BMH MEDICAL JOURNAL

BMH Medical Journal 2015;2(3):60-70 **Review Article**

Update on Movement Disorders - Five New Things in Parkinson's Disease

Thyagarajan Subramanian, MD[1], Kala Venkiteswaran, PhD[2]

[1]Professor of Neurology and Neural and Behavioral Sciences, Director, Central PA APDA Informational Center and Movement Disorders Program, Penn State College of Medicine, Hershey, PA 17033 USA

[2]Assistant Professor of Neurology and Neural and Behavioral Sciences, Director, Gene Therapy and Transcranial Ultrasound Core, Executive Director, Central PA APDA Informational Center, Penn State College of Medicine, Hershey, PA 17033 USA

Address for Correspondence: Thyagarajan Subramanian, MD, Penn State Milton S. Hershey Medical Center, Mail Code H109, Room C2846, 500 University Drive, Hershey, PA 17033. E-mail: tsubram@yahoo.com

Abstract

Movement disorders is a branch of neurology that deals with disorders of the extrapyramidal system. Most such disorders have pathology in the basal ganglia or the cerebellum or their connections to the rest of the brain. Parkinson's disease is perhaps the best known example of movement disorders. Another example is Huntington's disease, which has become one of the most well studied genetic disorder in neurology. Other common movement disorders include essential tremor, dystonia and Tourette syndrome. This article will focus on 5 new contributions to the field of movement disorders focusing on Parkinson's disease from our research group and how these have influenced the medical field.

Keywords: Dyskinesias, *Mucuna pruriens*, levodopa, cell transplantation

Disease Progression Markers in Parkinson's Disease

Parkinson's disease (PD) is characterized by the triad of symptoms: resting tremor, bradykinesia (slowness of movement) and rigidity. PD occurs usually in the population that is 65 years of age or older although a significant minority can manifest disease at a younger age. A few of the celebrities who have had PD at young age are the Hollywood actor Mr. Michael J. Fox and the former world heavy weight and Olympic boxing champion Mr. Mohammed Ali. PD responds well to dopaminergic medications in the initial years of disease, but they develop significant complications as the disease progresses beyond the first 5-10 years. These complications include drug-induced dyskinesias - disabling choreioform movements and "on/off" fluctuations when the medications could abruptly become ineffective for a short period of time before working again like an "on/off" switch [1, 2]. Although there are a large number of therapeutic options for PD including levodopa containing preparations, dopamine agonists, monoamino oxidase inhibitors (MAO-I), carboxy-o-methyl transferase inhibitors (COMT-I), deep brain stimulation (DBS) of the subthalmic nucleus or

the globus pallidus and intestinal gel infusions of levodopa, all these treatments are purely symptomatic and do not have proven disease modifying properties. Such disease modification is necessary ultimately to overcome the neurodegeneration that occurs in the nigrostriatal pathway in PD.

PD is classified into 5 stages and the first stage is characterized by the presence of symptoms only on one side of the body (hemiparkinsonism). Typically patients are in this stage for 3-10 years before evolving to stage II bilateral disease. However, this is only true if the diagnosis is made early. Also, young onset PD (<40 years of age) and early onset PD (EOPD, >40 <60 years of age) patients tend to have longer periods of stage I disease [3, 4]. Average age of onset of PD has gradually inched up as shown in many recent studies such that the mean age is 65 years. So, one idea that came from our group is the notion that if we are able to detect side to side differences in PD in stage I disease, then we may have a disease progression biomarker that will allow the clinician to detect progression of disease to stage II. This is a particularly important concept as patients in stage I will be symptomatically medicated with anti-PD medications and these medications will suppress manifestation of PD symptoms on the opposite side from clinical detection. Further, asking the patient to stop the anti-PD medication even temporarily would require 14-28 day drug holiday that would cause severe disability and would be unethical. Therefore, clinicians today are not able to reliably predict the advancement of stage I disease to stage II.

