Expert Opinion

Central & Peripheral Nervous Systems

Potential therapeutic targets for Parkinson's disease

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Background: Parkinson's disease is a common disorder that becomes more prevalent with advanced age. The cardinal features are related to dopamine deficiency, arising from loss of neurons projecting from the substantia nigra in the midbrain to the striatum. Therapies based on dopamine replacement are well established but while highly effective, leave a number of currently unmet needs. These include features of disease that are probably not related to dopamine deficiency and are unresponsive to dopamine-replacement therapy, as well as complications of long-term dopaminergic therapy itself. The most important gap is the availability of treatments that modify the inexorable progression of disease or that could prevent its onset in subjects at risk. *Objective*: To identify needs that are unmet or only partially addressed by currently available therapies for Parkinson's and select approaches that may be helpful for their management. *Methods*: Discussion of the mechanisms that may contribute to currently unmet needs in Parkinson's disease. Based on consideration of pathogenic mechanisms and a review of recent and previous relevant literature, identification of possible approaches that are in development, including pharmacological strategies and potential targets for gene therapy. *Conclusions*: Better treatments for levodopa-unresponsive aspects of Parkinson's will depend upon improved understanding of the pathophysiology of these complications. Dopamine-based therapies have been extensively developed and further improvements in treatment of established disease are likely to be based on modification of other neurotransmitter systems, including 5-hydroxytryptamine, adenosine receptors, amino acid receptors and possibly neuropeptides. The failure of neuroprotective and neurorescue strategies to keep pace with expectations probably reflects a combination of inadequate models of disease pathogenesis and poor biomarkers to assess the impact of these interventions. The development of novel therapies will be heavily dependent on improvements in these arenas. Most gene therapies under development will address the symptoms of Parkinson's disease but will at best only partially address the underlying disease.

Keywords: 5-hydroxytryptamine, adenosine receptors, animal models, apoptosis, biomarkers, dementia, dopamine, dyskinesias, excitatory amino acids, gene therapy, glutamic acid decarboxylase, kinase, mitochondria, neuroprotection, neurotrophins, Parkinson's, protein aggregation, ubiquitin-proteasome system

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1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, estimated to affect from 200 – 300 per 100,000 population and at least 1% of people age 65 or more. Although PD remains a serious source of disability, the ability to limit its impact was revolutionized nearly 50 years ago with the

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demonstration that the primary symptoms could be adequately treated by the dopamine precursor levodopa. Despite early difficulties with levodopa therapy, it is now firmly established as the most effective and probably overall best tolerated symptomatic treatment when combined with a peripheral decarboxylase inhibitor. However, despite (indeed probably because of) the profound impact levodopa has had on survival and quality of life in PD, its deficiencies have become more apparent as people live longer.

Levodopa is extremely effective for treating the rigidity and bradykinesia of PD and in many patients, the tremor as well. However, there remain core features that are minimally or not at all responsive to levodopa. These include speech and postural disturbances, autonomic dysfunction and cognitive impairment that may over time affect a majority of patients. To a certain extent, these problems may reflect involvement of non-dopaminergic neurons. For instance, while other mechanisms may play a role, dementia in PD is associated with loss of cholinergic innervation of cerebral cortex and may respond (as do visual hallucinations) at least in part to cholinesterase inhibitors. The basis for depression in PD is not established but could reflect loss of dopamine projections to limbic circuitry as well as involvement of other monoaminergic neurons such as serotonergic neurons of the brainstem. Other complications such as hypophonia and postural instability are probably multifactorial and while they may respond somewhat to dopamine replacement therapy, non-medical approaches such as voice training or physiotherapy may be more effective. Ultimately, however, better therapy for these important complications will require a better understanding of the underlying pathophysiological mechanisms.

Secondly, levodopa and other dopaminergic therapies are themselves associated with numerous complications over long-term usage [1,2]. Some of these, such as fluctuations in motor response and involuntary movements or dyskinesias, are probably related to an interaction between the disease itself and the therapy. Thus, dyskinesias are unlikely to occur in non-parkinsonian individuals exposed to levodopa. Others, such as confusion, hallucinations, psychosis and pathological behaviours such as punding, compulsive drug use and gambling [3], while quite possibly modified by the disease process, could presumably occur in any prone individual taking dopaminergic medication.

