Mitochondrial Toxins in Basal Ganglia Disorders: From Animal Models to Therapeutic Strategies

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Abstract: Current knowledge of the pathogenesis of basal ganglia disorders, such as Huntington’s disease (HD) and Parkinson’s disease (PD) appoints a central role to a dysfunction in mitochondrial metabolism. The development of animal models, based upon the use of mitochondrial toxins has been successfully introduced to reproduce human disease, leading to important acquisitions. Most notably, experimental evidence supports the existence, within basal ganglia, of a peculiar regional vulnerability to distinct mitochondrial toxins. MPTP and rotenone, both selective inhibitors of mitochondrial complex I have been extensively used to mimic PD. Accordingly, in human PD, a specific dysfunction of complex I activity was found in vulnerable dopaminergic neurons of the substantia nigra. Conversely, in HD a selective impairment of mitochondrial succinate dehydrogenase, key enzyme in complex II activity was found in medium spiny neurons of the caudate-putamen. The relevance of such finding is further demonstrated by the evidence that toxins able to primarily target mitochondrial complex II, such as malonic acid and 3-nitropropionic acid (3-NP), strikingly reproduce the main phenotypic and pathological features of HD.

Despite the advances obtained from these experimental models, a deeper understanding of the molecular and cellular mechanisms underlying such neuronal vulnerability is lacking.

The present review provides a brief survey of currently utilized animal models of mitochondrial intoxication, in attempt to address the cellular mechanisms triggered by energy metabolism failure and to identify potential therapeutic targets.

Key Words: Striatum, vulnerability, Parkinson’s disease, Huntington’s disease, progressive supranuclear palsy.

INTRODUCTION

The basal ganglia represent key structures involved in motor learning and memory. Growing experimental and clinical evidence supports the notion that these brain areas consist of distinct loops proceeding along parallel circuits linking cortical and subcortical regions [27]. They subserve the processing of sensorimotor, associative and affective tasks. Neurodegenerative disorders involving basal ganglia regions show a clinical heterogeneity, with predominant motor impairment. In spite of distinct clinical features, evidence has emerged in favour of a possible common pathogenic background. In particular, both dysfunction in mitochondrial energy metabolism and oxidative damage have been strongly implicated in the pathogenesis of Parkinson’s disease (PD), Huntington’s disease (HD) and progressive supranuclear palsy (PSP) [1,75].

Neurons have a high energy demand, hence they are particularly sensitive to ATP depleting conditions. Mitochondria play a crucial bioenergetic role in supplying the cellular energy reservoir and conditions that alter their function can trigger either the necrotic or the apoptotic pathway to cell death [48,28]. Compelling experimental and

clinical evidence support a key role for the specific impairment of complex I in the pathogenesis of PD, while complex II dysfunction has been shown to be linked to the occurrence of HD [2,12,64,75].

Notably, the neuronal loss observed in these neurodegenerative disorders is not homogeneous. In PD the primary pathological finding is the neurodegeneration of dopaminergic neurons of the substantia nigra pars compacta, whereas the striatum is mostly affected in HD. Such a selective involvement depends upon the differential vulnerability observed among brain regions and distinct neuronal subtypes. Indeed, the striatum is among the most vulnerable brain areas and, within the striatal population, projection neurons are selectively affected to energy metabolism impairment, compared to the population of cholinergic interneurons [22,56].

The development of animal models reproducing these neurodegenerative diseases was prompted by the reports on the occurrence in humans of neurological syndromes induced by accidental exposure to toxins, known to specifically interfere with the mitochondrial complexes of the mitochondrial transport chain [4,10,11,36]. Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone or 3-nitropropionic acid (3-NP), toxins inhibiting complex I or II, respectively, reproduces in rodents and non-human primates many histochemical and behavioural features of human PD or HD [9,33,34]. Both chronic or acute

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treatment and different routes of administration (systemic or local) have been utilized [19,24,67,68]. Although these models have proven useful, none of them reproduce all the features of the human disease. Such limitation has generated criticisms on the reliability of the models. However, it should be reminded that an animal model does not necessarily mimic its human counterpart. This concept would be indeed unrealistic. Conversely, the model should be intended as a means aimed at clarifying single pathogenic steps, or testing selective drugs.

Here we present a survey of the use of mitochondrial toxins to model human neurodegenerative disorders in animals. The most significant data obtained from these models will be discussed, taking into account their transferability to the human pathophysiology.