To solve this issue, we undertook a clinical biomarker trial. Using a human investigation committee approved protocol funded by the Charles Dana Foundation. We recruited 32 EOPD patients in stage I disease and had been seen for at least 24 months in our practice to ensure stability of PD diagnosis. They underwent MRI imaging of the brain at enrollment and at 12, 18 and 24 months post-enrollment that was analyzed without knowledge of the clinical status of the patient by a blinded MRI expert. We also performed blinded transcranial sonography (TCS) on all our subjects at each of these visits. The idea was that these imaging modalities will show clear asymmetry of pathology in the brain and that the hemisphere that corresponds to the side that is symptomatic will have more pathology. For example if the patient had right hand tremor at rest, rigidity and bradykinesia (right hemiparkinsonism) and did not have any parkinsonism on the left hand or leg then we predicted that this patient will have pathology predominantly on the left substantia nigra (left hemisphere controls right side of the body) and its connections. Indeed this is what we found. This was the first study to show that MRI abnormalities where unilaterally predominant in early stage I PD patients if they had early onset of disease. To perform this study, we used MRI techniques called transverse relaxation and diffusion tensor imaging (DTI). DTI is a method that detects overall freedom of water movement at the tissue level. This technique allows images to show mean diffusivity (MD) of water and direction of water diffusion that is called fractional anisotropy (FA). Transverse relaxation is a method to detect iron in the brain and in PD there is clear evidence of iron accumulation in the substantia nigra. Our study was able to show for the first time that there was clearly quantifiable and statistically significant difference between the effected hemisphere and the unaffected hemisphere in transverse relaxation at the level of the substantia nigra and in FA and MD at the level of the putamen - the target of the substantia nigra dopaminergic neurons [4].

The TCS studies were even more dramatic in these EOPD patients showing that TCS based hyperechogenicity (increase in signal) in the corresponding substantia nigra was much larger to meet the criteria for abnormal pathology. We also showed that hyperechogenicity in the less effected substantia nigra progressed slowly over the course of next 24 months to reach the threshold for pathology even before the clinician could detect the clinical change to stage II bilateral disease [5]. This suggests that we may have for the first time a way to detect disease progression in PD at least from stage I to stage II in EOPD patients. This is a small but significant gain in a disease where there are no disease progression markers that are reliable. To put in context, there have been numerous MRI studies, positron emission tomography (PET), single photon emission (SPECT) and TCS studies and they have failed to provide disease progression biomarkers. This is different from their ability to diagnose PD and differentiate it from other conditions. In this task many modalities

especially SPECT, PET and TCS have made a lot of strides. However, our study is the first promise as a disease progression biomarker in EOPD.

Pathophysiology of Drug Induced Dyskinesias in Parkinson's Disease

For many decades the pathophysiology of drug induced dyskinesias in PD have been attributed to the loss of dopamine producing cells in the substantia nigra due to neurodegeneration and the subsequent loss of axonal connections to the putamen and caudate nuclei (collectively called the striatum). This loss of continuous dopaminergic stimulation (CDS) has been attributed to the cause of drug induced dyskinesias. The argument goes as follows: Initially there is loss of a few substantia nigra neurons, this causes the dopamine receptors in the striatum to undergo changes (plasticity) to accommodate for the loss of dopamine. Then as the patient receives large doses of levodopa given 2 or 3 times a day orally as medications this causes an overwhelming amount of dopamine to flood the striatum intermittently followed by periods of very low levels when the medication effects wear off. Since the effective clinical half life of levodopa, the most powerful dopaminergic medication is only about 4 hours in most humans and much shorter in PD patients, they go through multiple upswings followed by downswings of dopamine in the brain on a daily basis. This causes the dopamine receptors to become supersensitive - a process caused by neuronal and receptor plasticity. This process is thought to take 3-5 years, which coincides roughly to the time period over which drug induced dyskinesias occur in PD patients. This hypothesis had received support from experiments performed in the early 1990's that suggested that intravenous or intrajejunal infusion of levodopa may solve the problem of drug induced dyskinesias and on/off fluctuations in later stages of PD [1, 2]. This is the basis of some of the newer treatments that have come for PD including longer acting dopamine agonists like Ropinirole, Rotigotine and Pramipexole. However, these longer acting agents are clearly not as potent as levodopa formulations and the idea that delaying the use of levodopa preparations allows for delaying disease progression has been roundly discredited in multiple clinical trials [6, 7].

However, this theory of loss of CDS followed by intermittent high dose levodopa therapy as the basis of drug induced dyskinesias and motor fluctuations has many drawbacks [1, 2]. The most prominent of these is the fact that DBS surgery and other functional surgeries in the brain for PD dramatically improve drug induced dyskinesias. The proponents of the CDS hypothesis have tried to explain this beneficial effect of DBS is due to reduction of levodopa doses in such patients. However, many patients who receive DBS or other functional surgery experience reduction in dyskinetic symptoms even without reducing the dose of levodopa [8]. To understand this issue further, we undertook a series of studies in animal models of PD and showed that traditional oral medical therapy with levodopa or even systemic injections of levodopa does not normalize electrophysiological changes that occur in the brain in PD [9]. Further, we showed in a study published in *Brain*, that dopamine producing grafts placed in the brain of parkinsonian rats can normalize the electrophysiological abnormalities whereas levodopa administered multiple times in a day or even round the clock for 24 hours is incapable of normalizing electrophysiological changes in the brain [10].