Finally, and most obviously, levodopa and other dopaminergic medications have no impact on the inexorable progression of PD, apart from their clear ability to prolong life. Previous concerns that levodopa might actually hasten the progression of PD have proved to be unfounded [4]. Likewise, claims that dopamine agonists may be associated with a slower rate of disease progression than levodopa have been based entirely on the use of neuroimaging as a surrogate marker of disease severity and have not been supported by the available clinical data [5,6]. Open-label follow-up has suggested that patients treated with the propargylamine monoamine oxidase-B inhibitor rasagiline following a 6-month delay in initiation of therapy did not attain the same degree of improvement as those treated with active medication from the start [7]. This may be interpreted as evidence of neuroprotective effects, in keeping with earlier suggestions of disease modifying effects from this class of drugs [8,9] but the latter trials have been confounded by concerns regarding symptomatic benefit.

In this review, the author will consider potential targets in each of these three categories.

2. Disability arising from non-dopaminergic mechanisms in PD

2.1 Speech

Speech is commonly affected in PD, with low volume, loss of prosody and a tendency to hasten, with progressive decline in volume and clarity ('festination' of speech). Although it is widely held that the speech deficits of PD are resistant to levodopa, some measures of clarity and prosody are improved with medication [10-12]. Surgery is similarly generally unhelpful and may indeed result in profound hypophonia or aphonia (particularly bilateral thalamotomy or pallidotomy) but in some cases, although usually deemed unhelpful [13], deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been beneficial [12,14]. Modification of speech (Lee Silverman Voice Therapy) is widely accepted and benefits have been demonstrated in a limited number of controlled trials [15,16]. As the basis for speech deficits in PD is poorly understood and the impairment of communication represents a major source of disability for both patients and those close to them, any therapeutic development in this area would be seen as a major development.

2.2 Postural instability

Postural instability is one of the cardinal features of PD but by definition does not appear until later stages. Although generally regarded as relatively unresponsive to dopaminergic therapy, its appearance is also universally seen as a clear indication for the initiation of levodopa in patients not already taking this medication. The basis for postural instability in PD is not well established. Secondary changes in inputs to other basal ganglia structures such as the pallidum and superior colliculus may be important, as are abnormalities in sensory and attentional processing. However, recent reports of benefit following high frequency stimulation of the pedunculopontine nucleus [17,18] suggest that impaired function of this structure, either primary or secondary, may play a key role. The pedunculopontine nucleus comprises both glutamatergic and cholinergic neurons. There is to date no convincing evidence that manipulation of either of these neurotransmitter systems with medication has any impact on postural control. The effects of subthalamic nucleus stimulation on gait and postural stability are much more variable [13,19].

2.3 Sleep disturbance

Sleep disturbance in PD is probably multifactorial. Immobility, akathisia and pain due to muscle rigidity are all presumably symptoms of dopamine deficiency and may respond to relatively minor modification of standard anti-Parkinson therapy. It is important to exclude depression as a basis for disrupted sleep. Although the pathophysiological basis for depression in PD is poorly understood, it is generally managed with standard antidepressants. In recent years, there has been a dramatic increase in the recognition of REM sleep behaviour disorder (RBD) as a common manifestation of PD that may indeed precede the appearance of motor abnormalities [20]. The usual therapies are clonazepam or gabapentin but some patients may be managed by adjusting the timing of their dopaminergic medication. It appears likely that RBD may be associated with reduced function in cholinergic projections from the pedunculopontine nucleus to the thalamus [21]. However, while some studies have reported benefits in response to cholinesterase inhibitors [22], others have reported either no benefit or even exacerbation.