PARKINSON'S DISEASE

MPTP Model

The dopaminergic neurotoxin MPTP was discovered about 20 years ago when an outbreak of idiopathic parkinsonism was observed among drug addicts consuming a contaminated "synthetic heroin" [38]. MPTP induced in humans a syndrome showing many features of classical PD [38]. Since then, MPTP has been successfully used to set up animal models of PD [13,34,55]. MPTP is mainly used in non-human primates and in mice. Systemic administration of MPTP induces a bilateral parkinsonian syndrome in monkeys. Either acute or chronic treatment of monkeys is used to induce parkinsonism; however chronic intoxication with low doses of MPTP causes a gradual neurodegenerative pattern better suited to modelling human PD. Indeed, the anatomical localization of degenerating nigrostriatal terminals is similar in low-dose MPTP treated monkeys and in PD [46].

Systemically administered MPTP crosses the blood-brain barrier and is converted by monoamine oxidase B (MAO-B) to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP) in glia and serotonergic neurons. MPDP then spontaneously oxidizes to a toxic metabolite, 1-methyl-4-phenylpyridinium (MPP⁺). Once released into the extracellular space, MPP⁺ can be taken up by the dopamine transporter (DAT) and enter the dopaminergic neurons, where it incorporates into synaptic vesicles by binding to the vesicular monoamine transporter (VMAT) [35,41]. MPP⁺ then accumulates into mitochondria utilizing the mitochondrial transmembrane potential where it inhibits complex I of the electron transport chain leading to impairment of respiration [57]. The inhibition of mitochondrial respiration could represent a key factor in the pathogenesis of PD. A partial inhibition (by 25%) of complex I, NADH ubiquinone oxidoreductase, in nerve terminals is able to cause a severe impairment of oxidative phosphorylation [75]. In mitochondria, a small amount of reactive oxygen species (ROS) is produced, mostly at the level of complex I and III, by leakage of electrons directly to molecular oxygen. Upon conditions of reduced oxidative phosphorylation, more electrons are available for oxygen reduction. Indeed, enhanced ROS generation was observed after prolonged complex I

inhibition, suggesting that a vicious cycle might be induced by complex I deficiency [75].

To date, the reasons for the selective vulnerability of dopaminergic neurons are still largely elusive. However, the accumulation of α -synuclein has been proven to play a central role in the vulnerability of dopaminergic neurons. Both α -synuclein and dopamine itself can generate ROS, and impair mitochondrial activity [84]. Thus, an aberrant interplay between α -synuclein and dopamine degradation has been proposed as a mechanism responsible for nigral dopaminergic neurons cell death [84].

In monkeys, the MPTP model remarkably reproduces several features of human PD, including tremor, rigidity, bradykinesia, and postural instability. Neuropathological features are also very similar [41,70,78]. The response to levodopa (L-DOPA) treatment and the development of motor complications during long-term therapy are also similar to those seen in PD patients. However, in spite of the consistent monoaminergic neuronal loss observed in PD, MPTP toxicity selectively affects dopaminergic cells [25]. Moreover, classical Lewy bodies have not been reported to occur in humans or monkeys acutely intoxicated by MPTP [25]. Recently, Fornai *et al.* [24] developed a mouse model of chronic MPTP infusion (1-30 mg/kg/day) *via* osmotic minipumps. These authors found that continuous MPTP infusion led to progressive behavioural changes, inhibition of the ubiquitin-proteasome system (UP), and formation of neuronal inclusions containing ubiquitin and α -synuclein. Interestingly, in mice lacking α -synuclein most of these alterations were reduced. A central role of UP dysfunction in the pathogenesis of PD has been suggested by observations reporting genetic defects or functional impairment of UP in inherited or sporadic forms of PD and the presence of various UP components within Lewy body neuronal inclusions [45]. Indeed, experimental inhibition of the proteasome was reported to induce selective damage of dopaminergic neurons both *in vivo* and *in vitro* [23,59]. The cellular mechanisms underlying the specific sensitivity of dopaminergic neurons are largely unknown. However, it has been proposed that they might be due either to their failure in inducing the chaperone Hsp70 in response to UP impairment, or to the potent activation of poly(ADP-ribose) polymerase (PARP) by MPTP, *via* nNOS and peroxynitrite nitration and inactivation of tyrosine hydroxylase (TH) [44,59]. Indeed, after chronic i.p. administration of MPTP, PARP knockout mice were spared from MPTP toxicity [44].

