Molecular Targets for Improvement of Parkinson's Disease Therapy

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Abstract

This paper is a concise overview of molecular targets whose attack can contribute to improve the therapy of Parkinson's disease. Particular attention is given to the merits of gene therapy and to the information that can be acquired, studying the hereditary forms of PD, on the pathogenetic mechanisms of Parkinsonism and putative targets for new therapeutical measures.

Keywords: Parkinson's disease; Neurodegenerative disease; Nervous system

Introduction

Parkinson's disease (PD), the most frequent ageing-progressive neurodegenerative movement disorder, 1-2% prevalence above 65 years, is caused by loss, above a threshold level, of dopamine producing neurons in substantia nigra pars compacta and involves dysfunction in dopamine excitable midbrain neurons as well as in other central and peripheral nervous system regions [1]. PD results from a complex interplay of genetic and environmental factors and the pathogenetic processes are not yet completely unveiled [2]. Two hundred years after its first description PD is still incurable. PD by itself can, in fact, represent an intrinsic ageing factor in the affected patients. Administration of levodopa (L-DOPA), which reaches substantia nigra neurons where it is decarboxylated to dopamine and is then released at the synapses of dopamine excitable neurons, is the effective drug for treatment of PD motor impairment symptoms. After a variable number of years treatment, the therapeutical efficacy of L-DOPA decreases, however, and adverse effects increase [3]. This demands for new therapeutical strategies.

Improving L-DOPA Therapeutical Treatment

Over time motor fluctuations and motor complicating dyskinesias develop in the later stages of L-DOPA PD therapy. These are primarily due to "wearing off " and "on-off" movement disorders which reflect the increasing short duration of action of the single doses of L-DOPA administration [4], worsened by intervening excessive glutamatergic basal ganglia neuronal excitability [5,6]. As a first measure to overcome these intervening drawbacks of L-DOPA treatment, different L-DOPA formulations have been developed to provide more continuous, neuronal supplementation of L-DOPA. These include use of soluble pro-drug compounds like L-DOPA methylester, gastro retentive L-DOPA formulations, microspheres of different sizes delivering L-DOPA at different rates (for example the PX-066, (Rytary) whose use has been approved in USA) [4]. Combined administration of L-DOPA with monoamine oxidase inhibitors or selective catechol-o-methyl transferase (COMT) inhibitor, are valuable adjuncts prolonging the duration of L-DOPA action [7]. Combined administration of L-DOPA with dopamine, eventually incorporated in biodegradable nanoparticles to ferry DA across the blood-brain barrier appears to improve the efficacy of L-DOPA therapy [8]. Antagonists of the NMDA glutamate receptor in basal ganglia, like amantadine or modulators of the metabotropic glutamate receptors are under trials [9].

Viral Vector-based Gene Therapy Approaches

Over the past decade, gene therapy (GT) approaches have been undertaken to treat PD. GT based on therapeutical application of DNA recombinant strategy consists of direct micro-surgical injection into the putamen of PD patients of viral-vector recombined genes encoding rate-limiting enzymes in L-DOPA biosynthesis like, aromatic L-aminoacid decarboxylase (AADC) [10-12], plus tyrosine hydroxylase and cyclohydrolase1 [13]. The remarkable advantage of this GT approach is provided by the continuous and stable expression of the enzyme proteins in the site of the injection with continuous dopamine production restricted to the dopamine depleted-striatum. This allows significant reduction of the dose of L-DOPA administration, thus avoiding drawbacks of excessive generalized dopamine production. A recombinant adeno-associated virus (AAV) serotype 2 vector, encoding human AADC (AAV-hAADC) [10-12] or a tricistronic lentiviral vector (ProSavin), that is based on the equine infectious anemia virus encoding the rate-limiting dopamine biosyntheic enzymes tyrosine-hydroxylase, AADC and cyclohydrolase I [13,14] have been used.

Phase 1 clinical trials on groups of six [11] and fifteen patients [13] confirmed controlled and persistent targeted expression of the engineered enzyme and dopamine production. A 12 months follow-up of the patients treated with ProSavin verified that the patients, besides mild and moderate adverse effects, did not present serious adverse events related to the study drug and surgical procedure [13]. These early phase clinical trials provide preliminary evidence for safety and potential clinical benefit of GT therapy for long-term treatment of PD patients and motivate longer-term clinical trials to confirm efficacy, tolerability, patient's acceptance and regulatory approval [15].

Delivery to the subthalamic nucleus of viral vector-mediated glutamic acid decarboxylase (GAD) has, also, been applied to enhance production of gamma aminobutyric acid to counteract glutamatergic excitability of basal ganglion motor system [16].

In the same years in which gene therapy was implemented, application of cell therapy (CT) to PD treatment has also been pursued. Review of CT is beyond the scope of the present paper. Data on this can be found in GT reviews [17,18].