2.4 Cognitive impairment

A majority of PD patients will exhibit deficits in frontal-subcortical executive function, even in the absence of dementia. Estimates of the frequency of dementia range considerably but while a figure of 20 – 30% is widely accepted, some investigators take the view that were they to live long enough, most PD patients would eventually succumb [23]. While there may undoubtedly be other factors, loss of cholinergic projections to cortex in PD with dementia is significant and indeed greater than that seen in Alzheimer's disease [24,25]. Cholinesterase inhibitors have been shown to help with the cognitive deficits and activities of daily living in dementia associated with PD, and may be particularly helpful for visual hallucinations [26]. Despite theoretical concerns of deterioration in motor manifestations, they have in general proved to be well tolerated apart from some exacerbation of tremor.

3. Complications of dopaminergic therapy

3.1 Fluctuations in therapeutic response

Although virtually all patients with PD (as opposed to those with parkinsonism arising from other conditions such as multiple system atrophy or progressive supranuclear palsy) will respond to levodopa, a significant proportion will develop fluctuations in the response to their medication within 5 years. Several medical approaches can be taken, including the adjunctive use of direct-acting dopamine agonists or medications that prevent the breakdown of levodopa (inhibitors of catechol *O*-methyltransferase) or dopamine (inhibitors of monoamine oxidase) [27]. The role of these approaches is well established and will not be considered further here. Although not practical for most patients, it is possible to infuse levodopa continuously [28]. Transdermal administration of a dopamine agonist may also allow for more continuous delivery of medication and should theoretically reduce motor fluctuations and dyskinesias [29].

An emerging approach is the modulation of other neurotransmitters that act downstream of dopamine in the striatal outflow pathways. A well-developed example of such an approach is the blockade of adenosine 2A (A_{2A}) receptors. The A_{2A} receptor is expressed on medium spiny neurons of the indirect pathway and thereby positioned to modify the response to dopamine D2 receptors [30]. Blockade of A_{2A} receptors has only a modest effect on PD symptoms on its own but as an adjunct, this approach significantly prolongs the therapeutic response to levodopa and dopamine agonists [31-34]. Evidence from animal models suggests that adenosine receptor blockade may also have a neuroprotective role in PD [35].

Chronic dopaminergic stimulation also modifies the phosphorylation state of striatal *N*-methyl-D-aspartate (NMDA) receptors [36]. In experimental models of PD, blockade of NMDA receptors prevents abnormal serine phosphorylation of GluR1 receptors and prolongs the behavioural response to an individual dose of levodopa [37,38].

3.2 Dyskinesias

Dyskinesias are thought to arise from pulsatile stimulation of dopamine receptors in both the direct and indirect striatal outflow pathways. Although there is no consistent change in expression of dopamine receptors (with the possible exception of D3 receptors in rodent models of sensitization [39]), there is an increase in the expression of the transcription factor deltaFosB [40,41] and of neuropeptides [42-45]. While it is clearly possible to manage dyskinesia either by reducing the dose of dopaminergic medication or by administering a dopamine antagonist, these strategies will generally result in worsening parkinsonism. In theory, potential approaches might include the use of a selective dopamine D1 or D3 receptor antagonist (or the use of a D3 partial agonist) [46-48] but these have not had demonstrated benefit in clinical trials to date.

Although studies in animal models suggested that stimulation of 5-hydroxytryptamine $(5HT)_{1A}$ receptors might be beneficial in dyskinesias and this appeared to be the case in open-label studies in humans [49], a large randomized, double-blind placebo-controlled trial failed to confirm this [50]. Indeed, the placebo effect in that study was considerable [51], highlighting the importance of controlling for placebo effects in the assessment of novel therapies. In monkeys with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism, stimulation of $5HT_{1A+B}$ receptors also appears promising [52]. Drugs that block $5HT_{2A}$ and/or $5HT_{2C}$ receptors are effective in rodent and primate models of levodopa-induced dyskinesia. However, many of the drugs currently available for use in humans act at multiple sites, including dopamine

receptors. Preliminary reports suggest that combined stimulation of $5HT_{1A}$ receptors and blockade of $5HT_2$ receptors with mirtazapine will suppress dyskinesias in PD patients [53] but such reports must be viewed with caution unless substantiated by larger controlled trials. The experience with sarizotan indicates that the placebo effect may be quite powerful in suppression of levodopa-induced dyskinesia [50].