What these studies mean is that in the case of the brain disorders like PD, effectiveness of pharmacotherapy cannot be adequately assessed based on behavioral or clinical improvements alone. Such an approach is likely to cause long-term negative side effects like drug induced dyskinesias and on/off phenomenon. Drugs for PD need to be evaluated using electrophysiology as much as pharmacology and clinical outcomes. Normalizing electrophysiology is equally important as pharmacological considerations and clinical behavioral improvements. This approach to experimental therapeutics is becoming better accepted in recent years with a number of papers using electrophysiological outcomes in addition to traditional clinical and pharmacological outcome studies.

Mucuna pruriens for Parkinson's disease

Mucuna pruriens, a legume that grows very well in many countries including India. It is known in Sanskrit as *Atmagupta* and in Malayalam as *Naikurna*. It is a medicinal plant that is frequently used in Ayurveda for the treatment of PD or other disorders characterized by tremor (Kampavata). Interestingly, PD had been described in Charaka Samhita and other Ayurvedic literature as early as 1000 B.C. and L-dopa had been isolated from *Mucuna pruriens* in 1937 well before synthetic L-dopa use in PD patients [11, 12]. Since Ayurvedic medications are often combinations of multiple plant products administered at variable doses it has not been clear how *Mucuna* is effective in PD. Anecdotal data from various Ayurvedic physicians suggest that PD patients that receive only Ayurvedic medications do not exhibit drug induced dyskinesias. However, it is also clear that this form of treatment is not standardized and the treatment effectiveness is highly variable [13, 14].

Mucuna endocarp powder when administered to PD patients at comparable doses as synthetic levodopa caused a lot of gastrointestinal discomfort and could not be well tolerated. Despite these difficulties with the endocarp powder, *Mucuna pruriens* has been investigated as a putative anti-dyskinetic and anti-PD treatment. A single dose small study of *Mucuna pruriens* endocarp powder failed to show anti-dyskinetic effects. But a growing body of literature has shown that *Mucuna pruriens* does appear to have anti-dyskinetic properties. It had been hypothesized that the beneficial effects of *Mucuna pruriens* are mediated via the natural presence of 4% by weight of levodopa. The claim had been that because of this low concentration of levodopa in *Mucuna pruriens*, the improved side effect profile is just an indication of under dosing.

To disprove this contention, we prepared a water extract of *Mucuna pruriens* and showed that this water extract retains all the beneficial effects of the endocarp powder. This water extraction technique removed all the gastrointestinal side effects of the endocarp powder of *Mucuna pruriens*. We also showed that the water extract has the same 4% by weight of levodopa that is present in the whole endocarp powder. Next we compared the effects of administering the same exact dose of levodopa in the synthetic form versus *Mucuna pruriens* water extract in animal models. These experiments showed that these animals had exactly equal improvement in parkinsonism, but *Mucuna pruriens* water extract treated animals had no drug induced dyskinesias while the synthetic levodopa treated animals had severe drug induced dyskinesias [13]. This finding suggests that *Mucuna pruriens* contains additional ingredients that are anti-dyskinetic and that the effects of this medicinal compound are not mediated solely by levodopa. In addition, *Mucuna pruriens* appears to normalize the electrophysiological changes in the brain that does not appear to occur when treatments are done with synthetic levodopa alone [12]. These experiments suggest that *Mucuna pruriens* water extract maybe an effective treatment for PD. However, additional studies need to be completed to determine the mechanism of action of these additional ingredients.

What does this mean for contemporary treatment of PD? Although there is promise, *Mucuna pruriens* is not ready for primetime treatment in patients with PD. This is so because there continues to be uncertainty on the correct dosing and standardization of this medicinal product. *Mucuna pruriens* is sold under various brand names over the Internet and we frequently get phone calls on how to use this medication. Our standard answer is that this medicinal product is not standardized and that it is quite variable in its efficacy. Therefore, we do not recommend taking it as a treatment at the present time for PD. However, we do think that this medicinal product holds enormous promise and that further development of this medicine can help it become a modern therapeutic in the near future.

Experimental Therapeutics for Disease Modification and Pharmacotherapy in Parkinson's disease

There is a long history of attempts to modify disease progression in PD. One of the best examples was the DATATOP study, which tested Deprenyl (Seligiline, a MAO-B Inhibitor) and vitamin E for

their disease modifying abilities in early PD. This study showed that the progression of PD symptoms were not influenced by either selegiline or vitamin E. Subsequently a number of other agents have been tested as possible disease prevention agents in PD. One of the latest such study that we participated was the co-enzyme Q10 double blind placebo-controlled study. This is one of the largest PD studies to date. Results showed that patients on placebo were doing better than patients receiving co-enzyme Q10 and the study had to be terminated early [15]. These studies are good examples of futility of trying vitamins and similar agents in PD. The recommendation to clinicians is to not prescribe any additional vitamins as preventative therapy in PD.

