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Neurological Disorders and the Blood Brain Barrier: 2. Parkinson and Other Movement Disorders

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Abstract

While the extent of our body of knowledge of Parkinson's disease (PD) and other movement disorders and the number of corresponding treatment drugs are both vast, there is still no cure for these chronic and relentlessly progressive diseases. The motor symptoms of PD are known to result from reduced dopamine production in the brain's basal ganglia. However, Dopamine does not cross the blood brain barrier (BBB) so it cannot be taken as a medicine to boost the brain's depleted levels of Dopamine. However a precursor of Dopamine, Levodopa, can pass through this barrier to the brain where it is readily converted to Dopamine. Administration of this drug temporarily diminishes the motor symptoms of PD. Unfortunately, only 5–10% of the drug crosses the barrier with much of the remainder being metabolized to Dopamine elsewhere in the body, where it causes a variety of side effects. I will first discuss the current treatments of motor symptoms prior to treatment with the mainstay L-dopa-based therapy and later as the disease progresses and other pharmacologic treatment options as well as the numerous pharmacotherapies for non-motor symptoms. For early to very advanced PD patients, I will also discuss deep brain stimulation (DBS), which leads to less motor or neuropsychiatric adverse effects, or both. I will next analyze the challenges faced when treating motor and neuropsychiatric symptoms of the disease. More emphasis will be placed on emergent new treatments, especially the use of high-intensity focused ultrasound (HIFUS) and its guidance under magnetic resonance imaging. HIFUS is sufficient to create a coagulation lesion in the brain with the goal of developing a substantially less invasive way to create stereotactic brain lesions. It can be employed to safely open the BBB for localized delivery of large therapeutics such as proteins, genes, and cells as a potentially restorative treatment. Emphasis will then be placed on two important advances that devolved from our most recent understanding of the genetics and neuropathology of the disease: the discovery of pathological alpha-synuclein-aggregations in the Lewy bodies and the Braak staging. Such advances are leading to research that is revolutionizing the understanding of PD. New research vistas are described, including in genetics, neural and stem cell transplantation, transcranial magnetic stimulation, and the search for disease-modifying therapies. The author's proposed therapy utilizing the principles of nanotechnology and anti-PD drugs embedded in nanoparticles to be delivered by nanodevices is also outlined.

Keywords: *Blood brain barrier; Cortico-basal degeneration; Deep brain stimulation; Dementia with Lewy bodies; Essential tremor; High intensity focused ultrasound; Lesional surgery; Movement disorders; Nanomedicine; Nanotechnology; Pallidotomy; Parkinson's disease; Parkinson-plus diseases; Progressive supranuclear palsy; Thalamotomy*

Abbreviations: APD: Anti-Parkinson Drugs; ASAE: Alpha Synuclein Autophagia Enhancer; ASAM: Alpha-Synuclein Aggregation Modulators; BBB: Blood Brain Barrier; B(CSF)B: Brain Cerebrospinal Fluid Barrier; B(iCSF)B: Brain-inner CSF barrier; B(oCSF)B; Brain-outer CSF Barrier; B(R)B: Brain Retinal Barrier; CNS: Central Nervous System; COMT: Catechol-O-Methyl Transferase; CSF: Cerebro-Spinal Fluid; DBS: Deep Brain Stimulation; DLB: Dementia with Lewy Bodies; ET: Essential Tremor; FDA: (U.S.) Food & Drug Administration; FDGPET: Fluoro-Desoxyglucose PET; FUS: Focused Ultra-Sound; GCS-A: Glucocerebrosidase gene A; GDNF: Glial Derived Neurotrophic Factor; Gpi: Globus Pallidus internal part; GWA: Genome Wide Association; HDCA: Hexa-Decyl-Cyano-Acrylate; HIFUS: High-Intensity FUS; IMDS: International Movement Disorder Society; iPSC: Induced Pluripotent Stem Cells; IT: Immuno-Therapy; LDL: Low Density Lipoprotein; MAO-B: Mono Amine Oxidase; MAPT: Microtubule-Associated Protein Tau; PMA: Peptidomimetic Monoclonal Antibodies; MEN: Magneto-Electric Nanoparticles; MR: Magnetic Resonance; MRI: MR Imaging; MRg-HIFUS: MR-guided HIFUS; MRT: MR Thermometry; MSA: Multiple System Atrophy; ND; Nano Devices; NIH: (U.S.) National Institute of Health; NINDS: (U.S.) National Institute of Neurological Disorders and Stroke; NM: Nanomedicine; NMS: Neuroleptic Malignant Syndrome; NMS: Non-Motor Symptoms; NMDA: N-Methyl-D-Aspartate; NP: Nano Particles; NT: Nano Technology; PACA: Poly-Alkyl-Cyano-Acrylate: PD: Parkinson Disease; PET: Positron Emission Tomography; PLGA: Poly-Lactic-Co-Glycolic Acid; PMA: Peptidomimetic Monoclonal Antibodies; REM: Rapid Eye Movement; RES: Reticulo-Endothelial System; RF: Radio Frequency; RP: Related Protein; RS: Radio-Surgery; RSBD: REM Sleep Behavior Disorder; SN: Substantia Nigra; SPECT: Single-Positron Emission Computed Tomography; TMS: Transcranial Magnetic Stimulation (TMS); VIN: Ventral Intermediate Nucleus.

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Disorders Mentioned: Akynesia; Autophagia; Choreatic dyskinesia; Cortico-basal degeneration; Dementia with Lewy bodies; Diabetes mellitus; Diplopia; Dysarthria; Dyskinesia; Dysphagia; Hallucinations; Heart failure; Hyposmia (a diminished sense of smell); Impulse control disorders; Lysosomal degradation; Metabolic syndrome; Multiple system atrophy; Orthopedic syndrome; Paresthesia; Parkinson's disease; Parkinson-plus diseases; Progressive supranuclear palsy; Proteasomal degradation; Psychosis; Punding; Stroke; System atrophy.

Drugs Listed: Amantadine; Apomorphine; Benserazide; Carbidopa; Casomorphion; Catechol-O-Methyl Transferase; Clozapine (an anti-psychotic); Donopezil; Droxidopa; Duloxetine; Entacapone (a short-acting COMT-inhibitor); Hexa-Decyl-Cyano-Acrylate; Istradethyl-line; Isradipine; L-dopa (L-Dihydroxyphenylalanine); Levodopa; Mannitol; Melevodopa; Naloxone; N-Methyl-D-Aspartate (a glutamate subtype); Opicapone; Oxycodone; Peptidomimetic; Monoclonal Antibodies Pimavanserin (an inverse agonist); Piribedil (only registered in Europe); Poly-Alkyl-Cyano-Acrylate; Poly-Lactic-Co-Glycolic Acid; Pramipexole; Quetiapine (an anti-psychotic); Rasagiline (a centrally active COMT inhibitor); Ropinirole; Rotigotine; Rytary (XPO66); Safinamide; Selegiline (a centrally active COMT inhibitor); Tolcapone (an intermediate-acting COMT-inhibitor); Tozadenant.

Introduction

In an earlier, companion article (1), I discussed Parkinson's disease (PD) and other movement disorders, dwelling on motor and neuropsychiatric (non-motor) symptoms, their genetic and environmental causes, categorization, diagnosis, staging, complementary and supportive therapies, rehabilitation and palliative care, prediction and prevention, management, prognosis, and risk and protective factors. While there are currently no blood or laboratory tests to diagnose sporadic PD, it is also difficult to diagnose it accurately early on in the course of the disease. I devoted substantial time to medical treatments that involve drugs that have the following effects: (a) increase the level of dopamine (the mainstay being Levodopa/Carbidopa), (b) mimic the presence of dopamine (agonists), (c) inhibit dopamine breakdown (with MAO-B inhibitors, COMT inhibitors), or else (d) decrease the reaction of acetylcholines (anticholinergics), as well as other drugs of unknown mechanism of action, and (e) drugs that help control the non-motor symptoms of the disease. I also discussed surgical treatments including (a) lesional surgery, (b) deep brain stimulation (DBS), and (c) intentional formation of lesions

(pallidotomy, thalamotomy). Both types of treatment (medications and surgery) can provide substantial improvements. Other diseases and conditions resembling PD were also covered such as (a) multiple system atrophy, (b) dementia with Lewy bodies, (c) progressive supranuclear palsy, (d) cortico-basal degeneration, and (e) PD-plus diseases.

However, while the extent of our body of knowledge of PD and other movement disorders and the number of treatment drugs are both vast, we still have no cure for these chronic and relentlessly progressive diseases. The motor symptoms of PD are known to result from reduced dopamine production in the brain's basal ganglia. However, Dopamine does not cross the blood brain barrier (BBB) so it cannot be taken as a medicine to boost the brain's depleted levels of Dopamine. However a precursor of Dopamine, Levodopa, can pass through this barrier to the brain where it is readily converted to Dopamine. Administration of this drug temporarily diminishes the motor symptoms of PD. Unfortunately, only 5–10% of the drug crosses the barrier with much of the remainder being metabolized to Dopamine elsewhere in the body, where it causes a variety of side effects. The purpose of this article is to review and discuss the multiple emerging treatments of PD and the several lines of investigation currently pursued as well as suggest new research vistas in the search for a cure.