There are close interactions between nigrostriatal dopamine projections and excitatory corticostriatal projections onto dendritic spines in the striatum. Dyskinesias are associated with altered expression of NMDA receptors in the striatum [54], decreased D1/NMDA receptor complexes [55] and loss of synaptic depotentiation [56]. Although non-selective blockade of NMDA receptors is a potential approach to the problem (and weak NMDA antagonists such as amantadine have indeed been consistently demonstrated to be helpful for dyskinesias in clinical studies [57]), this strategy is potentially difficult owing to the widespread expression of the NMDA receptor, as well as the possibility of interfering with crucial NMDA-mediated functions such as learning. The NR2B subtype is preferentially expressed in the striatum and in experimental models selective antagonists reduce dyskinesias without exacerbating parkinsonism [58], even though the ratio of NR2A to NR2B receptors is increased in association with dyskinesia [54,59]. Interactions between the NR2B receptor and membrane-associated guanylate protein kinase (MAGUK) proteins are altered in dyskinesia [59]. A related and interesting target for treatment of dyskinesias is the NMDA receptor-associated scaffolding protein postsynaptic density protein 95 (PSD-95), possibly via an interaction with dopamine D1 receptors [60].

Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and metabotropic glutamate receptor subtypes are also an interesting target for levodopa-induced dyskinesias [61], particularly in view of recent evidence suggesting that the emergence of dyskinesias is associated with alterations in synaptic plasticity.

4. Approaches to the modification of disease progression

This is the most important and potentially most fruitful area for development but also the area most abounding in failures. To date, there has been no convincing evidence that any of the multitude of approaches suggested has had any effect on the underlying progression of PD.

Although taken together, genetic forms represent only a minority of all PD, the mechanisms involved may be relevant to sporadic disease. Of particular importance is an understanding of the common mechanisms that link the various monogenic forms of disease. However, as it is already clear that there are several causes of PD, the idea that a single therapeutic approach may successfully modify disease may be somewhat naive. On the other hand, it is entirely possible that several different aetiologies could unleash a cascade of events resulting in initial and regionally selective death of dopamine neurons but a relatively limited number of non-selective mechanisms could explain the progressive loss of neurons rendered more susceptible.

The first dominantly inherited mutation in PD to be described was an A53T modification of the gene encoding α-synuclein [62]. Mutated α-synuclein forms aggregates and renders dopamine neurons more prone to oxidative stress. Although α-synuclein mutations represent only a small proportion even of dominantly inherited PD, sporadic disease is generally associated with the deposition of α-synuclein-positive aggregates in dopamine neurons of the substantia nigra and other brainstem neurons. It is still a matter of some controversy as to whether aggregated α-synuclein is toxic or whether the formation of aggregates is a protective mechanism [63,64]. If the former, a compound that inhibits protein aggregation, possibly the formation of protofibrils, would be an interesting target for disease progression [65]. To date, no such molecule has been developed and there remain concerns that such an approach could actually hasten disease by interfering with endogenous mechanisms to sequester toxic proteins. Recent evidence suggests that α-synuclein toxicity is mediated by Sirtuin-2, a member of the histone deacetylase family of proteins and that sirtuin-2 inhibitors inhibit toxicity both *in vitro* and in an *in vivo* transgenic Drosophila model of PD [66].

The other major dominantly inherited basis for PD is one of several mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2, the protein also known as dardarin, from the Basque for tremor) [67,68]. LRRK2 is an extremely large protein with multiple domains but it currently appears that toxicity may be mediated by a gain of kinase function [69]. Several attempts are currently underway to develop kinase inhibitors but it remains to be seen whether these will be selective enough to avoid unwanted effects. Furthermore, LRRK2 may have numerous other functions besides its role as a kinase and this approach may be overly simplistic [70].