We have also participated in several clinical trials that validated new therapeutics in PD. One example is the PRESTO study in which subjects were given Rasagiline mesylate, a MAO-B inhibitor or placebo as add-on therapy against "on/off" fluctuations. This study showed that Rasagiline could be used as add-on therapy in PD patients to mitigate on/off fluctuations [16-20]. Although its effects are mild and it is an expensive medication, in selected patients, this add-on therapy can be beneficial and can improve quality of life. This is especially true for people who have difficulty in taking multiple doses of medications as Rasagiline is a once a day medication and does not have the amphetamine like metabolites produced by Seligiline (Deprenyl). We are currently involved in experimental therapeutic trials for drug induced dyskinesias mitigation and disease modification in PD.

Cell transplantation for Parkinson's disease

One of the methods by which dopamine can be replenished in PD is by transplanting dopaminergic cells into the striatum. One of the first attempts to do such experiments was to use the patient's own adrenal medulla as an autograft. Such transplants required initial abdominal surgery to remove one adrenal gland and then stereotactic placement of autologous adrenal medullary cells into the striatum. After initial enthusiasm there was a major disappointment when there was excessive morbidity from such surgical interventions and failure in blinded placebo controlled studies. To overcome this morbidity and to improve cell survival we cografted sural nerve that contains schwann cells that provide nerve growth factor (NGF) along with the adrenal medullary issue as cotransplants. In this small clinical trial we showed that such cografts could potentially improve cell survival and clinical outcome [21]. This technique never took off because of the excessive morbidity associated with these open surgeries. Next there was an attempt to transplant fetal mesencephalic tissue (the precursors to the substantia nigra in the fully developed brain) that contained dopaminergic cells in patients with PD. This tissue was obtained from first semester abortions. Several lines of research including seminal clinical trials showed that such fetal grafts provide limited benefits and that several graft recipients had to undergo DBS surgery because they developed graft-induced dyskinesias - a disabling side effect that occurred 5-10 years after transplantation of fetal mesencephalic tissue [22, 23]. Later a few of these patients died from natural causes and came up for brain pathological examination. These autopsy studies showed that the newly grafted fetal tissue had acquired pathological changes that are normally seen in PD. This finding has the lead to a hypothesis that PD is a prion like disease. Conceptually, the notion here is that abnormal folded proteins like alpha-synuclein that are seen in fully established PD can be released from degenerating dopaminergic neutrons and such released abnormally folded proteins can act as infective particles. This allows for disease to spread within the substantia nigra and beyond. This brings caution to surgical procedures as prion like spread could potentially be an iatrogenic issue [24-27].

To overcome this disadvantage, and also to minimize the ethical objections to using tissue derived from human abortions we have tried to use human fetal retinal pigment epithelial cells (RPEC) as transplants in PD [22, 23]. This approach has numerous advantages and in particular it was attractive in that RPEC derived from a single fetal donation could be expanded in tissue culture such that over

50,000 patients can be transplanted from such a single source. We were the first to demonstrate this new technique where RPEC attached to microcarriers were transplanted into the dopamine-depleted striatum for PD [28]. After initial success in animal models we performed the first human trial [29]. In this trial we were able to demonstrate safety and efficacy for such transplants. Immediately after the results of this study came out, this technology was purchased by Schering AG, a German pharmaceutical company for further development. German regulations did not permit the use of fetal tissue of any kind and as a poor chemical alternative; they substituted postnatal RPEC for the definitive trials without any preclinical animal experimentation. Unfortunately this change created major issues, as postnatal RPEC do not appear to have the same survival and neurochemical properties as prenatal RPEC. In this pivotal study both placebo in the test groups demonstrated clinical benefit that where not statistically different [30]. One subject enrolled in this study came up for autopsy because of death unrelated to the study. Interestingly, these pathologists only published the analysis of a single hemisphere, which showed very poor cell survival [31]. For reasons that are not clear the results from the second hemisphere were never published. However, reports filed by Titan Pharmaceuticals indicate that the second hemisphere autopsy did show much better survival of transplanted RPEC attached to microcarriers. Several other studies that used fetal RPEC attached to microcarriers have shown that such transplants are efficacious and without any side effects [32-38]. Additional work from our laboratory shows that the key to successful involvement of RPEC is to use first or second trimester fetal origin cells as we did in all our animal studies and in the first human trial. It is difficult to explain the rather surprising finding that the placebo group that received sham surgery (patients actually got general anesthesia and stereotactic frame placement and burr holes but never got the transplants) had remarkable improvement in symptoms. This placebo effect has come to the attention of the PD research community and PD patients. There has been intense debate whether such placebo controlled surgical trials are necessary? [39]. Also there is debate if PD therapies have such great benefits from placebo, can we take advantage of such placebo effects to help our patients or to preselect patients who may be susceptible to placebo effects from clinical trials especially if they are double blind placebo controlled such that a placebo bias does not negate any real effects of the experimental surgery [40, 41].