Current Treatments of Motor Symptoms

In 1960, the lack of Dopamine in brains of patients with PD was discovered. The first rationally- derived therapy was then introduced as intravenous L-dopa, a precursor of Dopamine that passes, at least in a small part, through the BBB. It yielded a "miraculous" improvement of the motor symptoms. The symptoms of PD are caused by a Dopamine deficit, leading to an imbalance of the motor, cognitive, and emotional loops in the basal ganglia circuitry.

Although hard to believe, 57 years after its discovery, L-Dopa is still the gold standard for any of the available multiple symptomatic therapies for PD. However, because of L-Dopa's short plasma half-life (1–2 hours), repeated intake is necessary, resulting in a pulsatile plasma profile. With progressing neurodegeneration of the nigrostriatal pathway, the storage capacity of the central nervous system (CNS) for L-Dopa and Dopamine declines, thus, in the intermediate-to-advanced stages of PD, the duration of the central L-Dopa effect will mimic the pulsatile plasma profile of the medication. The longer the disease lasts, the more patients with PD experience "motor complications", consisting of motor fluctuations [a change between phases of akinesia ("off": no or low therapeutically ineffective L-Dopa level) and normal movement ("on": therapeutically effective L-Dopa level) and also of an excess of movements (choreatic "dyskinesia") at the peak of the L-Dopa curve in the blood and, thus, in the CNS.

To delay or ameliorate these L-Dopa therapy-associated motor complications, several other classes of drugs are available to be prescribed before the use of, or in combination with, L-Dopa. The L-Dopa effect can be enhanced and prolonged in either of the following two ways. One, the combination with peripheral inhibitors of degrading enzymes such as decarboxylase (i.e., Benserazide or Carbidopa, a standard combination) and Catechol-O-Methyl Transferase (COMT) (i.e., by adding the short-acting COMT-inhibitor Entacapone or intermediate-acting Tolcapone). Next, with centrally active inhibitors of the degrading enzyme Monoamino-Oxidase B (MAO-B), such as Selegiline or Rasagiline.

As an advanced therapeutic option, L-Dopa emulsion can be applied by an external pump via a percutaneous tubing into the jejunal cavity in order to provide a nearly constant continuous supply of L-Dopa to the blood and thus to the CNS. In addition, physicians have at hand five non-ergot dopamine agonists, mainly of the dopamine-2-receptor type: Pramipexole, Ropinirole and Piribedil (only registered in Europe) for oral intake, Rotigotine by 24-hour transdermal application, and Apomorphine, which needs a parenteral administration (i.e., subcutaneous: bolus or pump assisted infusion). Besides the standard release formulation, Pramipexole and Ropinirole are available as slow release preparations, four non-ergot agonists can offer continuous Dopamine-2-receptor stimulation for a 24-hour period. Ergot dopamine agonists are indicated only as a second-line choice, as they run the risk of inducing fibrosis of the heart valves or the retroperitoneum. Furthermore, the N-methyl-D-aspartate (NMDA) (a glutamate subtype) receptor antagonist Amantadine is considered to improve PD motor symptoms and at the same time to reduce motor complications, especially dyskinesia.

Finally, numerous pharmacotherapies are available for individual non-motor symptoms (NMS) though mostly by employing a given compound approved to treat the symptom or disease *per se* and not a particular symptom as part of the spectrum of NMSs in PD (for example, using an antidepressant for depression in PD).

The introduction of deep brain stimulation (DBS) further increased the therapeutic options for patients with PD. This procedure has been shown to be effective not only in very advanced PD patients but also in PD patients who just have started to develop motor complications. In turn, DBS allows one to decrease the amount of pharmacotherapy and this measure leads to less motor or neuropsychiatric adverse effects, or both, which can occur with the above-mentioned combination of different pharmaceuticals in all stages of PD.

In addition, other therapies include physical exercise, physiotherapy, dance interventions, and logopedic training of dyspagia for the neurological symptoms of PD patients and their quality of life.

Challenges of Treating Motor and Neuropsychiatric Symptoms

Neurologists can choose from a large number of compounds to treat the motor symptoms in PD effectively for several years, if not decades. In addition, owing to advances in internal medicine, anesthesia and surgery, patients with PD live longer but within the optimal therapeutic balance between motor- and non-motor symptoms (NMS) with a minimum of adverse effects.

With advancing age and duration of PD, gait problems (which do not respond to dopamimetic therapy) combined with the increased risk of falls and fractures develop with other NMS (including autonomic dysfunctions such as urinary incontinence and severe obstipation, sleep impairment, pain syndromes and neuropsychiatric symptoms such as depression, impulse control disorders, punding, hallucinations, overt psychosis in part induced by dopamimetic therapy, and cognitive impairment progressing to dementia). Still further, the extent of comorbidity increases (orthopedic syndromes, diabetes mellitus, metabolic syndrome, heart failure, and stroke).

Thus, the weight of therapeutic need has shifted from "just" making or keeping the PD patient mobile to the challenge of fine-tuning a therapeutic combination of drugs for (1) the treatment of motor and non-motor symptoms, (2) motor and non-motor complications and, in accordance with other medical treatments and care, (3) treatment that is acceptable to the patient and the caring partner(s).

Present Therapy Is Still Symptomatic

Despite all of the above-mentioned achievements, until today, we still treat PD at an entirely symptomatic level as further discussed below.

Pharmacological therapy

Pharmacological therapy of PD, based on Levodopa (L-dopa), Dopamine agonists, Dopamine breakdown inhibitors, decrease of acetylcholine reactions, and control of non-motor symptoms is well established, specific, and effective. However, therapy resistance, fluctuations, and dyskinesias may develop over time. To provide treatment to these chronically and severely affected patients, surgical approaches have been developed since the 1950s and 1960s. They comprise radiofrequency (RF) lesion and high-frequency stimulation (HFS) at the level of the motor thalamus, subthalamic nucleus, or the internal part of the globus pallidus (Gpi). HFS of the subthalamic nucleus has been mostly used in the last few years.

Surgical therapy

In the 1960s, RF lesioning in the subthalamus was proposed and explored to provide symptom relief. Recently, the new technology of magnetic resonance (MR)-guided focused ultrasound technology (MRg-FUS) has allowed performing accurate therapeutic thermocoagulations in the thalamus to treat chronic and therapy-resistant neuropathic pain. Starting in 2011, this technology has been applied to the treatment of neuropathic pain, PD, and essential tremor (ET).

Oriental practices

There is no evidence to substantiate that acupuncture, and practice of Qigong, or T'ai chi have any effect on the course of the disease or symptoms.

Dietary elements

Fava beans and velvet beans are natural sources of Levodopa and are eaten by many people with PD. Their intake is not risk-free as life-threatening adverse reactions have been described such as the neuroleptic malignant syndrome (NMS).

Emerging Treatments

Emerging treatments of PD are the use of high-intensity focused ultrasound (HIFUS) and its guidance under magnetic resonance imaging.

High-Intensity Focused Ultrasound

Although diagnostic ultrasound is a well-established modality, it is less well known as a potential therapy for neurologic diseases. With the recent U.S. Food and Drug Administration (FDA) approval (July 2016) of essential tremor (ET) as the first neurologic condition for focused ultrasound (FUS) treatment, both preclinical and clinical research are expanding rapidly for several neurologic indications. Much of this progress is due to improving technology to provide controlled levels of ultrasonic energy that can be focused onto a brain target, non-invasively through the skull, and guided by magnetic resonance imaging (MRI). High-intensity-FUS (HIFUS) is sufficient to create a coagulation lesion in the brain with the goal of developing a substantially less invasive way to create stereotactic brain lesions. Moderate levels of FUS energy, delivered in pulse (p) mode, can be employed to safely open the BBB for localized delivery of large therapeutics such as proteins, genes, and cells as a potentially restorative treatment of neurodegenerative diseases such as PD.

Principle of operation under image-guidance

Similar to light waves, ultrasound waves can be focused using either single-element concave transducers or electronically-controlled phased-arrays of many smaller piezoelectric transducers (somewhat analogous in principle to a Gamma Knife in nuclear medicine). Through this focusing, the energy can be concentrated up to 3 orders of magnitude into a small and elongated ellipsoid volume (typical focus area ~ 2 × 7 mm). As a result, the rates of energy deposition generally used clinically are capable of raising the temperature of the tissues within seconds to 60°C or greater to induce denaturation of cell proteins and ultimately coagulative necrosis. In the intervening tissues of the pre- and post-focal regions, lower intensities of sonic energy are found. As a result, energy absorption is also lower, and the deleterious effects of the exposures (i.e. thermal damage) do not occur [2].

The above-mentioned devices employ multi-element, phased-array transducers for fast, accurate electronic beam steering. Current technology allows for more precise correction of aberrations in the sonic beam path that occurs as it passes through the skull. This capacity to create a targeted thermal lesion within the brain through the intact skull allows for the application of HIFUS to a variety of neurologic conditions, including PD and other movement disorders.

High-intensity focused ultrasound guidance for movement disorders treatment

The current standard for image-guided HIFUS for minimally invasive, non-incisional treatments in the brain employs magnetic resonance imaging (MRI) as the imaging modality. Magnetic resonance guided HIFUS (MRg-HIFUS) enables high-resolution soft tissue imaging for treatment planning, whereas magnetic resonance thermometry (MRT) also allows for quasi real-time brain temperature monitoring. This validates the treatment region as having received the designated thermal dose, while also ensuring that regions outside of the treatment zone are not adversely affected.