The best-studied recessively inherited mutations leading to PD are those of parkin, phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-induced putative kinase 1 (PINK1) and DJ-1. Parkin is an ubiquitin E3 ligase important for proteasomal function [71]. Administration of proteasome inhibitors has been reported to result in selective loss of nigral dopamine neurons [72] but this finding has not been readily reproduced [73-76]. The implication is that promotion of proteasome function or alternatively, the identification of protein partners not adequately handled by the ubiquitin proteasome system (UPS) might be of benefit in preventing progression of PD. Parkin has functions besides its role in the UPS. For example, it is responsible for monoubiquitylation of epidermal growth factor receptor pathway substrate 15 (Eps15), which in turn inhibits

the ability of Eps15 to interact with ubiquitinated EGF receptor (EGFR) and promote EGFR internalization. Impairment of parkin function will disrupt this relationship and lead to increased endocytosis and degradation of the EGF receptor and could thereby potentially contribute to decreased neuronal survival [77]. Enhancement of the non-proteasomal ubiquitinating functions of parkin could potentially be of benefit.

In addition to the UPS, misfolded or aggregated proteins can be handled by lysosome-mediated autophagy and by molecular chaperones. There is a significant body of evidence to suggest that overexpression of molecular chaperones such as heat-shock proteins [78,79] or treatments that upregulate chaperone expression [80] may be beneficial in models of PD. This potential approach has been the topic of a number of recent reviews [81-84].

Although its precise function is unknown, PINK1 resides in the mitochondrial inter-membrane space and recent evidence suggests that it too may protect against oxidative stress by phosphorylation of the mitochondrial chaperone TNF receptor-associate protein 1 (TRAP1) [85]. In Drosophila, mutation of PINK1 results in a phenotype virtually identical to that seen with mutation of parkin. Both mutants can be rescued by expression of wild type parkin, while expression of wild type PINK1 will not rescue parkin mutants, suggesting that parkin (which is located in the cytosol) is acting downstream of PINK1 [86,87] and that therapies based on restoration or overexpression of parkin function may be of therapeutic benefit. DJ-1 is also a molecular chaperone that can inhibit the aggregation of α-synuclein in a redox-dependent fashion [88,89]. These properties of PINK1 and DJ-1 lend further support to the potential for developing therapies based on enhanced chaperone function.

Regardless of the upstream mechanism, nigral dopamine neurons are thought to undergo apoptotic death. Inhibitors of apoptosis therefore remain a therapeutic target of interest for PD as well as for other neurodegenerative disorders. To date, there has been no evidence that such agents confer an increased risk of malignancy. However, there has also been no evidence for therapeutic benefit. Initial enthuasiasm for the propargylamine monoamine oxidase (MAO)-B inhibitor selegiline has largely dissipated, as it became apparent that the majority of the benefits could be attributed to symptom relief rather than disease modification [90]. In a study of the newer propargylamine rasagiline, the group assigned to placebo followed by open-label active drug did not derive the same benefit as the group treated with rasagiline from study outset [91]. This difference in the delayed-start group could reflect a modest effect of medication on disease progression but must be verified by a prospective, fully blinded study, currently underway. For both selegiline and rasagiline, any disease-modifying effects are presumed to be related to their capacity to interfere with GAPDH-mediated apoptosis rather than inhibition of MAO-B. Another propargylamine devoid of MAO inhibitory properties recently failed to demonstrate any effect on disease progression in PD, despite ample benefits in animal models [92]. There was similarly no evidence of benefit (or harm) associated with the mixed lineage kinase inhibitor CEP 1347 [93].

Other mechanisms that might contribute to neuronal loss and progression in PD include mitochondrial failure, oxidative stress, excitotoxicity and inflammation. Attempts to boost mitochondrial function and limit oxidative stress have included the use of Coenzyme Q10. In the only properly controlled trial, the largest dose (1200 mg daily) of Coenzyme Q10 resulted in very modest reduction of the rate of clinical worsening in the first few months in patients who did not yet require symptomatic therapy, but no benefit was seen at lower doses, nor was there any significant delay in the requirement for levodopa [94]. A futility analysis conducted by the NET-PD group suggested that creatine and minocycline are worthy of further testing as neuroprotectants in PD, although the benefit seen in that study was marginal [95].