As a result of these experiments a moratorium on cell transplantation trials was placed in 2011 [42]. Soon, thereafter, a large meta-analysis of all transplantation studies showed that at least in the case of fetal tissue transplantation there is considerable optimism. Therefore, the moratorium on fetal tissue testing has been lifted and a European consortium of scientists, neurologists, and neurosurgeons have opened up a patient registry to track outcomes in continued clinical research studies [43]. In the meantime, many new techniques that allow stem cells to be created or derived in such a fashion as to make them dopaminergic have been accomplished. Another new technology that allows skin cells from patients with PD called inducible pluripotent stem cells (IPSC) to be converted into dopaminergic neurons has also been successful. These new technologies need to be meticulously tested in preclinical and clinical trials. Such studies are underway. In addition, studies that allow remote control of grafted issue, using optogenetics and chemogenetics are also ongoing. Therefore, there is tremendous potential for these new techniques to translate into new forms of therapies in PD.

In summary, the field of movement disorders is making tremendous progress. We have touched upon only a small fraction of research studies that are making an impact in this field. There is progress in other movement disorders beyond PD that we have not touched upon in this brief review. Disorders like Huntington's disease, dystonia and Tourette syndrome have you benefited from that introduction of tetrabenazine and its derivatives. Newer studies show that combination of pharmacotherapy and chemodenervation using botulinium toxins can be highly effective in these movement disorders [44]. The incremental progress we have made in understanding the pathophysiology of PD and in experimental therapeutics for PD has been the most impressive. A modern era of pharmacotherapy that is closely monitored with electrophysiology and modulated by optogenetics or chemogenetics is just round the corner. We believe that this is one of the most exciting times to be involved in movement disorders, a field that did not had a large number of interventions, which is rapidly changing for the better.

Although challenges remain there is a clear path for drug discovery and experimental therapeutics that gives hope for modern medicine to overcome the disabilities associated with movement disorders.

Acknowledgements

This work was supported in part by research grants from the National Institutes of Health (NIH) - National Center for Complementary and Alternative Medicine (NCCAM) R21AT001607 and National Institute of Neurological Disorders and Stroke (NINDS) R01NS42402, Health Resources and Services Administration DIBTH0632, and Pennsylvania Tobacco Settlement Funds Biomedical Research grants to Dr. T. Subramanian The Pennsylvania Department of Health specifically disclaims responsibility for any analyses, interpretations, or conclusions. Penn State University Brain Repair Research Fund provided additional funding.

Conflict of Interest Disclosure: Dr. Subramanian has served in the speaker's bureau for Teva Pharmaceuticals as a paid consultant and received financial compensation for his services after the approval of Rasagiline as a therapeutic in PD. Teva pharmaceuticals currently holds worldwide patent on Rasagiline. Dr. Subramanian is also the founder and President of StereoRx, which holds intellectual property rights on modified RPEC. Dr. Subramanian is currently serving and/or has in the past served on advisory boards for Novartis, UCB Pharma, Allergan, Merz, Boehringer-Ingelhiem and GlaxoSmithKline. He is currently funded for part of his research studies by Merz, Ipsen, NIH and Allergan. Dr. Subramanian is married to Dr. Venkiteswaran. There are no separate conflicts to disclose for Dr. Venkiteswaran.

References

1. Deogaonkar M, Subramanian T. Pathophysiological basis of drug-induced dyskinesias in Parkinson's disease. Brain research Brain research reviews. 2005;50(1):156-68.

2. Lieu CA, Shivkumar V, Gilmour TP, Venkiteswaran K, Nolt MJ, Deogaonkar M, Subramanian T. Pathophysiology of Drug-Induced Dyskinesias. In: Rana AQ, editor. Symptoms of Parkinson's Disease. Rijeka: InTech Open Access Publisher; 2011. p. 83-114.

3. Lieu CA, Subramanian T. The interhemispheric connections of the striatum: Implications for Parkinson's disease and drug-induced dyskinesias. Brain research bulletin. 2012;87(1):1-9.