To date, despite the lack of a precise knowledge of the molecular mechanisms underlying its effect, the MPTP model is still the most authoritative model of PD.

Rotenone Model

Rotenone, a natural pesticide widely used in vegetables garden and in lake and reservoirs to kill or sample fish populations, is a well characterized, high-affinity, specific inhibitor of complex I, one of the five enzyme complexes of the inner mitochondrial membrane involved in oxidative phosphorylation (Fig. 1). Because it is extremely hydrophobic, rotenone easily crosses the biological membranes and enters the cytoplasmatic space, independently of dopamine trans-

Fig. (1). Toxins acting on mitochondrial electron transport chain. Schematic representation of the site of action of mitochondrial toxins used to model human neurodegenerative diseases.

porter activity. Therefore rotenone (unlike MPTP) could be well suited to produce a systemic inhibition of complex I in experimental animal models [4]. Of interest, post-mortem studies on tissue samples obtained from PD patients revealed a selective complex I deficiency [64].

After chronic systemic infusion of rotenone (2,5 mg/kg per day over a period of days to weeks) rats develop a syndrome characterized by rigidity and akinesia. Furthermore nigral neurons have been reported to show cytoplasmic inclusions, like typical parkinsonian Lewy body inclusions, containing α -synuclein and ubiquitin [4,68]. However, there is not general consensus about the use of rotenone-intoxicated animals as an experimental model of PD [33,39]. Höglinger *et al.* [33] claimed that rotenone infusion reproduces a pattern of multisystem degeneration, therefore affecting not only the nigrostriatal pathway but also the striatum and brainstem nuclei. A recent *in vitro* study provided evidence that acute application of rotenone on rat striatal slices induced a widespread degeneration of striatal neurons, reported to be spared in human PD [5]. Hence, further work is required to ameliorate this model, by targeting selective brain areas.

6-Hydroxydopamine Model

6-Hydroxydopamine (6-OHDA), a hydroxylated derivative of dopamine, was introduced in the sixties as the first animal model of PD [76]. Contrary to MPTP and rotenone, 6-OHDA does not cross the rat blood-brain barrier, therefore it is administered by stereotaxic injection along the pathway of the dopaminergic fibers into the substantia nigra pars compacta, the median forebrain bundle, or the striatum. The stereotaxic injection allows a unilateral lesion, thus avoiding generalized symptoms and providing a contralateral control [18,50]. The extent of the lesion can be quantified *in vivo* by measuring the asymmetric turning behaviour of the rats induced by systemic administration of dopamine receptor agonists, L-DOPA or dopamine releasing drugs [32,77]. However, caution is required in evaluating the turning behaviour as unilateral lesions may also cause contralateral modifications at striatal level or in the subthalamic nucleus

[51,62]. In various animal species, rodents, cats and non-human primates, 6-OHDA-induced toxicity is relatively selective for monoaminergic neurons, as a result of its uptake by dopamine and noradrenaline transporters [43]. Once inside neurons 6-OHDA produces toxic effects by inhibiting mitochondrial complex I and causing oxidative stress [26,61].

The 6-OHDA model has provided considerable amount of information. Dopamine denervation is known to affect corticostriatal glutamatergic neurotransmission [16,21,67]. *In vitro* studies from 6-OHDA lesioned rats showed that striatal projection neurons undergo severe functional impairment, as they loose both forms of long-term synaptic plasticity, potentiation (LTP) and depression (LTD) [14]. In the parkinsonian rat, the symptomatic L-DOPA therapy is able to revert the alterations observed in glutamatergic neurotransmission [14,53]. As in humans, however, after prolonged L-DOPA treatment dyskinesias appear. The cellular mechanism underlying these involuntary movements has been proposed to reside in the loss of bidirectional synaptic plasticity by striatal projection neurons [52]. Though L-DOPA is able to restore LTD and LTP of corticostriatal transmission, in dyskinetic animals striatal projection neurons are no more able to revert glutamatergic transmission to baseline after the induction of LTP.

Neuropathologic findings observed after 6-OHDA lesions do not show cytoplasmatic inclusions; moreover, neuronal cell loss appears restricted to the substantia nigra.