One very interesting and relatively straightforward approach has received recent attention in animal models. Dopamine neurons have an intrinsic pacemaker function that is determined by expression of the $Cav_{1.3}$ channel, which is only expressed later in development. Blockade of $Cav_{1.3}$ channels using isradipine results in temporary loss of pacemaker activity but within less than 2 h, this returns, now mediated by sodium channels. Isradipine was effective in reducing loss of dopamine neurons in MPTP and rotenone models in rodents but has not yet been tested in primate models or in less acute models of disease [96].

Attempts to interfere with excitotoxic nigral cell death have included the use of inhibitors of sodium-dependent glutamate release such as riluzole, which, however. failed to modify progression of early disease [97]. A number of AMPA receptor antagonists are currently in clinical trials as neuroprotectants. As lesions of the STN protect against the effects of 6-hydroxydopamine toxicity [98], there was some hope that deep brain stimulation of the STN might delay disease progression in human Parkinson's disease in addition to the beneficial effects on symptoms but the limited evidence available to date does not support this [99]. Gene therapy to modify the phenotype of the STN is discussed below.

There is ample evidence for abnormal inflammation in PD, based on animal models, as well as *in vivo* imaging and postmortem histological studies in humans [100]. There have been no prospective studies of anti-inflammatory therapies as neuroprotectants in PD. However, numerous case-control studies have demonstrated a lower risk of PD in people taking anti-inflammatory agents [101,102].

Neurotrophic factors held great hope for the prevention of disease progression, despite numerous technical challenges, not the least of which is adequate delivery of the molecule to the target site (striatum or substantia nigra).

The agent that has until recently attracted the greatest attention was glial cell line derived neurotrophic factor (GDNF). Initial studies of intracerebroventricular administration demonstrated no benefit and significant side effects [103]. However, based on studies in non-human primate models of MPTP toxicity, the effects of direct intraputaminal administration have been investigated. Unfortunately, initial promising results from open-label studies [104] were not substantiated in a double-blind placebo-controlled trial [105]. The related agent neurturin is currently being administered using recombinant adeno-associated virus gene therapy. Open-label studies have demonstrated considerable benefit [106] and a double-blind sham-surgery-controlled study is currently underway. Another approach to neurotrophic factor administration is to give a small molecule that crosses the blood–brain barrier and stimulates the production of endogenous neurotrophic factors. SR 57667B (paliroden) is such a molecule that was recently tested in a double-blind trial in early Parkinson's disease but there was no clinical or imaging evidence of disease modification [107].

5. Potential gene therapy targets for PD

Gene therapy for PD has received considerable attention in the last several months. As is the case for medical therapies, gene therapy may target either the symptoms of PD or attempt to modify disease progression. Treatment of symptoms has largely been based on the use of viral vectors to introduce either tyrosine hydroxylase [108] or aromatic amino acid decarboxylase [109], the latter is already in clinical trials. Attempts to interfere with disease progression have included viral delivery of GDNF or the related molecule neurturin [110]. Neurturin has demonstrated some promise in open-label studies but the results of an ongoing double-blind sham-surgery-controlled trial are required. GDNF was not beneficial in a double-blind study but some investigators feel the design was flawed. Gene therapy can also be used to modify the effects of aberrant protein aggregation. Thus, viral delivery of parkin [111,112] has been shown to reduce the toxicity of α-synuclein overexpression and it has been suggested that lentiviral delivery of beta-synuclein may confer similar benefit [113].