4. Wang J, Yang QX, Sun X, Vesek J, Mosher Z, Vasavada M, Chu J, Kanekar S, Shivkumar V, Venkiteswaran K, Subramanian T. MRI evaluation of asymmetry of nigrostriatal damage in the early stage of early-onset Parkinson's disease. Parkinsonism & related disorders. 2015 (In Press).

5. Krishnaiah B, Venkiteswaran K, Shivkumar V, Gilmour T, Harid N, Saadi R, Subramanian T. Hyperechogenicity of Substantia Nigra: A Potential Disease Progression Marker in Early Onset Parkinson's Disease (EOPD)(P6. 065). Neurology. 2014;82(10 Supplement):P6. 065-P6.

6. Fahn S, Oakes D, Shoulson I, Kieburtz K, Rudolph A, Lang A, Olanow CW, Tanner C, Marek K, Parkinson Study G. Levodopa and the progression of Parkinson's disease. The New England journal of medicine. 2004;351(24):2498-508.

7. Kieburtz K, Parkinson Study Group Prami BIDI. Twice-daily, low-dose pramipexole in early Parkinson's disease: a randomized, placebo-controlled trial. Movement disorders : official journal of the Movement Disorder Society. 2011;26(1):37-44.

8. Oyama G, Foote KD, Jacobson CEt, Velez-Lago F, Go C, Limotai N, Zeilman PR, Romrell J, Wu SS, Neal D, Okun MS. GPi and STN deep brain stimulation can suppress dyskinesia in Parkinson's disease. Parkinsonism & related disorders. 2012;18(7):814-8.

9. Gilmour TP, Lieu CA, Nolt MJ, Piallat B, Deogaonkar M, Subramanian T. The effects of chronic levodopa treatments on the neuronal firing properties of the subthalamic nucleus and substantia nigra reticulata in hemiparkinsonian rhesus monkeys. Experimental neurology. 2011;228(1):53-8.

10. Gilmour TP, Piallat B, Lieu CA, Venkiteswaran K, Ramachandra R, Rao AN, Petticoffer AC, Berk MA, Subramanian T. The effect of striatal dopaminergic grafts on the neuronal activity in the substantia nigra pars reticulata and subthalamic nucleus in hemiparkinsonian rats. Brain : a journal of neurology. 2011;134(Pt 11):3276-89.

11. Ovallath S, Deepa P. The history of parkinsonism: descriptions in ancient Indian medical literature. Movement disorders : official journal of the Movement Disorder Society. 2013;28(5):566-8.

12. Manyam BV. Paralysis agitans and levodopa in "Ayurveda": ancient Indian medical treatise. Movement disorders : official journal of the Movement Disorder Society. 1990;5(1):47-8.

13. Lieu CA, Kunselman AR, Manyam BV, Venkiteswaran K, Subramanian T. A water extract of *Mucuna pruriens* provides long-term amelioration of parkinsonism with reduced risk for dyskinesias. Parkinsonism & related disorders. 2010;16(7):458-65.

14. Lieu CA, Venkiteswaran K, Gilmour TP, Rao AN, Petticoffer AC, Gilbert EV, Deogaonkar M, Manyam BV, Subramanian T. The Antiparkinsonian and Antidyskinetic Mechanisms of *Mucuna pruriens* in the MPTP-Treated Nonhuman Primate. Evidence-based complementary and alternative medicine : eCAM. 2012;2012:840247.