Essential tremor (ET) was the first neurological disorder evaluated for treatment with Mrg-HIFUS for several reasons: (a) It is a common disorder where medical therapy is frequently inadequate for patients with severe disability tremor; (b) The VIM of the thalamus is a well-established target for both lesioning and DBS for reduction in tremor in medically refractory patients with either

ET or PD; (c) The anatomical target-the VIN-is centrally located within the brain, which reduces the distortional effects of the skull on focusing the ultrasound energy; (d) Treatment of the VIN in ET not only results in tremor reduction but also substantially reduces disability in selected patients with only unilateral treatment, such as patients with severe tremor in the dominant hand. This approach has been validated by several published clinical studies that have shown significant improvement after treatment using standardized scales rating both tremor amplitude and tremor-related disability. The VIN has also been targeted with Mrg-HIFUS for relief of tremor associated with PD. Targets other than VIN have been treated with Mrg-HIFUS for relief of other aspects of PD besides tremor [3-6].

Comparison with deep brain stimulation

Essential tremor and especially PD are progressive conditions where motor symptoms worsen over time. Patients treated with deep brain stimulation (DBS) are seen at regular intervals where the parameters of stimulation are adjusted to compensate for worsening symptoms. Unfortunately, disease progression may eventually result in worsening symptoms in many patients in spite of reprogramming. When significant worsening occurs after lesional surgery, repeat surgery may be performed, which has usually been successful in regaining the original clinical response. The potential efficacy and safety of re-treatment of any aspect of a movement disorder with Mrg-HIFUS remain to be determined.

Although effective, lesioning for movement disorders has been largely replaced by DBS surgery, which is similar in strategy to stereotactic lesioning but has two significant advantages: (1) Unlike lesional surgery, DBS does not create any intentional brain injury. Suppression of motor abnormalities such as tremor is accomplished through continuous high-frequency (130–180 Hz) stimulation, although the mechanism of its lesion-like inhibitory effect is still debated. Unlike stereotactic lesions, bilateral DBS can be safely performed to improve the larger number of patients with bilateral motor symptoms. Adjustability of these devices is a major advantage of this approach over lesional surgery. When side effects of bilateral stimulation such as dysarthria occur, these can usually be mitigated by lowering the intensity of stimulation [7]. DBS is not, however, without its own complications, including surgical complications such as intracerebral hemorrhage (0.5%–2.0%) and infection (1%–3%), as well as DBS-specific issues such as lead migration and fracture (1%–3%) and device malfunction (1%–3%). DBS also introduces added procedures and costs over surgical lesioning, including surgical implantation and periodic replacement of the programmable pulse generator, as well as device programming visits.

Comparison with radiosurgery

The goal of all forms of surgery for movement disorders is maximal relief of motor symptoms (tremor, bradykinesia, rigidity, and dystonia) without intrusion of symptoms associated with damage or stimulation of adjacent (off-target) brain regions such as dysarthria, paresthesia, weakness, diplopia, or visual field defects. The limitations of DBS cited previously provided a rationale for investigating less invasive surgical methods such as radiosurgery (RS). RS uses contemporary stereotactic methods to localize the brain target and a focused array of emitters that have been extensively used to treat brain tumors (Gamma Knife). This less invasive method has also been applied to relieve symptoms of both essential tremors ET and PD, with the best results seen in lesioning of ventral intermediate nucleus (VIN) for ET. A major issue preventing widespread acceptance of this method for functional neurosurgery is the delayed effect of ionizing radiation–based lesioning. The extent of the treatment is determined solely by a calculation of dose because the effects of radiation occur with a variable delay. Although the rate of off-target effects for RS is relatively low, these effects can occur with a delay of days to months. These previous studies of RS along with the real-time effects of sonic energy have in fact added to the rationale for the study of Mrg-HIFUS as a treatment of movement disorders.

Recent Advances

Two hundred years after their description, the three cardinal motor symptoms of PD (akinesia in combination with either tremor at rest or rigidity) are still the basis of the clinical diagnosis. For more than 100 years, we have known the neuropathological hallmarks of the disorder: the so-called Lewy bodies (proteinaceous intra-cytoplasmic inclusion bodies) containing aggregations of the protein alpha-synuclein and the loss of pigmented-melanin-containing neurons in the midbrain. The latter reflects the neurodegeneration of dopaminergic neurons in the substantia nigra (SN) leading to a marked dopamine deficit in the striatum.

Since 1961, L-Dihydroxyphenylalanine (L-Dopa), a symptomatic dopamine replacement therapy, has been available for PD for more than 50 years. As L-Dopa, the precursor of dopamine and subsequent dopamine agonists are highly effective in reducing motor symptoms, PD was-for a long time-predominantly considered as a movement disorder. This focus was even enforced by the unraveling of the motor circuitry of the basal ganglia, of its imbalances in PD, and the dramatic therapeutic effect of DBS of the subthalamic nucleus or the globus pallidus. These symptomatic therapeutic achievements may explain why the development of therapies for the wide range of disabling non-motor symptoms (NMSs) that the PD patient experiences throughout the course of the disease has been neglected. In addition, research efforts on the development of disease-modifying drugs were largely performed in acute toxin-induced rodent models. The neuro-scientific results of these efforts failed to translate into clinically successful drugs. Thus, apart from few cases of toxin-induced Parkinson syndromes, firm knowledge at the molecular level on the etiopathogenesis of PD was lacking until the year 1996.

Two discoveries between 1996 and 2006 have changed the field: (1) The discovery of a mutation in the gene for the protein alpha-synuclein (a duplication of the gene for normal wild-type alpha-synuclei, that is the presence of three alleles instead of two alleles leads to the production of 150% of normal alpha-synuclein) causes a rare form of autosomal-dominant PD. It is the cause of PD and the explanation of the presence of pathological alpha-synuclein-aggregations in the Lewy bodies in the SN; and (2) The publication of the Braak staging of PD combined with the "dual hit theory" proposing that the manifestation of motor PD symptoms is a late-stage phenotype preceded for years, if not decades, by three prodromal stages.

Up to 2016, we still had no treatment to stop or even slow down the progression of the disease. As already emphasized, available therapy so far has been symptomatic. Now, however, and for the first time in the history of the disease, as a result of our greater understanding of the genetic and neuropathology of the disease, substances with a potentially disease-modifying effect are under development. This enhanced understanding is leading to research that is revolutionizing the understanding of PD.

Based on these findings, the majority of cases with PD (the so-called idiopathic form of PD) were assumed to present an alpha-synucleinopathy. Drug development thus shifted its focus from transmitters, transmitter-related receptor agonists and antagonists, and transmitter-synthesizing and -degrading enzymes to the protein chemistry, synthesis, transport, aggregation, and degradation of alpha-synuclein and other proteins related to neurodegenerative disorders, such as MAP-Tau or beta-amyloid. A 20-year-long effort in neuroscience and drug development appears to provide the first results.

New Therapeutic Developments

With this situation in mind, efforts over the last 20 years to develop new therapies for PD can be divided in two categories: (1) improving symptomatic therapy of motor and non-motor symptoms and (2) addressing potential causes of PD, with a focus on the protein alpha-synuclein, its chemistry, synthesis, aggregation, degradation, and interaction with other proteins in order to develop a disease modifying treatment.

Symptomatic therapy of motor systems

To improve the available symptomatic therapy for motor symptoms, several drugs have recently been approved or are still under testing. These developments include: (1) Improvement of the pump device for infusing L-Dopa in the jejunal cavity (likely available in 2017-18), and (2) approval of a long-acting (5- to 6-hour duration of action) L-Dopa (currently available in the USA under the tradename Rytary). Table 1 lists the newly developed symptomatic therapy for motor systems and motor complications by means of a dopaminergic mode of action including four newly approved drugs for PD.

Compound/ Trade name (Company [C], Sponsor [S])	Indication	Mode of action	Phase of development	Commentary and approved dose	Reference
Melevodopa/ Carbidopa Sirio (Chiesi [C])	Motor	Modified form of L-Dopa soluble tablet	Approved	Marketed in Italy	Zangaglia., *et al*. (2010) Fasano., *et al*. (2014)
Opicapone Ongentys (BIAL [C])	Motor wearing-off	COMT-inhibitor, long-acting, add-on to L-Dopa	Approved	Reimbursed in EU 50 mg/day	Ferreira., *et al.* (2015) Roccaet al. (2016) Fabbri., *et al*. (2016)
Safinamide Xadago (Zambon [C])	Motor wearing-off	MAO-B-inhibitor, glutamate modulator, add-on to L-Dopa	Approved	Reimbursed in EU, active comparator study to other MAO-B-inhibitors not available 50 or 100 mg/day	Stocchi + Torti Cattaneo., *et al*. (2016) Borgohain., *et al*. (2014) Borgohain., *et al*. (2014) Schapira., *et al*. (2013) Stocchi., *et al.* (2012)
"XP066" Rytary (Impax [C])	Motor wearing-off	L-Dopa/Carbidopa (4/1) long-acting, extended release	Approved	Reimbursed in USA 95, 145, 195, 245mg L-Dopa capsules	Yao., *et al*. (2016) Mao + Modi (2016) Waters., *et al*. (2015) Hsu., *et al*. (2015) Stocchi., *et al*. (2014) Pahwa., *et al*. (2014) Hauser., *et al*. (2013)

Source: Oertel and Schulz (2016)

Table 1: *Newly developed symptomatic therapy for motor systems.*

Symptomatic therapy for motor and non-motor systems

Likewise, Tables 2 list the symptomatic therapy developed for motor symptoms and their complications or non-motor symptoms by means of a non-dopaminergic mode of action (a) in case of drugs approved for PD and (b) for drugs approved in anther indication but now tested in PD. Included in the former category are five newly developed drugs and three in the latter category, all of which being in advanced stages of clinical trials.