It can be noted, at any event, that GT involves the use of well characterized viral-mediated constructs of genes coding known enzymes acting on the biosynthetic pathway of dopamine or GABA. Furthermore procedures to evaluate, at molecular level, the successful targeted production of the neurotransmitters are available. CT, which is based on transplantation in the PD striatum of pluripotent embryonic, bone marrow-derived or mesenchimal stem cells, or induced pluripotent stem cells from adult cells of the same patient, clearly involves interaction of the transplanted cells with the local environment at the site of their introduction and complex interplay with differentiation and grow factors. Furthermore there is the risk that the transplanted stem cells, besides differentiation in dopaminergic cells, could result in tumour formation [17].

How Familial Cases of PD Help to Identify Pathogenetic Mechanisms and Possible Molecular Targets for Therapy

Important clues for identification of additional molecular targets for improvement of PD therapeutical treatment come from the study of hereditary forms of PD. Whilst 90% of PD cases are sporadic, rare mendelian hereditary forms, resulting from a variety of autosomal mutations in more than 10 PARK genes, represent all together 10% of PD cases [19,20]. Mutations in mitochondrial DNA have also been found to contribute to PD development and clinical course [21]. Elucidation of the pathogenetic mechanism(s) of PD familial cases, contributes to identify susceptible sites and networks whose acquired alterations might be involved in sporadic PD [2]. Mutations in PARK2 gene, coding for parkin, are the most common cause of autosomal recessive PD and more than 100 different PARK2 pathogenetic mutations have, so far, been identified [20]. Parkin belongs to the ring between ring fingers (RBR) protein family of E3 ubiquitin ligases [22]. It is generally accepted that loss of the ligase activity, or even loss of the entire parkin protein, in patients with PARK2 mutations impair protein degradation by the ubiquitin-proteasome system with accumulation of toxic products and aggregates leading to degeneration and death of dopaminergic neurons [23-25]. Parkin appears to exert its effects in different cellular compartments including in particular mitochondria [26]. Parkin can translocate into mitochondria upon phosphorylation by the PINK1, protein kinase encoded by the PARK6 gene [27] and exert there a critical role in degradation of altered proteins and induction of elimination of dysfunctional mitochondria by a process defined as 'mitophagy' [28]. PARK2 mutations which abrogate this parkin clearing function results in a progressive general mitochondrial dysfunction [24]. Investigations on transfected cell cultures has, however, provided evidence that parkin can also up- or down-regulate the expression of different genes [29]. Extensive investigation in the authors' laboratory in primary cultures of fibroblasts from patient's skin biopsies have shown that different mutations of PARK2, in independent patients affected by autosomal recessive PD, resulted in depression of mitochondrial respiratory chain enzymes, in particular complex I, inhibition of the mitochondrial respiratory activity and ATP production [30]. These effects were associated with defect in the PGC1□ mastered transcription cascade of mitochondrial respiratory proteins [30]. Pathogenetic mutations in PARK6, coding for PINK1 serine/threonine kinase, represent the second most common cause of autosomal recessive PD. Also in the fibroblasts of a PARK6 PD patient a marked depression of respiratory chain complex I, mitochondrial respiration and ATP production was observed [31,32]. In both PARK2 and PARK6 patient's fibroblasts a marked increase in the level of oxygen free radicals was detected thus setting up a damaging vicious cycle. It was, in particular, found that the inhibition of respiration was caused

by loss of cytochrome c from mitochondria [32,33]. There is, in fact, convincing evidence and consensus that defects in mitochondrial respiratory ATP production associated to increased oxygen free radical production is a major cause of functional impairment of dopaminergic neurons [34,35]. Proteomic analysis of PARK2 patient's fibroblasts from our laboratory (Rosa Lippolis; Rosa A Siciliano; Consiglia Pacelli; Anna Ferretta; Maria F Mazzeo; Salvatore Scacco; Francesco Papa; Antonio Gaballo; Claudia Dell'Aquila; Michele De Mari; Sergio Papa; Tiziana Cocco (in press) BBA-Molecular Basis of Disease) shows a general decrease in cellular Ca^{2+}-binding proteins including the S100A-4, S100A-6 and S100-A10 members of the S100A family of proteins which are involved in modulation of intracellular Ca^{2+} level [36], calreticulin and annexin A5. It has, in fact, been reported that parkin deficiency in cellular model leads to calcium level increase that makes cells more vulnerable to neurotoxins [37].