15. Parkinson Study Group QEI, Beal MF, Oakes D, Shoulson I, Henchcliffe C, Galpern WR, Haas R, Juncos JL, Nutt JG, Voss TS, Ravina B, Shults CM, Helles K, Snively V, Lew MF, Griebner B, Watts A, Gao S, Pourcher E, Bond L, Kompoliti K, Agarwal P, Sia C, Jog M, Cole L, Sultana M, Kurlan R, Richard I, Deeley C, Waters CH, Figueroa A, Arkun A, Brodsky M, Ondo WG, Hunter CB, Jimenez-Shahed J, Palao A, Miyasaki JM, So J, Tetrud J, Reys L, Smith K, Singer C, Blenke A, Russell DS, Cotto C, Friedman JH, Lannon M, Zhang L, Drasby E, Kumar R, Subramanian T, Ford DS, Grimes DA, Cote D, Conway J, Siderowf AD, Evatt ML, Sommerfeld B, Lieberman AN, Okun MS, Rodriguez RL, Merritt S, Swartz CL, Martin WR, King P, Stover N, Guthrie S, Watts RL, Ahmed A, Fernandez HH, Winters A, Mari Z, Dawson TM, Dunlop B, Feigin AS, Shannon B, Nirenberg MJ, Ogg M, Ellias SA, Thomas CA, Frei K, Bodis-Wollner I, Glazman S, Mayer T, Hauser RA, Pahwa R, Langhammer A, Ranawaya R, Derwent L, Sethi KD, Farrow B, Prakash R, Litvan I, Robinson A, Sahay A, Gartner M, Hinson VK, Markind S, Pelikan M, Perlmutter JS, Hartlein J, Molho E, Evans S, Adler CH, Duffy A, Lind M, Elmer L, Davis K, Spears J, Wilson S, Leehey MA, Hermanowicz N, Niswonger S, Shill HA, Obradov S, Rajput A, Cowper M, Lessig S, Song D, Fontaine D, Zadikoff C, Williams K, Blindauer KA, Bergholte J, Propsom CS, Stacy MA, Field J, Mihaila D, Chilton M, Uc EY, Sieren J, Simon DK, Kraics L, Silver A, Boyd JT, Hamill RW, Ingvoldstad C, Young J, Thomas K, Kostyk SK, Wojcieszek J, Pfeiffer RF, Panisset M, Beland M, Reich SG, Cines M, Zappala N, Rivest J, Zweig R, Lumina LP, Hilliard CL, Grill S, Kellermann M, Tuite P, Rolandelli S, Kang UJ, Young J, Rao J, Cook MM, Severt L, Boyar K. A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. JAMA neurology. 2014;71(5):543-52.

16. Elmer L, Schwid S, Eberly S, Goetz C, Fahn S, Kieburtz K, Oakes D, Blindauer K, Salzman P, Oren S, Prisco UL, Stern M, Shoulson I, Parkinson Study Group T, Investigators P. Rasagiline-associated motor improvement in PD occurs without worsening of cognitive and behavioral

symptoms. Journal of the neurological sciences. 2006;248(1-2):78-83.

17. Marras C, Lang AE, Eberly SW, Oakes D, Fahn S, Schwid SR, Hyson C, Shoulson I, Parkinson Study Group D, investigators P. A comparison of treatment thresholds in two large Parkinson's disease clinical trial cohorts. Movement disorders : official journal of the Movement Disorder Society. 2009;24(16):2370-8.

18. Parkinson Study G. A randomized placebo-controlled trial of rasagiline in levodopa-treated patients with Parkinson disease and motor fluctuations: the PRESTO study. Archives of neurology. 2005;62(2):241-8.

19. Ravina B, Tanner C, Dieuliis D, Eberly S, Flagg E, Galpern WR, Fahn S, Goetz CG, Grate S, Kurlan R, Lang AE, Marek K, Kieburtz K, Oakes D, Elliott R, Shoulson I, Parkinson Study Group L-PDI. A longitudinal program for biomarker development in Parkinson's disease: a feasibility study. Movement disorders : official journal of the Movement Disorder Society. 2009;24(14):2081-90.

20. Schwarzschild MA, Marek K, Eberly S, Oakes D, Shoulson I, Jennings D, Seibyl J, Ascherio A, Parkinson Study Group PI. Serum urate and probability of dopaminergic deficit in early "Parkinson's disease". Movement disorders : official journal of the Movement Disorder Society. 2011;26(10):1864-8.

21. Watts RL, Subramanian T, Freeman A, Goetz CG, Penn RD, Stebbins GT, Kordower JH, Bakay RA. Effect of stereotaxic intrastriatal cografts of autologous adrenal medulla and peripheral nerve in Parkinson's disease: two-year follow-up study. Experimental neurology. 1997;147(2):510-7.

22. Subramanian T. Cell transplantation for the treatment of Parkinson's disease. Seminars in neurology. 2001;21(1):103-15.

23. Subramanian T, Deogaonkar M. Cell transplantation and Gene therapy for the treatment of Parkinson's disease. In: Galvez-Jimenez N, editor. Scientific basis for the treatment of Parkinson's disease: CRC Press; 2013.

24. Cersosimo MG. Invasive procedures in Parkinson's disease: Let's be aware we are dealing with prion-like proteins. Journal of the neurological sciences. 2015;351(1-2):200-1.

25. Schapira AH, Olanow CW, Greenamyre JT, Bezard E. Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. Lancet. 2014;384(9942):545-55.

26. Olanow CW, Brundin P. Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? Movement disorders : official journal of the Movement Disorder Society. 2013;28(1):31-40.

27. Olanow CW, Prusiner SB. Is Parkinson's disease a prion disorder? Proceedings of the National Academy of Sciences of the United States of America. 2009;106(31):12571-2.