Compound	Indication	Mode of action	Phase of development	Commentary	Reference
Amantadine Extended release (Adamas [C])	Motor dyskinesia off-time	NMDA-receptor antagonist long-acting	Phase III completed	Likely to be registered 2017 or 2018 as 340 mg/day	Pahwa., *et al*. (2016) Pahwa., *et al*. (2015)
Droxidopa L-Threo-3,4-Dihydroxy-Phenylserine Northera (Lundbeck [C])	Motor and Non-Motor freezing Neurogenic orthostatic hypotension	Noradrenaline precursor		Approved in Japan, USA Capsules: 3 × 100 mg max. 3×6 (max. daily dose 1,800 mg)	Hauser., *et al*. (2014) Espay., *et al*. (2014) Mathias., *et al*. (2001)

Istradefylline Nouriast (Kyowa-Hakko-Kirin [C])	Motor wearing-off	Adenosine 2A receptor antagonist	Phase III positive Phase III ongoing in EU	Approved in Japan 20 mg/once daily (40 mg/daily possible)	Vorovenci + Antonini (2015) Kondo., *et al*. (2015) Pinna (2014) Mizuno., *et al*. (2013) Pourcher., *et al*. (2012) Factor., *et al*. (2010) Mizuno., *et al*. (2010)
Tozadenant (Biotie [C])	Motor dyskinesia wearing-off	Adenosine 2A receptor antagonist	Phase III ongoing		Michel., *et al*. (2015) Hauser., *et al*. (2014) Perez-Lloret + Morello (2014)
Pimavanserin Nuplazid (Acadia [C])	Non-motor psychosis	5HT2A inverse agonist	Phase III positive	Approved in USA 2 x 17 mg/ once daily	Cummings., *et al*. (2014) Hacksell., *et* al. (2014)

Source: Oertel and Schulz (2016).

Table 2a: *Newly developed symptomatic therapy for motor and non- motor systems - Drugs approved for Parkinson's disease.*

Compound	Indication	Mode of action	Phase of development	Commentary	Reference
Donepezil Eisai (C)	Non-motor falls, gait disorder, dementia in Parkinson's disease (PD)	Acetylcholinesterase-inhibitor	Phase IIIb ongoing	Approved for therapy of Alzheimer dementia	Chung., *et al*. (2010) Ravina., *et al*. (2005)
Duloxetine Cimbalta, Xeristar (University of Toulouse [S])	Non-motor pain	SSNRI	Phase III ongoing	Approved for therapy of pain and of depression	
Oxycodone/ Naloxone Targin (MundiPharma [C])	Severe pain syndrome in PD	Opioid	Phase III positive	Approved for therapy of pain	Trenkwalder., *et al*. (2015)

Source: Oertel and Schulz (2016)

Table 2b: *Newly developed symptomatic therapy for motor and non- motor systems - Drugs approved in another indication, now tested in Parkinson's disease*

Symptomatic therapy for non-motor systems

In 2011, the International Movement Disorder Society (IMDS) published a comprehensive evidence-based medicine review on the therapy of NMS of PD [9] to provide guidance for how to treat the individual NMS in PD. Since then, increased efforts in this field have led to the approval of new therapies. For the treatment of dopamimetic-induced psychosis in PD, the 5HT2A inverse agonist Pimavanserin has been approved in the USA. Thus, for the first time, an alternative to the currently employed antipsychotic compounds Quetiapine or Clozapine is available. In regard to severe pain syndromes, the slow-release preparation of Oxycodone/Naloxone has been successfully tested in PD. Furthermore, the precursor of noradrenaline Droxidopa, also known as L-threo-DOPS, has been approved for the treatment of neurogenic orthostatic hypotension, one of the troubling autonomic symptoms in advanced PD and even more so in

multiple system atrophy (MSA), another alpha-synucleinopathy. In regard to DBS, a large recent study has shown beneficial effects of this neurosurgical procedure on NMS. Given the major impact of NMS and therapy-related non-motor complications on the quality of life for PD patients and their partners, this field clearly needs priority in future clinical trials.

In summary, these compounds and techniques allow fine tuning of the available symptomatic therapy of motor and in part of NMS in PD. However, they do not represent a major innovation. True, highly needed innovations would be (1) a compound with disease-modifying properties in order to slow down, if not stop, the progressive pathophysiology of PD (that is, most likely the spreading of the alpha-synucleinopathy in the central, peripheral, autonomic, and gastrointestinal nervous system of patients with PD) and (2) the use proposed by this author of nanomedicine principles and technology to cross the BBB and deliver the appropriate compound(s) at the right location and in the right dose (as discussed later in this article).

New Research Vistas

Beginning in 1997, the world of PD research began changing dramatically with contemporaneous advances in genetics and nanomedicine.

Genetic research and the search for Parkinson's disease modifying therapy

For the first time, though very rare, an autosomal dominant mutation (termed PARK1) responsible for the protein alpha-synuclein was described (10). Shortly after this discovery, alpha-synuclein aggregates were identified in the Lewy bodies in the post-mortem SN samples of patients with idiopathic PD. Therefore, the majority of patients with idiopathic PD are now considered to suffer from an alpha-synucleinopathy. By 2016, at least eight monogenic causes for PD had been evidenced. The autosomal dominant forms relate either to a mutation of alpha-synuclein or to LRRK2, whereas autosomal recessive forms (PARK2, PINK1, DJ1) cause mitochondrial dysfunction. The third major discovery was the fact that 3–7% of patients with idiopathic PD carry a heterozygous mutation for the gene glucocerebrosidase A (GCS-A). Further, besides the role of alpha-synuclein, genome-wide association (GWA) studies have confirmed the importance of the microtubule-associated protein tau (MAPT) in the etiopathogenesis of PD. Still further, at least 28 genetic risk (susceptibility) factors have been identified, and it is likely that this number will further increase (11). These discoveries have already had a major impact on the development of new therapies, especially in regard to potentially disease-modifying compounds.

Gene therapy typically involves the use of a non-infectious virus (i.e., a viral vector such as the adeno-associated virus) to shuttle genetic material into a part of the brain. The gene used leads to the production of an enzyme that helps to manage PD symptoms or protect the brain from further damage. In 2010, there were four clinical trials using gene therapy in PD. So far, no important adverse effects in these trials have been reported although the clinical usefulness of gene therapy has not yet been established.

The Braak staging of Parkinson's disease

Based on the distribution of the Lewy bodies in the nervous system, Braak., *et al*. [12] postulated that, as defined with its motor symptoms, PD is most likely a late-stage phenotype of a disease which has been going on for decades.

The advantage of the Braak hypothesis is that it can clinically be tested. By carefully screening and following up patients at risk for PD, clinicians can identify a subgroup of patients with prodromal PD who present with and develop the sequence of symptoms related to the postulated prodromal PD stages. This type of study may lead to the discovery of endpoints for future neuroprotective trials in prodromal PD. However, the hypothesis has its own limitation for the post-mortem analysis of PD brains has shown, for example, that the density of Lewy bodies in the medullary areas is lower than in the cortex. In addition, a similar distribution of Lewy neuropathology is observed in patients with incidental Lewy body disease (that is, individuals with the hallmark Lewy pathology in brain who did not present when alive with motor Parkinson features). This observation does not appear to be consistent with a caudorostral spreading of alpha-synuclein aggregates. But if Lewy bodies are considered a mechanism to reduce the amount of soluble toxic alpha-synuclein oligomers in the cell, then the density of Lewy bodies in a given brain area may reflect its "defense" capability. In addition, if the caudorostral ascending process is tightly linked to the connectome of the involved structure, the locus coeruleus with its lack of connections

to the basal ganglia and, on the other hand, its strong projections to cortical areas, might drive the alpha-synuclein load of cortical areas many years longer than the SN might influence the alpha-synuclein load of the basal ganglia. This speculation is again testable in animal models and in post-mortem studies.

The spreading hypothesis

Combined with the "dual hit theory" [13], the Braak hypothesis proposes that PD starts either in the olfactory bulb and related areas or in the gastrointestinal system. Thus, a pathological agent would retrogradely reach the SN via an only recently discovered connection between the olfactory bulb and the SN [14]. Alternatively, it may move retrogradely from the gastrointestinal system up to the dorsal motor nucleus of the vagal nerve and would then propagate upwards in the brainstem reaching the locus coeruleus complex. Over the next 5 to 10 years, it would finally affect the SN.