An interesting approach that primarily addresses symptoms but may also have an impact on disease progression has recently been reported. Kaplitt and colleagues have used rAAV2 to deliver glutamic acid decarboxylase (GAD) to the subthalamic nucleus, thereby converting it from a primarily excitatory (glutamatergic) structure to an inhibitory (GABAergic) one. In an open-label study conducted in 12 patients, this manipulation of STN phenotype resulted in symptom improvement [114]. This was associated with reversal of Parkinson-associated metabolic abnormalities on [^{18}F]2-fluoro-2-deoxyglucose positron emission tomography (PET) [115]. There were no significant treatment-related adverse events. However, apart from the lack of a placebo control, there were a number of other observations that suggest that caution is indicated before embracing this approach. Although the viral vector was introduced unilaterally, bilateral improvement was evident. Furthermore, unlike other surgical manipulations of STN function, improvement was seen in function while patients were on medication – usually, significant improvement is restricted to the off-medication state and surgical therapies do not further augment the benefit of optimal medication. There was no apparent effect of viral dose. The long-term effects of converting a structure that is usually excitatory into one that has an inhibitory function are not fully appreciated. Taking all these caveats into consideration, there is some preclinical evidence to suggest that the effects of STN rAAV-GAD therapy may be neuroprotective in a rodent model of PD [116]. This has not yet been demonstrated in human or non-human primate models and as noted above, there is to date no evidence to suggest that STN DBS is neuroprotective in human PD.

It should be noted that even if these provocative therapies ultimately prove to be beneficial, they are focused on either dopamine deficiency itself, or associated downstream changes in circuitry subserving motor functions of the basal ganglia. Thus, none of these approaches (with the possible exception of parkin overexpression) is likely to have an impact on degeneration of non-dopaminergic neuronal populations or its sequelae.

6. Other needs for therapeutic target development

Although the imperfections and deficiencies of dopaminergic medications are manifold, one should not lose sight of the fact that levodopa remains the single most effective and well tolerated medication for treating the cardinal manifestations of Parkinson's disease. Attempts to develop therapies that address the non-motor complications of advanced PD will depend to a large degree on a better understanding of the pathophysiology underlying these complications. However, the development of disease-modifying therapies has been stymied by two other significant deficiencies. One is the lack of well-validated biomarkers. Although functional imaging using either PET or single-photon emission computed tomography (SPECT) can provide valuable information on the level of striatal dopamine innervation, the expression of several of the markers used may be subject to regulation by the treatments that are being used, or by processes that serve to compensate for the disease [117]. Regardless of the reason, the correlations between imaging and clinical outcome measures in numerous trials of putative disease modifying therapies has been very poor. Until a more reliable biomarker can be developed, it will be very difficult to assess the effects of novel therapies on disease progression [118,119]. The second major barrier to the development of novel therapies has been the lack of animal models that successfully predict

the response to disease modification. Thus, most models involve the acute or subacute toxin-induced death of dopamine neurons and are primarily useful for predicting the effects of symptomatic therapies. The questionable relevance of such models to the insidiously progressive nature of PD and their failure to account for non-dopaminergic lesions in advanced PD is highlighted by the consistent failure to translate into clinical benefit neuroprotective strategies that were highly effective in these models. The future successful development of neuroprotective therapies will be heavily dependent upon the use of better validated animal models of PD. Early promising indications for the use of systemic proteasome inhibitors have not been replicated in many labs and currently the most promising approach is the use of animals in which expression of key genes such as α-synuclein has been modified.

7. Expert opinion

The major challenges for the treatment of PD remain modification of the underlying disease and its progression, as well as the management of advanced disease, particularly complications reflecting dysfunction in non-dopaminergic systems. At this point, numerous approaches to the management of dopaminergic dysfunction have been developed and further advances are likely to be incremental, with the possible exception of therapies that selectively target specific dopamine receptor subtypes, such as antagonists or partial agonists for the dopamine D3 receptor, for the management of dyskinesias.

