sleep, wakefulness, and is associated with the syndrome of REM behavior disorder.


LB are found in the midbrain substantia nigra where dopamine is made. As LB accumulate here, inflammation occurs and neurons are lost.
Braak stage III

Braak stage IV

LB are found in the BG, where dopamine is used and stored.


more limbic structures and cortex contain LBs, associated with mood and higher cortical function.
Striatum implant

We can apply Dr. Braak’s findings to a group of PD patients who underwent brain tissue implant beginning in 1990.

These patients were implanted with healthy neurologic tissue with the goal of making dopamine and correcting parkinsonism.

For several years the grafts survived and patients showed sustained benefit. Some of the patients did so well that they did not require dopamine containing medication during that time.
Lewy bodies in grafted neurons up to 16 years later

However, by 2008 some of the patients had expired and brains were examined revealing the presence of LBs in grafted neurons.
Lewy bodies in grafted neurons

Researchers concluded that LB had spread from the patients’ unhealthy neurons to the transplanted healthy neurons without any virus in evidence.

Cell-to-cell spread of Lewy bodies

It seemed that normal proteins were warped by diseased proteins resulting in LBs, akin to one bad apple spoiling the bunch.
Lewy bodies are collections of abnormal proteins

So, where do these LB come from?
LB are made up of proteins. Let’s start with that.
Your cells contain DNA
DNA contains genes which code for amino acids.
Amino acids string together like links in a chain to form proteins

There are many different types of proteins
Individual proteins have special properties
Proteins form specific 3D shapes unique to the type of protein
Amino acids $\rightarrow$ proteins

Shape is part of what allows a protein to do a particular job, similar to a key fitting into a lock. If somehow the shape of the protein is changed, there is a "loss of function:" a protein that does not work. Or, there may be a "gain of function:" a protein that does something new, usually something bad.

For eg., many proteins are supposed to dissolve inside of the liquid environment of cells. To do this proteins shape themselves by folding in a very specific way.
Misfolded protein-loss of function

When they are not folded correctly, referred to as "misfolding," a protein may be unable to dissolve. Think about a raw egg white, which is liquid and filled with proteins. Cooking the egg denatures, or changes of shape of the proteins, thus the folding, and the protein becomes hard white insoluble mass. Lewy bodies are like cooked egg whites where there should be raw egg. And, the most prevalent component found in LB is a misfolded version of the protein called alpha-synuclein (ASN).
Alpha-synuclein (ASN)

ASN, the star of my talk today and in its native state is a naturally-occurring protein found at the
The synapse, home of alpha-synuclein

where communication between one neuron and another occurs with release of neurotransmitters such as dopamine
 ASN stabilizes collections of dopamine which come to the synapse in tiny bags called vesicles.
Alpha-synuclein at the synapse

ASN is also believed to help control synaptic plasticity, or the formation of new neural pathways.

DA = dopamine
Misfolded alpha-synuclein 😞

If ASN is misfolded and does not work the dopamine is not released, new pathways are not made, and bad things happen.
One of these bad things is that under stress conditions, such as when a neuron is unhealthy with LBs, the neuron may release misfolded ASN from the cell.
Misfolded alpha-synuclein may be released
Misfolded alpha-synuclein is taken up by another neuron
Misfolded alpha-synuclein causes Lewy bodies in a chain reaction from cell to cell.

Calo et al., Synaptic Failure and Alpha-Synuclein. Movement Disorders. 2016:31(2); 169-77
One bad alpha-synuclein

spoils... the whole bunch
Lewy bodies contain misfolded alpha-synuclein
Researchers have demonstrated this by injecting misfolded ASN into lab animals.

and later demonstrating LB in the brain.
Injecting ASN into the DMN of medulla - stage I of the Braak system,
the abnormal ASN will travel along the lengthy axon high up the brainstem to other neurons.
Similar results have been demonstrated with olfactory bulb, the gut, or even the leg, all of which were followed by LBs in the brainstem.
Can misfolded alpha-synuclein be used to diagnose PD?

• Yes.
  – The frustrating reality of PD is that the only way to definitively diagnose disease currently is brain biopsy to demonstrate LBs.
  – The problem with brain biopsy is of course, brain biopsy. You are using your brain, all of it, all the time.
Misfolded alpha-synuclein may start in the gut and climb the vagus nerve to the brainstem.

A better target is the gut. The Braak hypothesis indicates pathology starts in the gut and climbs up the very long vagus nerve to the lower brainstem. There is evidence to support this idea.
Misfolded alpha-synuclein may start in the gut and climb the vagus nerve to the brainstem

ASN has been seen in biopsy of the colon up to 8 years prior to development of motor symptoms

Misfolded alpha-synuclein may start in the gut and climb the vagus nerve to the brainstem.

ASN has been found in the appendix of asymptomatic patients.

Gray, et al., Alpha-synuclein in the appendiceal mucosa of neurologically intact subjects. Mov Disord 2014;2
Misfolded alpha-synuclein may start in the gut and climb the vagus nerve to the brainstem.

And in stomach wall biopsy in patients with clinical PD.

Lab animals fed Rotenone

Putting all this together, investigators used a toxin that may trigger PD, the pesticide rotenone. When rotenone is fed to lab animals chronically, ASN can accumulate in nerves of the gut.