Neuroprotective treatments

Investigations on neuroprotection are at the forefront of PD research. Several molecules have been proposed as potential treatments. However, none of them have been conclusively demonstrated to reduce degeneration. Agents currently under investigation include (a) anti-apoptotics (Omigapil, CEP-1347), (b) antiglutamatergics, (c) monoamine oxidase (MAO) inhibitors (Selegiline, Rasagiline), (d) promitochondrials (coenzyme Q10, creatine), (e) calcium channel blockers (Isradipine) and (f) growth factors (GDNF). Preclinical research also targets alpha-synuclein. A vaccine that primes the human immune system to destroy alpha-synuclein, PD01A was developed by the Austrian company Affris) and has entered clinical trials in humans.

Neural transplantation

Since the early 1980s, fetal, porcine, carotid or retinal tissues have been used in cell transplants in which dissociated cells are injected into the SN in the hope that they will incorporate themselves into the brain in a way that replaces the dopamine-producing cells that have been lost. Although there was initial evidence of mesencephalic dopamine-producing cell transplants being beneficial, double-blind trials to date have indicated that cell transplants produce no long-term benefit. An additional significant problem was the excess release of dopamine by the transplanted tissue, leading to dystonias.

Stem cell transplants

Stem cell transplants are a recent research target because stem cells are easy to manipulate and, transplanted into the brains of rodents and monkeys, have been found to survive and reduce behavioral abnormalities. Nevertheless, use of fetal stem cells is controversial. It has been proposed that effective treatments may be developed in a less controversial way by use of iPSCs taken from adults.

Transcranial magnetic stimulation

Repetitive transcranial magnetic stimulation (TMS) temporarily improves Levodopa-induced dyskinesias. Its usefulness in PD is an open research topic.

Nutrients

Several nutrients have been proposed as possible treatments; however, there is no evidence that vitamins or food additives improve symptoms.

The search for prodromal stages of Parkinson's disease

In the clinical situation, according to Braak., *et al*. manifest PD is preceded by years, if not decades, by prodromal phases (the phases related to an early or premonitory symptom of the disease). To screen for prodromal (premotor) phases, the NMS hyposmia, constipation, depression, and the REM sleep behavior disorder (RSBD) are now considered prodromal indicators. Whereas the first three are sensitive but not specific, RSBD is now accepted as the most specific phenotype of the PD prodromal phases with a risk of more than 80% to convert into PD, or dementia with Lewy bodies (DLB) or less frequently into multiple system atrophy (MSA) 10 to

15 years later. Similar research on prodromal stages takes place with "at-risk relatives" of Parkinson patients, who are either heterozygous for the LRRK2 gene or are homozygous for one of the autosomal-recessive genes for a mitochondrial dysfunction in PD (PAKR2, PINK1, DJ1). For a comprehensive review of Mendelian and non-Mendelian inheritance of PD, see Hernandez., *et al*. [15-18].

The search for disease-modifying therapy

Two therapeutic strategies are currently followed. The first is based on epidemiological findings and large clinical prospective trials reporting a correlation between a reduced occurrence or prevalence (or both) of PD and the consumption of compounds such as caffeine or nicotine [19]. Table 3 cites examples of these generic substances with a postulated disease-modifying potential for PD.

Compound	Indication	Mode of action	Phase of development	Reference
Caffeine (University of Montreal, Canada [C])	Motor early Parkinson's disease (PD)	Adenosine-receptor antagonist	Phase IIIb ongoing	Wills., *et al*. (2013) Postuma., *et al*. (2012)
Inosine (Michael J. Fox Foundation MJFF [C])	Motor early PD	Precursor of urate, antioxidant	Phase Iib ongoing	Bhattacharyya., *et al*. (2016) Ascherio., *et al*. (2009)
Isradipine – STEADY-PDIII (NIH-NINDS, Novartis, University of Chicago [C])	Motor early PD	Dihydropyridine calcium channel blocker	Phase IIIb ongoing	Simuni., *et al*. (2016) Simuni., *et al*. (2013)
Nicotine - NIC-PD (German Parkinson Study Group, Parkinson Study Group USA; MJFF, IPF, NP, DPG, Novartis Germany [C])	Motor de novo PD	Cholinergic, modulation of α-synuclein aggregation?	Phase IIIb completed	Oertel., *et al*. (2016) Quik., *et al*. (2008) Hong., *et al*. (2009)

Source: Oertel and Schulz (2016)

Table 3: *Therapy with compounds of disease-modifying potential (generic substances).*

The second approach relates to the groundbreaking genetic discoveries in PD (see previous sub-section). In fact, a dramatic shift in the strategy for developing a new PD therapy has taken place: pharmaceutical efforts now target alpha-synuclein protein synthesis, degradation (such as autophagia, lysosomal, or proteasomal degradation), protein aggregation, and propagation in the nervous system. Finally, 20 years after the discovery of PARK1, the academic and pharmaceutical industrial scientific community can offer the first candidates with a potential for a disease-modifying effect in PD.

Three different principles of therapeutic action are addressed: (1) active or passive immunotherapy, (2) modulation of alpha-synuclein aggregation, and (3) enhancement of autophagy of alpha-synuclein (Table 4).

The first approach mimics a strategy that has been followed in Alzheimer's disease for the last decade: active and passive immunizations are being developed as therapeutic approaches. This immunotherapeutic strategy relies on the assumption that (a) alpha-synuclein is accessible in the extracellular space (trans-synaptic spreading), (b) antibodies against alpha-synuclein reach the brain in sufficient quantity, and (c) they trap alpha-synuclein aggregates when these are released ("spread") into the extracellular synaptic space. Today, active and passive immunization trials are under way in phases I and II. These treatments have passed the safety level testing, and the first data on phase II trials are awaited in 2017- 2019. One limitation of active and passive immunotherapy, the low amount of antibodies passing the BBB, may be overcome by coupling antibodies to the peptide penetratin, as has recently been reported in a mouse PD model [20].

In the modulation of alpha-synuclein aggregation approach, modulating the aggregation of alpha-synuclein aims to block or reduce the aggregation of its monomers to oligomers or later on to fibrils. Two drugs are close to or under very early development. The first

compound (called ANLE138b) has been demonstrated to be able to reduce the aggregation of alpha-synuclein. In addition, in a mouse model with an A30P alpha-synuclein mutation, the compound extends survival. For the second drug (NPT200-11), only abstracts on its efficacy in preclinical testing are in the public domain. This latter compound again reduces aggregation of alpha-synuclein at least in vitro and, according to public information, should have reached the very first safety testing in humans. A third compound (NPT100-18a) has been reported to displace alpha-synuclein from membranes, but is still in preclinical testing. The advantage of these small molecules is that, in variance to antibodies employed in immunotherapeutic attempts, they readily pass the blood-brain barrier.

In the third approach, other newly developed compounds promise to enhance autophagy of alpha-synuclein. They are still in preclinical testing, although screening of libraries of registered compounds may well reveal further potential members of this group.

Compound	Indication	Mode of action	Phase of development	Reference
Immunotherapy (IT)				
Active immunization (Affiris [C])	Motor	IT	Phase II ongoing	Schneeberger., *et al.* (2016) Manoutcharian., *et al.* (2016)
Passive immunization (PI) (Biogen [C]) (Parthena/Roche [C])	Motor	IT	Phase II in preparation Phase II in preparation	Weihofen., *et al.* (2016) Bergström., *et al.* (2016) Kalia., *et al.* (2015) Games., *et al.* (2014) Spencer., *et al.* (2016)
Alpha-synuclein aggregation modulators (ASAM) NPT200-11 (UCB/Neuropore [C])	Motor? likely in de novo Parkinson's disease (PD)	ASAM	Phase I in planning	Koike., *et al.* (2014) Szoke., *et al*. (2014)
NPT100_18a (Neuropore [C])	Not applicable	ASAM	Preclinical testing	Wrasidlo., *et al.* (2016)
ANLE 138b (MODAG [C])	Motor? likely in de novo PD	ASAM	Phase I in planning	Deeg., *et al.* (2015) Levin., *et al.* (2014) Wagner., *et al.* (2013)
Alpha-synuclein autophagia enhancer (ASAE)				
Nilotinib Tasigna off-label use (Georgetown University, Washington, DC, USA MJFF [C])	Motor non-motor	"Tyrosine kinase inhibitor" ASAE	Investigator initiated trial– open-label small pilot study randomized controlled trial in planning (MJFF-USA, Cure PD Trust, UK)	Pagan., *et al.* (2016) Hebron., *et al.* (2014) Hebron., *et al.* (2013)

Source: Oertel and Schulz (2016)

Table 4: *Therapy with compounds targeting alpha-synuclein.*

In summary, the field has steadily shifted from developments on symptomatic therapy to preventive therapy, with at least five different options: (1) active immunization, (2) passive immunization, (3 and 4) two small molecules that function as alpha-synuclein aggregation modulators, and most recently (5) an autophagy enhancer with a known adverse profile (which is already registered in the field of oncology). Thus, for the very first time, the possibility of a disease-modifying therapy appears to be testable in PD. Taking together the discoveries on the genetic background of PD and the Braak staging hypothesis, new avenues for drug development and clinical testing have opened up. In the next few years of clinical testing, potential disease-modifying compounds will be tested in the early stage of motor PD. Here, *de novo* PD patients who never received a symptomatic therapy will be recruited and should present with a unilateral asymmetric very mild motor symptomatology.