More Molecular Targets for PD Therapy

Hurley et al. [38] have found increased expression of $Ca_v1,3$ voltage-gated calcium channel in post-mortem brain of early stage Parkinson disease together with reduced level of Ca^{2+} binding proteins. Both of these changes are expected to contribute to enhance cellular Ca^{2+} concentration and consequent Ca^{2+} excitotoxicity [39]. Increased cytosolic concentration of $Ca^{2+} > 10^{-5}$ M results in a large accumulation of Ca^{2+} in mitochondria [40], where it induces a sequential series of deleterious events including: opening of the cyclosporine-sensitive permeability transition pore and loss of cytochrome c and other pro-apoptotic factors from mitochondria [41]. The decrease in the level of Ca^{2+}-binding/buffering proteins and increased expression of $Ca_v1,3$ voltage-gated calcium channel [38] lend support to the concept that Ca^{2+} excitotoxicity plays a central role in Parkinson disease [38,42]. An historical cohort study in Denmark [43] has indicated that the exposure of patients to dihydropyridine Ca^{2+}-channels blockers, largely used in therapeutical treatment of hypertension, is associated with a reduced risk of incident PD and reduced mortality among PD patients. These observations warrant further basic and clinical studies aimed to develop and test blockers of Ca^{2+} channels in prevention and treatment of Parkinson disease. A main target of Ca^{2+} excitotoxicity is represented by mitochondria. Large accumulation of Ca^{2+} in these organelles results in a deleterious cascade of events, which ultimately cause impairment of respiration and ATP production and enhancement of ROS production [30,44]. Impaired mitochondrial energy metabolism, oxidative stress conditions and age-related progressive accumulation of oxidatively damaged and misfolded proteins, which in turn aggregate and impair cellular house keeping and specialized functions, all together contribute to PD pathogenesis [45,46]. All this supports an adjuvant therapeutical action of natural antioxidant phenols (see the case of resveratrol [44] and hydroxytyrosol [47]) which can promote the expression and/or the activity of gene products involved in cell energy metabolism and protection against oxidative stress and productions/accumulation of oxidized/misfolded proteins [48,49]. In this respect the neuropeptide CART (cocaine-amphetamine-regulated transcript)which is mainly expressed in the brain, particularly in dopaminergic midbrain regions, and has antioxidant activities, qualifies as another therapeutic candidate for PD [50,51]. The list of molecular candidates for PD therapy has in fact grown in the last few years. Results in animal models of PD indicates that retinoic acid reduces degeneration and loss of dopaminergic neurons induced in rat by exposure to 6-hydroxydopamine, a toxic catabolite of dopamine [52,53]. Injection in the striatum of rats, with MPTP-induced parkinsonism, of retinoic acid encapsulated in polymeric nanoparticles has been shown to be particularly effective in reducing death of dopaminergic neurons [54]. Lactoferrin nanoparticles, with

encapsulated human glial cell neurothophic factor gene (hGDNF), injected intraperitoneally, was also found to improve locomotor activity and reduce dopaminergic neuron loss in rotenone-induced PD rats [55]. Accumulation of α-synuclein Lewy Bodies in dopaminergic neurons appears as one of the factors involved in neuronal degeneration and clinical evolution of at least certain cases of PD [56]. Based on this and related observations vaccines constituted by α-synuclein peptides [56] have been developed by the research EU consortium SYMPATH. Experimental controls have verified the efficacy of □-synuclein vaccines [56], and they are now undergoing, with much hope, to phase 1 clinical trials.

Finally a compound which appears to be another L-DOPA adjuvant in PD therapy is safinamide [57]. Safinamide inhibits monoamine oxidase, MAO-B and prevents excessive release of glutamate by blocking Na$^+$ Channels [58]. In phase 3 clinical trials safinamide was found to prolong the time of L-DOPA positive effect on movement impairment without increasing complicating dyskinesias [57].

Conclusions

Good perspectives are today emerging for improvement of L-DOPA therapeutical treatment of PD. Phase 1 clinical trials have shown that gene therapy, based on delivery to selected brain areas of viral vector-mediated genes coding for enzyme involved in dopamine and gamma-aminobutyric synthesis, provide safe, controlled and persistent generation of the biologically active proteins in the targeted structures of human brain. More extensive and prolonged clinical trials and predictive animal models are waited to verify reliable and safe, long-term efficacy of gene therapy, as well as of cell therapy. L-DOPA therapy can be improved by the use of different formulations of the drug, that allow more continuous and controlled dopamine supply to the defective neurons. PD therapy can also be improved by combined administration of L-DOPA with dopamine and inhibitors of dopamine decarboxylation. Experimental work on animal PD models indicate a potential therapeutical action of retinoic acid. α-synuclein vaccines have been produced. Their effect in removing toxic Lewy bodies from dopaminergic neurons is being checked. It is hoped that clinical trials will verify the therapeutical efficacy of these vaccines. What we are learning on the pathogenesis of autosomal familial cases of PD are, on the other hand, disclosing molecular targets for compounds like Ca^{2+}channel blockers and natural and synthetic antioxidants, which, in addition to the other known L-DOPA adjutants, can contribute to improve PD therapy, prolonging L-DOPA positive effect on movement impairment and reducing dyskinesias. All these new therapeutical approaches may result in a better psycho-physical condition of the PD patients and prolong their life expectancy.

Acknowledgements

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