28. Subramanian T, Marchionini D, Potter EM, Cornfeldt ML. Striatal xenotransplantation of human retinal pigment epithelial cells attached to microcarriers in hemiparkinsonian rats ameliorates behavioral deficits without provoking a host immune response. Cell transplantation. 2002;11(3):207-14.

29. Stover NP, Bakay RA, Subramanian T, Raiser CD, Cornfeldt ML, Schweikert AW, Allen RC, Watts RL. Intrastriatal implantation of human retinal pigment epithelial cells attached to microcarriers in advanced Parkinson disease. Archives of neurology. 2005;62(12):1833-7.

30. Gross RE, Watts RL, Hauser RA, Bakay RA, Reichmann H, von Kummer R, Ondo WG, Reissig E, Eisner W, Steiner-Schulze H, Siedentop H, Fichte K, Hong W, Cornfeldt M, Beebe K, Sandbrink R, Spheramine Investigational G. Intrastriatal transplantation of microcarrier-bound human retinal pigment epithelial cells versus sham surgery in patients with advanced Parkinson's disease: a double-blind, randomised, controlled trial. The Lancet Neurology. 2011;10(6):509-19.

31. Farag ES, Vinters HV, Bronstein J. Pathologic findings in retinal pigment epithelial cell implantation for Parkinson disease. Neurology. 2009;73(14):1095-102.

32. Bu L, Li R, Liu H, Feng W, Xiong X, Zhao H, Vollrath D, Shen B, Cheng Z. Intrastriatal transplantation of retinal pigment epithelial cells for the treatment of Parkinson disease: in vivo longitudinal molecular imaging with 18F-P3BZA PET/CT. Radiology. 2014;272(1):174-83.

33. Xue Y, Li X, Pang S, Wang Y, Yao J, Zhou J, Hao P. Efficacy and safety of computer-assisted stereotactic transplantation of human retinal pigment epithelium cells in the treatment of Parkinson disease. Journal of computer assisted tomography. 2013;37(3):333-7.

34. Yin F, Tian ZM, Liu S, Zhao QJ, Wang RM, Shen L, Wieman J, Yan Y. Transplantation of human retinal pigment epithelium cells in the treatment for Parkinson disease. CNS neuroscience & therapeutics. 2012;18(12):1012-20.

35. Ming M, Li X, Fan X, Yang D, Li L, Chen S, Gu Q, Le W. Retinal pigment epithelial cells secrete neurotrophic factors and synthesize dopamine: possible contribution to therapeutic effects of RPE cell transplantation in Parkinson's disease. Journal of translational medicine. 2009;7:53. PMCID: 2709608.

36. Flores J, Cepeda IL, Cornfeldt ML, O'Kusky JR, Doudet DJ. Characterization and survival of long-term implants of human retinal pigment epithelial cells attached to gelatin microcarriers in a model of Parkinson disease. Journal of neuropathology and experimental neurology. 2007;66(7):585-96.

37. Cepeda IL, Flores J, Cornfeldt ML, O'Kusky JR, Doudet DJ. Human retinal pigment epithelial cell implants ameliorate motor deficits in two rat models of Parkinson disease. Journal of neuropathology and experimental neurology. 2007;66(7):576-84.

38. McKay BS, Goodman B, Falk T, Sherman SJ. Retinal pigment epithelial cell transplantation could provide trophic support in Parkinson's disease: results from an in vitro model system. Experimental neurology. 2006;201(1):234-43.

39. Katsnelson A. Experimental therapies for Parkinson's disease: Why fake it? Nature. 2011;476(7359):142-4.

40. Ko JH, Feigin A, Mattis PJ, Tang CC, Ma Y, Dhawan V, During MJ, Kaplitt MG, Eidelberg D. Network modulation following sham surgery in Parkinson's disease. The Journal of clinical investigation. 2014;124(8):3656-66. PMCID: 4109544.

41. Espay AJ, Norris MM, Eliassen JC, Dwivedi A, Smith MS, Banks C, Allendorfer JB, Lang AE, Fleck DE, Linke MJ. Placebo effect of medication cost in Parkinson disease A randomized double-blind study. Neurology. 2015;84(8):794-802.

42. Albanese A. Cell therapy for Parkinson's disease: have the glory days gone? The Lancet Neurology. 2011;10(6):492-3.

43. Abbott A. Fetal-cell revival for Parkinson's. Nature. 2014;510(7504):195-6.

44. Brin MF, Comella CL, Jankovic J, Lai F, Naumann M, Group CDBS. Long-term treatment with botulinum toxin type A in cervical dystonia has low immunogenicity by mouse protection assay. Movement disorders: official journal of the Movement Disorder Society. 2008;23(10):1353-60.