However, for "true" neuroprevention (that is, the prevention or delay of the conversion of a prodromal stage to the motor stage of PD), parameters and biomarkers which reflect the progression of the alpha-synucleinopathy in the prodromal stage have yet to be discovered. In addition, such a parameter must be responsive to therapy, even in the prodromal stage, in order to qualify as a primary endpoint for pivotal registration trials. At present, such a parameter has not been identified. Respective research ranges from studies on biomarkers in the CNS, peripheral blood, saliva, and sweat and in biopsies of the colonic enteric nervous system, the salivary gland, or the skin. Major efforts are placed into different imaging techniques with sophisticated MR methods, nuclear medical ligands for the dopamine transporter, MR single-positron emission computed tomography (SPECT) or fluoro-desoxyglucose positron emission (FDG-PET). At least in the next few years of clinical testing, potential disease-modifying compounds are and will be tested in the early stage of motor PD.

Mitochondria

Several lines of research suggest that mitochondria may play a role in the development of PD. Mitochondria are the energy-producing components of the cell and abnormalities in the mitochondria are major sources of free radicals (molecules that damage membranes, proteins, DNA, and other parts of the cell). This damage is often referred to as oxidative stress (OS). OS-related changes, including free radical damage to DNA, proteins, and fats have been detected in the brains of individuals with PD. Some mutations that affect mitochondrial function have been identified as causes of PD. While mitochondrial dysfunction, oxidative stress, inflammation, toxins, and many other cellular processes may contribute to PD, the actual cause of the cell loss death in PD is still undetermined.

Scientists funded by the U.S. National Institute of Neurological Disorders and Stroke (NINDS, see the Appendix), a component of the U.S. National Institutes of Health (NIH), have found that hundreds of genes involved in mitochondrial function are less active in people with PD. Drugs that target genes involved in mitochondrial function could perhaps slow progression of the disease.

Animal models

PD is not known to occur naturally in any species other than humans, although animal models which show some features of the disease are used in research. In the early 1980s, the appearance of parkinsonism in a group of drug addicts who consumed a contaminated batch of the synthetic opiate MPPP led to the discovery of the chemical MPTP as an agent that causes parkinsonism in non-human primates as well as in humans. Other predominant toxin-based models employ the insecticide rotenone, the herbicide paraquat and the fungicide maneb. Models based on toxins are most commonly used in primates. Transgenic rodent models that replicate various aspects of PD have been developed. Using the neurotoxin 6-hydroxydopamine, also known as 6-OHDA, a model of PD in rats was created by targeting and destroying dopaminergic neurons in the nigrostriatal pathway when injected into the substantia nigra.

The Nanomedicine Approach

In a separate article [21], I discussed the fact that of the approximately 400 known neural disorders, a number of these may be due to a disruption or failure of the BBB, including Parkinson, Alzheimer, epilepsy, brain abscess, cerebral edema, De Vivo, HIV encephalitis, meningitis, multiple sclerosis, neuromyelitis optica (Devic's disease), prion and prion-like diseases, progressive multi-focal leukoencephalopathy, rabies, systematic inflammation, tripanosomasis, and others (see Table 1 of 21). In the case of Parkinson, the penetration mechanism of the BBB is still rather unknown.

The brain protective barriers

The brain has five protective barriers (BPB) that hinder the delivery of therapeutic drugs. They describe the five main interfaces between the central nervous system (CNS) and the periphery. These include: the blood brain barrier (BBB) that extends down the spinal cord; the brain cerebrospinal fluid (CSF) barrier [B(CSF)B]; the brain-inner CSF barrier [B(iCSF)B]; the brain-outer CSF barrier [B(oCSF)B]; and the brain retinal barrier [B(R)B]. We shall mostly be concerned with the BBB.

All interfaces are physical and metabolic barriers that serve to regulate and protect the microenvironment of the brain. Composed of a monolayer of brain capillary endothelial cells, they are formed by tight junctions. In the case of the BBB, the tight junctions are

between the endothelial cells of the primary vasculature with primary manifestation being the impermeability of the capillary wall due to the presence of the junctions and a low endocytic activity. There is a relative paucity of fenestrae and pinocytotic vesicles that restrict brain uptake of circulating molecules.

Thus, the BBB limits access to the brain to small nonpolar molecules by passive diffusion or catalyzed transport of large and/or polar molecules. It hinders the delivery of most pharmaceuticals (diagnostic, therapeutic agents) to the brain. ABC efflux transporters at the BBB influence the brain uptake of a variety of therapeutic agents.

Possible links between Parkinson's disease and the blood brain barrier

In the case of PD, we have already reached three important conclusions. One, Dopamine does not cross the BBB so it cannot be taken as a medicine to boost the brain's depleted levels of Dopamine. However a precursor of Dopamine, Levodopa, can pass through this barrier to the brain where it is readily converted to Dopamine. Administration of this drug temporarily diminishes the motor symptoms of PD. Unfortunately, only 5–10% of the drug crosses the barrier with much of the remainder being metabolized to Dopamine elsewhere in the body, where it causes a variety of side effects. Next, the immunotherapeutic strategy for PD therapy relies on the assumption that (a) alpha-synuclein is accessible in the extracellular space (trans-synaptic spreading), (b) antibodies against alpha-synuclein reach the brain in sufficient quantity, and (c) they trap alpha-synuclein aggregates when these are released ("spread") into the extracellular synaptic space. However, one important limitation of active and passive immunotherapy is that the low amount of antibodies passing the BBB, may be overcome by coupling antibodies to the peptide penetratin, as has recently been reported in a mouse PD model. Lastly, modulating the aggregation of alpha-synuclein aims to block or reduce the aggregation of its monomers to oligomers or later on to fibrils. Three drugs are close to or under very early development (ANLE138b, NPT200-11, and NPT100-18a). The advantage of these small molecules is that, in variance to antibodies employed in immunotherapeutic attempts, they readily pass the BBB. Thus, being able to traverse or bypass the BBB while delivering therapeutic compounds at the right locations in the right dosage amounts would herald a new approach to the treatment of PD. This is what nanomedicine (NM) and nanotechnology (NT) promise to do [22-23]. However, while the technology is now well known, its application to PD has not yet been undertaken.

Drug resistance and the possible role of the BBB obviously remain an important research focus. Additionally, a compromised BBB has been associated with seizures in a number of disorders. Not only congenital defects, such as GLUT1 deficiency, but acquired deficiencies, like those resulting from brain tumors, head trauma, etc., often result in seizure disorders. More recently systemic and immune triggers have been implicated in a leaky BBB and neuroinflammation. Understanding the nature of the role of BBB in these disorders is imperative in the treatment of the associated disease, but the fundamental question of whether the compromised integrity of the BBB is a component of the etiology of PD or a consequence of it remains unanswered.

Diagrammed in Figure 2 of [21] are selected connections between epilepsy and the BBB, highlighting topics of interest , including: 5-HT: 5-hydroxytryptamine (serotonin); T cell (T); Mast cell (M); tumor necrosis factor (TNF); multi-drug resistance-1 protein (MDR1); RalA Binding Protein 1 (RLIP76); multi-drug resistance protein family (MDRP); glucose transporter 1 (GLUT1); and BBB disruption. Can a similar diagram be drawn in the case of PD?

Bioavailability of therapeutic drugs

Permeability of the BBB is one of the factors determining the bioavailability of therapeutic drugs and resistance to chemically different anti-Parkinson drugs (APD). It becomes particularly relevant in drug-resistant patients. There are no known theories describing drug resistance in PD. BBB disruption after acute head trauma is a well-known pathologic finding in humans and also animals. This disruption may persist for weeks to years after the injury and may be associated with abnormal EEG activity. Whether this abnormal activity develops into PD is currently unknown, but observations have suggested BBB disruption in conjunction with a slowing in EEG activity may be a precursor to seizures or tremors. Others have observed persistent BBB disruption in the absence of any evidence of active seizure/tremor foci. It has been demonstrated that with relatively severe loss of BBB function there is extravasation of serum

albumin into capillary endothelial cells, basal lamina and neuropil. Thus, the BBB integrity is closely correlated to the electrophysiological properties of the tissue as evaluated by intra-operative EEG.

Drug Delivery across the Blood Brain Barrier

Approaches

Several approaches are available for drug delivery across the BBB. These have been described in detail and illustrated elsewhere [21-24]. They will not be reviewed again here but, for convenience, will be summarized. Table 5 provides a partial listing of therapeutics for drug delivery across the BBB while Table 6 summarizes the delivery systems employed.