Lab animals fed Rotenone

Following this ASN can be found in the brainstem.
ASN in the medulla DMN - again the site of Braak’s stage I
ASN spreads to the Pons
ASN spreads to the midbrain and so on
Lab animals fed Rotenone

The same progression can be halted by cutting the vagus nerve, in which case abnormal ASN may still accumulate in the gut, but it does not arrive in the brain….
Lewy bodies are also in the salivary glands
Salivary gland needle biopsy

- PD of less than 5y
- average age 69
- positive in 74% (14 of 19) of the PD patients

Adler et al. Peripheral synucleinopathy in early Parkinson's disease: submandibular gland needle biopsy findings. Movement disorders. 2016:31 (2) 250-56
Salivary gland needle biopsy

- PD mean disease duration of 11.8 years
- 75% had positive staining.
  - The FDA is considering approving this as a diagnostic test.
three cardinal features of PD pathology:

• Aggregation of alpha-synuclein into LB
• Abnormal immune system
• Loss of neurons
  – programmed cell death
  – devoured by microglia
What about therapies?

• Each of these issues are potential targets for therapy
• I am focusing on alpha-synuclein.
• One way to target ASN is with monoclonal antibodies (Abs)
  – Abs function to clear pathologic proteins by binding, and therefore identifying them to your immune system.
  – Passive immunization uses monoclonal Abs, which are clones of a single Ab directed against a single target, such as ASN
Passive immunization

- 6 studies have been published since 2011 re animals injected with antibodies against alpha-synuclein
  - successful eradication of
    - pathology
    - abnormal behavior
Monoclonal antibodies target just the misfolded ASN

3 clinical trials of passive immunotherapy

- Prothena Biosciences and
- Hoffmann-La Roche (NCT02095171)
  - 40 healthy volunteers
    - several increasingly larger single doses of PRX002
    - all doses except the lowest were able to reduce levels of free ASN in plasma
    - highest dose made ASN undetectable
      - (presumably completely bound by antibodies).
3 clinical trials of passive immunotherapy

- phase I study with PRX002 ongoing
  - (NCT02157714)
  - 60 patients with mild to moderate iPD
  - measure ASN and AB in CSF
  - Estimated to conclude this fall
3 clinical trials of passive immunotherapy

• BIIB054 (Biogen)
  – 40 healthy volunteers
  – 5 different doses

• LOCATIONS
  • Evansville, IN
  • Dallas, TX
main drawbacks of MCP

- High cost
- Infusion frequency
  - requiring boosters probably every six months
Active immunization, aka Vaccine

There is also “active immunization”, with vaccine, in which the idea is that you break apart and show the abnormal proteins of misfolded ASN to the immune system, which will then find and destroy misfolded ASN in your brain and body.

Vaccines cause you to make your own antibodies
Vaccination has a greater potential for large-scale use

- production is easier than MCP
- less expensive
- administration much less time consuming
Downsides of vaccinations

• need for a normal immune system
• only 0.1-1% of AB will cross the BBB
• risk of serious adverse events
  – AD vaccine AN1792 trials were stopped
    • 6% of patients developed meningoencephalitis
  – Investigators did learn from that terrible lesson, and second generation vax attempts have had a generally favorable safety profile.
There have been multiple animal studies.
AFFiRis (Vienna, Austria) clinical human trial

• began in 2012
• phase 1 PD01A
  – 32 early PD patients
  – 12 months
  – favorable toxicity profile
  – no pt in the Tx groups deteriorated over controls

AFFiRis (Vienna, Austria) clinical human trial of active

• Patients were offered booster vaccinations and long term follow up with a Phase 1b.

• Ongoing trial with PD03A vax.

Nilotinib

- FDA approved from the treatment of chronic myelogenous leukemia
- shown in a mouse model to reduce the activity of a tyrosine kinase enzyme called c-Abl
- c-Abl is activated in PD patients and is associated with overproduction of alpha-synuclein
Nilotinib

- Animal studies have shown that injection of either ASN or c-Abl will increase levels of the other.
- And, excess of either can lead to LB formation and cell death.
  - targeting c-Abl decreases alpha-synuclein
    • which decreases LB

Nilotinib

- phase 1 clinical trial for the treatment of PD and DLB (NCT02281474) at Georgetown U.
- 36 iPD patients
  - measure changes in alpha-synuclein and tau
Summary

• alpha-synuclein can misfold and aggregate into Lewy bodies
• alpha-synuclein can be used to diagnose disease
• alpha-synuclein is a target of new therapeutic trials
Combination trials

Finally, these trials are encouraging. And, I think they should be taken with cautious optimism. We have all heard about failed trials which attempted to slow down or modify PD. But these trials might have asked the wrong question. Maybe each is not a failure, but a tool that should be tested in another way and could be used appropriately. Maybe CoQ10 is helpful, for eg, but for only part of the problem, and cannot on its own slow down disease with so many other problems untreated.

I think of PD like a house on fire. You can very effectively put out the fire in the kitchen, but if the rest of the house is still burning the overall rate of the building going up in smoke may not change. But does that mean putting out the fire in the kitchen was a bad idea? This is what PD studies are sometimes like, trying to fix a single problem and then asking about the overall rate of disease progression without addressing multiple other issues. It is like asking if putting out the kitchen fire slows or stops the rest of the house from burning. The effect is either negligible, or it only works when the fire is started and stopped in the kitchen before it has a chance to spread.
Combination trials

If we stop misfolded alpha synuclein very early in disease it might stop progression.

If a patient is already symptomatic, the fire has gotten out of the kitchen and now we have to stop neuro inflammation, the progression of disease, the loss of cells. What we need are trials that attack PD from more than one angle. This is not to say that the trials I've just discussed aren't very important. I am very optimistic about these studies at any stage of disease, but I think we need to combine therapies that strike more than one issue at a time. If rasagiline protects against cell death and fluoxetine against inflammation, why not try them both together? There are many other examples. And, there is precedence. Combining therapies in trials has worked in infectious disease, it has worked in some types of cancer, and they might just in work in PD.