Non-dopaminergic mechanisms in PD are still poorly understood. Blockade of striatal adenosine receptors is of demonstrated benefit in motor response fluctuations. Manipulation of striatal 5HT receptors may be useful for levodopa-induced dyskinesias but most available agents, particularly receptor antagonists, are somewhat non-specific and target dopamine receptors as well. Furthermore, 5HT receptors are widely distributed and treatment may result in numerous unwanted effects. This is perhaps even more the case for therapies based on modification of excitatory amino acid transmission, although the preferential distribution of specific NMDA receptor subtypes, coupled with the development of subtype-specific antagonists, may circumvent this problem. In recent years, there has been a resurgence of interest in cholinergic mechanisms in PD. The classical view of an altered balance between dopaminergic and cholinergic function in the striatum is overly simplistic and not therapeutically useful in view of the unacceptable cognitive side effects associated with non-selective blockade of muscarinic receptors. In the future, more selective agents may be of benefit. While stimulation of nicotinic receptors may result in dopamine release, its role in PD is unclear. A recent report suggests potential benefit for dyskinesias in MPTP-treated monkeys [120], but this finding must be substantiated. Cholinergic stimulation has an increasingly accepted role in the management of cognitive and behavioural disturbances associated with PD but the role in other complications such as sleep disturbance and postural instability that may arise in part from degeneration of brainstem cholinergic neurons is unclear. Although there is ample evidence for altered neuropeptide transmission in PD, and in association with levodopa therapy, this has not yet led to clear therapeutic strategies.

Deep brain stimulation of the subthalamic nucleus (STN DBS) is now widely accepted as established therapy for advanced PD. Although there are individual reports of improvement in complications that are only partially responsive to medication, it is generally felt that STN DBS can only result in the best function seen when patients are on medication [13]. However, as reduction in medication dose may be possible, some dose-related complications of medication use, including dyskinesias and psychosis, may improve. When the internal segment of the globus pallidus is stimulated, the dose of medication is less likely to be reduced but there may be a substantial reduction in dyskinesias. The role of pedunculopontine stimulation for postural and gait impairment is not yet established. Cognitive and behavioural side effects of DBS, including disorders of impulse control [121], may be considerable, the surgery is invasive, batteries and hardware may fail and the stimulator adjustments require expertise. This form of therapy is therefore likely to remain an adjunct to be used in the management of carefully selected patients rather than a replacement for optimized medication, even though short-term outcome is better in patients who undergo surgery [122]. Theoretical considerations that STN DBS might delay disease progression by interfering with excitatory outputs from this nucleus have not been supported by observations to date [99].

Disease modification is ultimately the most important therapeutic goal but has proved to be an enormous challenge. Despite theoretical evidence that oxidative stress, excitatory amino acid transmission and apoptosis may all play a role in Parkinson's, therapies based on these approaches have not to date proved useful. Anti-inflammatory treatments would also be of potential utility but have not been adequately tested. To date, neurotrophic therapies have demonstrated only limited, if any, benefit but difficulty delivering drug to the target site may play a greater role here.

Gene therapies for Parkinson's may prove useful in the future but this will depend upon the ability to express an appropriate molecule in a regionally selective fashion. It seems unlikely that gene therapy based on correction of dopamine deficiency will confer much advantage over available pharmaceutical agents. The role of STN GAD therapy and of viral delivery of genes encoding neurotrophic factors is still not clear. Even if these treatments prove helpful, they are unlikely to address the distressing non-motor, non-dopaminergic complications of advanced disease. Gene therapy to interfere with aberrant protein aggregation may

indeed be of interest but whether this will indeed have an impact on disease progression is far from certain.

One of the greatest sources of frustration has been the consistent failure of therapies that were promising in animal models to translate into meaningful modification of disease in humans. In some cases, this may reflect difficulties with drug delivery or toxicity arising from stimulation of unintended target sites. These are essentially technical hurdles that can theoretically be solved. However, a much bigger question is the relevance of the preclinical models to Parkinson's in humans. Thus, significant advances in this domain will require careful study of the condition in humans to try and gain further insights into etiopathogenetic mechanisms, as well as the development of animal models that more faithfully mimic the situation in human disease. Until this is done, we are likely to witness many more disappointments in the future. Once promising strategies are identified, their efficacy must of course be demonstrated in humans and this will require the development of better and more efficient trial designs with careful avoidance of potential confounding factors, as well as validated biomarkers that permit independent, objective measures of outcome. The importance of the placebo effect in PD should not be forgotten [123-130]. It is therefore crucial that all new therapies be subjected to adequately controlled trials in early stages, as many of the benefits reported from preliminary studies have disappeared when placebo controls were used.

Declaration of interest

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