Approach	Mediating factors	Results
Mannitol intracarotid diffusion	• Vasodilatation • Shrinkage of cerebrovascular endothelial cells • Modulation of the contractile state of the endothelial skeleton • Junction proteins by increased intercellular calcium	Marked increase in apparent BBB permeability to intravascular substances (factor 10) due to both increased diffusion and bulk fluid flow across the tight junctions
Use of immunosuppressants	• Drugs, metabolic derangements or hypoxic-ischemic injury • Concomitant hemodynamic disturbances (intracerebral hemorrhage or embolic stroke) • Loss of autoregulation of cerebral blood flow • Changes in intracranial pressure due to edema • Inflammation	• Seizures (transient and easily treated) • Lack of EEG data may actually underestimate the true impact of BBB failure on the breakdown of neuronal control
Physiological approach(es)	LDLRP/Epic (a low density lipoprotein/related protein) with engineered peptide compound	Improves transcytosis capacity of specific receptors expressed across the BBB

Source: Fymat (2017), (Reference 24)

Table 5: *Therapeutics for drug delivery across the blood brain barrier.*

Approach	Mediating factors
Chemical systems	• Lipid-mediated transport • Pro-drugs • Lock-in systems
Biological systems	Specific endogenous transporters located within the brain capillary endothelium
Other systems	• Receptor-mediated transport systems, e.g., endogenous peptides (insulin, transferin) • Solid lipids • Polymers • Mesoporous silica • Inorganic

Source: Fymat (2017), (Reference 24)

Table 6: *Delivery systems for drug delivery across the blood brain barrier.*

Nanotechnology applications

Both nanoparticles (NP) and nanodevices (ND) for delivering therapeutics are outlined below:

Nanoparticles

The layered NP consists of three components: (a) a core vesicle with a double-layered membrane. It is filled with water and hydrophilic and/or hydrophobic drugs; (b) a multi-layered shell; and an exterior shell that targets the NPs to areas identified as PD sites. The purposes of a multi-layered shell are: to stabilize the NPs; prevent drug leakage; target the NPs to the PD site; minimize the interactions of the NPs non-PD sites; and pass unnoticed by the immune system. The multi-layered NP can also transport drugs that are not easily stored in the core (e.g., highly charged nucleic acids). These molecules can be separated from drugs in the core that could inactivate their therapeutic effects (e.g., plasma drugs). The basic process to use drug delivery involves at least three steps: (i) Encapsulation of the drugs; (ii) Successful delivery of said drugs to the targeted region of the body; and (iii) Successful release of that drug there. NP-based delivery enables sophisticated tactics to fight disease. With their small size and intricate engineering design, they can improve control over drug release profiles, both spatially as well as temporally, and can reduce harmful side effects.

There are several clinical advantages to these NPs. Specifically, they:

- Circulate throughout the bloodstream without being attacked by the immune system;
- Are non-toxic as the platelet membranes are nanoparticle cores made of a biodegradable polymer that can be safely metabolized by the body; and
- Can be packed with many small drug molecules that diffuse out of the polymer core and through the platelet membrane onto their targets.

Several nanocarriers have been developed for drug delivery at the right address. However, challenges still remain, including: How not to let the medicine(s) act before they reach the right place. Carriers usually encapsulate drugs through long-range electrostatic interactions wherein the carrier attracts oppositely-charged medicines. Other tools are available to trigger the release of drugs, e.g. magnetic fields, different pH-values, etc., but, in each case, the problem of efficiency of the drug release remains. Nonetheless, work is still needed to determine the most effective NTs for brain tumors. Table 7 summarizes the various types of NPs and their indication(s):

Nanoparticle type	Indication(s)
Microspheres	
Bionanocapsules	
Radiolabeled polyethylene glycol-coated: • HexaDecylCyanoAcrylate; (HDCA) • PolyAlkylCyanoAcrylate (PACA) • PolyLacticCoGlycolic Acid (PLGA) • Peptidomimetic Monoclonal Antibodies; (PMA)	• Not be ready for clinical trials because of accumulation • Coated with polysorbate 80 or poloxamer 188
Magneto-Electric Nanoparticles	Wireless stimulation of cellls deep in the brain
Bioavailability-improved nanoparticles and molecules	
Maximization of bioavailability both at specific places in the body and over a period of time	Molecular targeting by nano-engineered device targeting molecules and delivering drugs with cell precision

Nutshells: • Platelet-coated NPs • Biocompatible/biodegradable gelatins • Shape-shifting engineered nanopaticles • Nanogels	Targeted by conjugated antibodies or peptides: • Can deliver higher doses of medication drugs to targeted sites in the body • Can de;liver multiple drugs bypassing the BBB • Can be tailored to specified sites and nowhere else • Non-sticking, with responsive shell permeability
Liposomes	
Peptides	• Able to cross the BBB through various mechanisms, e.g., Casomorphion (a heptapeptide)

Source: Fymat (2017), (Reference 24)

Table 7: *Various types of available nanoparticles able to contain therapeutic drugs.*

Table 8 lists the various delivery systems for new nanodevices being under current investigation and to become available in the future

Nanodevices	Action(s)
Engineered devices: • Improved pharmacokinetic strategies of drug molecules (biodistribution, bioavailability, controlled and site-specific drug release) • Decreased peripheral toxicity • Influence manufacturing factors (type of polymers and surfactants, particle size and size distribution, drug molecules) • Limitations of drug amount delivered, and physiological factors [phagocytic activity of the reticulo-endothelial system (RES), protein opsonization]	Potential to be engineered to efficiently and more safely deliver drug treatments directly to the location of diseased cells while helping avoid harm to healthy cells that fall victim to toxic drugs administered by conventional means
Miniaturized carriages: • Protein cages • Microbubbles • Multi-shell hollow nanogels with responsive shell permeability	• Created but challenge remains how not to let the medicine act before it gets to the right place in the brain • Triggers for drug release can be: external magnetic field, different pH values, etc.

Source: Fymat (2017), (Reference 24)

Table 8: *Nanoscale devices for drug delivery.*

Conclusions

The discovery of a mutation in the gene for the protein alpha-synuclein has simultaneously provided the cause of Parkinson disease (in both its motor and neuropsychiatric manifestations) and the explanation of the presence of pathological alpha-synuclein-aggregations in the Lewy bodies in the substantia nigra. The majority of Parkinson patients, even at the very early stage of neurological diagnosis, actually present a late-stage phenotype of an alpha-synucleinopathy. Consequently, the field has steadily shifted away from developments on symptomatic therapy to preventive therapy, with several different options: active immunization, passive immunization,

development of small molecules that function as alpha-synuclein aggregation modulators and, most recently, an autophagy enhancer with a known adverse profile. Thus, for the very first time, the possibility of a disease-modifying therapy appears to be testable. Taking together the discoveries on the genetic background and the Braak staging hypothesis, new avenues for drug development and clinical testing have opened up. In the next few years of clinical testing, we predict that potential disease-modifying compounds will be tested in the early stages of motor PD. However, the diagnostic methodology should identify a primary endpoint for clinical neuroprotective trials, not only in early motor PD but also in the prodromal stages of PD. For "true" neuroprevention (i.e., the prevention or delay of the conversion of a prodromal stage to the motor stage of PD), parameters and biomarkers which reflect the progression of the alpha-synucleinopathy in the prodromal stage have yet to be discovered. In addition, such a parameter must be responsive to therapy, even in the prodromal stage, in order to qualify as a primary endpoint for pivotal registration trials. At present, such a parameter has not been identified.

Deep brain stimulation and high-intensity focused ultrasound guided by magnetic resonance still have their place in the therapeutic armamentorium. However, the benefits of neural and stem cell transplantation need to be established. The proposed nanotechnological approach seems to overcome previous limitations of drugs crossing the blood brain barrier, namely (a) Dopamine does not cross the blood brain barrier so it cannot be taken as a medicine to boost the brain's depleted levels of Dopamine; (b) Levodopa, a Dopamine precursor, can pass through this barrier to the brain where it is readily converted to Dopamine. Unfortunately, only 5–10% of the drug crosses the barrier with much of the remainder being metabolized to Dopamine elsewhere in the body, where it causes a variety of side effects; (c) the immunotherapeutic strategy relies on the critical assumptions that alpha-synuclein is accessible in the extracellular space (trans-synaptic spreading), antibodies against alpha-synuclein reach the brain in sufficient quantity, and they trap alpha-synuclein aggregates when these are released ("spread") into the extracellular synaptic space. However, one important limitation of active and passive immunotherapy is that the low amount of antibodies passing the barrier may be overcome by coupling antibodies to the peptide penetratin. Three drugs are close to or under very early development (ANLE138b, NPT200-11, and NPT100-18a). The advantage of these small molecules is that, in variance to antibodies employed in immunotherapeutic attempts, they readily pass the BBB. Thus, being able to traverse or bypass the BBB while delivering therapeutic compounds at the right locations in the right dosage amounts would herald a new approach to the treatment of Parkinson's disease. This is what nanomedicine and nanotechnology promise to do (22-23). However, while the technology is now well known, its application to Parkinson has not yet been undertaken.

Appendix

Research Programs of the U.S. National Institute for Neurological Disorders and Stroke (NINDS)

The NINDS is a component of the (U.S.) National Institutes of Health (NIH). Its mission is to seek fundamental knowledge about the brain and nervous system and to use this knowledge to reduce the burden of neurological disease. It conducts and supports three types of research: basic (scientific discoveries in the laboratory), clinical (developing and studying therapeutic approaches to neurological disorders and stroke), and translational (focused on tools and resources that speed the development of therapeutics into practice. The goals of NINDS-supported research are to better understand and diagnose neurological disorders (including PD) and stroke, develop new treatments, and ultimately, prevent these disorders (including PD). NINDS also supports training for the next generation of researchers and clinicians, and serves as an important source of information for people with neurological disorders and stroke and their families. In January 2004, it hosted the conference themed "Parkinson's Disease 2014: Advancing Research, Improving Lives" to discuss the highest research priorities, ranging from laboratory discoveries to developing new therapies.

The Parkinson's Disease Biomarkers Programs (PDBP)

This is a major NINDS initiative aimed at discovering ways to identify individuals at risk for developing PD and to track the progression of the disease. Identifying biomarkers (signs that may indicate risk of a disease and improve iagnosis) will speed the development of novel therapeutics. Projects are actively recruiting volunteers at sites across the U.S. The NINDS also collaborates with the Michael J. Fox Foundation for Parkinson's Research (MJFF) on BioFIND, a project collecting biological samples and clinical data from healthy volunteers and those with PD.

The Morris K. Udall Centers of Excellence for Parkinson's Disease Research program

This is a central component of NINDS' PD research. It was established in 1997 to find the fundamental causes of PD and better diagnose and treat people with PD. Ten such Centers are currently funded across the U.S., where esearchers are examining PD disease mechanisms, the genetic contributions to PD, and potential therapeutic targets and treatment strategies.

Parkinson's Disease Clinical Studies

Clinical studies offer an opportunity to help researchers find better ways to safely detect, treat, or prevent PD and therefore hope for individuals now and in the future. NINDS conducts in-house clinical studies and supports PD studies at medical research centers throughout the U.S.

Genetic studies

A better understanding of genetic risk factors is playing a critical role in elucidating PD disease mechanisms. A 2011 NINDS workshop led to an analysis of data from PD GWA studies around the world, to correlate genetic variants and common traits among people with PD. The workshop contributed to the development of NeuroX, the first DNA chip that can identify genetic changes in persons at risk for a number of late-onset neurodegenerative diseases, including PD. Another NINDS collaborative, the Consortium on Risk for Early-Onset Parkinson's Disease (CORE PD), hopes to identify the genetic factors that contribute to the development of early-onset PD. Current clinical studies include the genetic connection to memory and motor behavior, the search for genes that may increase the risk of PD and related neurodegenerative disorders, and identifying biomarkers for PD.

Gene therapy and nerve growth factors

Nerve growth factors are proteins involved in nervous system formation and are of interest to researchers studying neurodegenerative diseases. One small clinical trial will assess the safety, tolerability, and potential clinical effects of gene therapy with Glial Derived Neurotrophic Factor (GDNF), a protein that may help protect dopamine-producing nerve cells.

Stem cell transplants

Scientists are exploring various types of cells, including induced pluripotent stem cells (iPSCs), as opportunities for PD drug discovery. iPSC technology is used to define disease mechanisms and discover the most promising treatments for sporadic PD. To pursue this area of research, NINDS established a PD cell research consortium in 2009 in collaboration with the Michael J. Fox Foundation and the Parkinson's Disease Foundation.

Neuroprotective drugs

NINDS supports basic, clinical, and translational research aimed at protecting nerve cells from the damage caused by PD. The NINDS-funded Neuro Next Network is designed to test new therapies and to validate biomarkers in a number of neurological disorders, including PD.

Motor complications

Involuntary movement, including dyskinesia (difficulty controlling intended muscle movement), as well as tremor, dystonia (involuntary muscle contractions), freezing of gait (inability to start walking), and other motor complications become evident as PD progresses. These symptoms are often difficult to treat. NINDS scientists have studied the safety and effectiveness of drugs and interventions in alleviating motor symptoms in persons with PD.

Deep Brain Stimulation

NINDS has been a pioneer in the study and development of DBS, which is now considered a standard treatment option for some people living with PD whose symptoms no longer respond to PD medications. While NIH supported research on brain circuitry was critical to the development of DBS, NINDS research continues to fine-tune the optimal site within the brain to implant the DBS electrodes to help even more people with PD regain function. Further, NINDS researchers are continuing to study DBS and develop ways of improving it.

Other clinical studies hope to determine the best part of the brain to receive stimulation and to determine the long-term effects of this therapy. In addition, NINDS-supported researchers are developing and testing improved mplantable pulse generators and conducting studies to better understand the therapeutic effect of neurostimulation on the brain.

Cognition and Dementia

Mild cognitive impairment is common in PD, sometimes in its early stages, and some people develop dementia in the disease's later stages. The NINDS has funded research using neuroimaging to predict which individuals with PD might develop cognitive impairment.

Animal models

These are valuable tools for scientists studying disease mechanisms to develop new treatments for people with PD. For example, a study of the drug Isradipine (which had been shown in animal models to have a protective effect on dopaminergic neurons) is being tested for a similar neuroprotective effect in humans.

Environmental studies

Risk factors such as repeated occupational exposure to certain pesticides and chemical solvents may influence PD's development. A NINDS-funded research consortium is hunting for environmental risk factors that increase susceptibility to developing PD before age 50.

Exercise

Exercise routines (including resistance training, stretching, and tai chi) are often recommended to help individuals with PD maintain movement and balance necessary for everyday living. A recent NINDS-funded study concluded that tai chi led to the greatest overall improvements in balance and stability for people with mild to moderate PD.

References

1. Fymat AL. "Parkinson and other movement disorders: A Review". *Current Opinions on Neurological Science* 2.1 (2017): 316-343.
2. Moser D., *et al*. "MR-guided focused ultrasound technique in functional neurosurgery: Targeting accuracy". *Journal of Therapeutic Ultrasound* 1.3 (2013).
3. Fishman PS and Frenkel V. "Treatment of movement disorders with focused ultrasound". *Journal of Central Nervous System Disease* 9 (2017).
4. Bertrand C., *et al*. "Stereotactic surgery for Parkinsonism". *Progress in Neurological Surgery* 5 (1973): 79-112.
5. Jeanmonod D., *et al*. "Transcranial magnetic resonance imaging-guided focused ultrasound: noninvasive central lateral thalamotomy for chronic neuropathic pain". *Neurosurgical Focus* 32.1 (2012): E1.
6. Vykhodtseva N., *et al*. "MRI detection of the thermal effects of focused ultrasound on the brain". *Ultrasound Medical Biology* 26.5 (2000): 871-880.
7. Rodriguez-Oroz M., *et al*. "Bilateral deep brain stimulation in Parkinson's disease: A multicentre study with 4 years follow-up". *Brain* 128 (2005): 2240-2249.
8. Oertel W and Schulz JB. "Current and experimental treatments of Parkinson disease: A guide for neuroscientists". *Journal of Neurochemistry* 139 Suppl.1 (2016): 325-337.
9. Seppi K., *et al*. "The MovementDisorder Society Evidence-Based Medicine Review Update: Treatments for the non-motor symptoms of Parkinson's disease". *Movement Disorders* 26.Suppl 3 (2011): S42-80.
10. Polymeropoulos MH., *et al*. "Mutation in the alpha-synuclein gene identified in families with Parkinson's disease". *Science* 276.5321 (1997): 2045-2047.
11. Dafsari HS., *et al*. "Beneficial effects of bilateral subthalamic stimulation on non-motor symptoms in Parkinson's disease". *Brain Stimulation* 9.1 (2016): 78-85.
12. Braak H., *et al*. "Staging of brain pathology related to sporadic Parkinson's disease". *Neurobiology of Aging* 24.2 (2003): 197-211.

13. Hawkes CH., *et al*. "Parkinson's disease: A dual-hit hypothesis". *Neuropathology and Applied Neurobiology* 33.6 (2007): 599-614.
14. Hoglinger GU., *et al*. "A new dopaminergic nigro-olfactory projection". *Acta Neuropathologica* 130.3 (2015): 333-348.
15. Hernandez DG., *et al*. "Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance". *Journal of Neurochemistry* 139. Suppl 1 (2016): 59-74.
16. Feng LR and Maguire-Zeiss KA. "Gene Therapy in Parkinson's Disease: Rationale and Current Status". *CNS Drugs* 24.3 (2010): 177-192.
17. Lewitt PA., *et al*. "AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial". Lancet Neurolology 10.4 (2011): 309-319.
18. Redmond DE. "Cellular replacement therapy for Parkinson's disease – where we are today?" *The Neuroscientist* 8.5 (2002): 457-488.
19. Hellenbrand W., *et al*. "Smoking and Parkinson's disease: A case-control study in Germany". *International Journal of Epidemiology* 26.2 (1997): 328-339.
20. Hauser RA., *et al*. "Tozadenant (SYN115) in patients with Parkinson's disease who have motor fluctuations on Levodopa: A phase 2b, double-blind, randomised trial". Lancet Neurology 13.8 (2014): 766-767.
21. Fymat AL. "Neurological disorders and the blood brain barrier: 1. Epilepsy". *Current Opinions on Neurological Science* 1.6 (2017): 277-293.
22. Fymat AL. "Nanoneurology: Drug delivery across the brain protective barriers". *Journal of Nanomedicine Research* 5.1 (2017):1-4.
23. Fymat AL. "Therapeutics delivery Behind, through and beyond the blood brain barrier". *Open Access Journal of Surgery* 5.1 (2017): 1-8.
24. Fymat AL. "Neurological Disorders and the Blood Brain Barrier: 1. Epilepsy". *Current Opinions on Neurological Science* 1.6 (2017): 277-293.