HUNTINGTON'S DISEASE

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by a progressive development of involuntary choreiform movements, behavioural changes and cognitive impairment [58]. HD has been classified among the translated polyglutamine disorders, as an abnormal expansion of CAG trinucleotide repeats (36-240) in the gene coding for huntingtin, results in an increased series of glutamines in the N-terminus of the protein. Several studies on animal models suggest that this

disease is, at least partly, due to a toxic gain of function of the mutated protein [30,83].

The predominant neuropathological feature of HD is a progressive striatal neuronal loss [79], which parallels disease progression. Indeed, within the striatal neuronal population, GABAergic projection neurons, which constitute nearly 95% of the entire striatal neuronal population, are the most vulnerable in HD. Conversely NADPH-diaphorase and cholinergic interneurons, representing a minority of striatal neuronal population, seem relatively spared [20].

Notably, similar behavioural deficits, motor impairment and selective neuronal damage can be reproduced after mitochondrial complex II inhibition both in animals and humans [9,10,42,49]. In rodents and primates the selective inhibition of mitochondrial complex II subunit succinate dehydrogenase (SDH) by the mitochondrial toxins 3-nitropropionate (3-NP) or malonate reproduced the striatal-selective lesions seen in human HD, thus emphasizing the relevance of SDH defects to the HD phenotype. Moreover, *in vitro* electrophysiological recordings from 3-NP-treated corticostriatal slices showed an abnormal, long-lasting enhancement of synaptic activity in striatal medium spiny projection neurons (Fig. 2), that has been suggested to underlie their peculiar vulnerability to SDH defects [15]. This hypothesis has been further strengthened by more recent experimental observations in presymptomatic R6/2 HD transgenic mice, carrying the human mutated huntingtin gene. During *in vitro* electrophysiological recordings from striatal neurons, acute treatment with 3-NP induced a membrane depolarization of larger amplitude in medium spiny neurons from R6/2 mice than in wild-type mice, indicating an increased susceptibility to mitochondrial impairment. Interestingly, the 3-NP-induced membrane response of cholinergic interneurons did not differ between the two groups of animals [63]. These findings indicate a peculiar regional vulnerability occurs in HD, indicating a major involvement of SDH in its pathogenesis.

Fig. (2). Long-term synaptic modification induced by 3-NP in striatal neuronal subtypes. 3-NP treatment of rat striatal slices selectively induces a long-term potentiation of synaptic transmission in medium spiny neurons (top). The amplitude of postsynaptic potential is unchanged in striatal cholinergic interneurons (bottom).

PROGRESSIVE SUPRANUCLEAR PALSY

Progressive supranuclear palsy (PSP) is a neurodegenerative disorder whose incidence is about 0.005 % among elderly people. It is characterized by parkinsonism, subcortical dementia, axial rigidity, repeated falls, and supranuclear gaze palsy. Another distinctive feature is a poor response to dopamine replacement therapy. Despite these seemingly unique characteristics, an accurate clinical diagnosis is rather difficult, because of phenotypic heterogeneity. Hence, neuropathological diagnosis remains so far the gold standard. Compelling evidence favours a generalized impairment of mitochondrial function in PSP. Moreover, deficits in the cholinergic systems are thought to be responsible for both the postural instability and cognitive impairment observed in PSP patients [80]. Interestingly, recent studies demonstrate that the levels of the striatal vesicular acetylcholine transporter, a reliable marker of cholinergic activity, is severely reduced in brain tissue from PSP patients, a finding consistent with the loss of striatal cholinergic interneurons [73]. In line with these data, prior investigations of PSP neurochemical changes found reductions of striatal ChAT, indeed suggesting that a loss of cholinergic neurons is a peculiar feature of PSP neuropathology [60]. Despite such evidence the cellular and molecular mechanisms underlying neurodegenerative processes is unknown.

The effects of mitochondrial inhibition have been recently investigated in striatal cholinergic interneurons [5]. Inhibition of mitochondrial complex I by acute application of the toxin rotenone produced an early membrane hyperpolarisation coupled to a fall in input resistance and a rise in intracellular Na^+ . In a later phase, a membrane depolarisation coupled to a rise in intracellular Ca^{2+} was observed. Interestingly, the pharmacological analysis suggested that rotenone acted by rapidly disrupting the ATP content, leading to a decreased $\text{Na}^+-\text{K}^+\text{ATPase}$ function and, in turn, to intracellular Na^+ overload. The opening of both ATP-sensitive and Na^+ -activated K^+ channels would then cause the membrane hyperpolarisation response. Notably, cell counts from corticostriatal slices acutely treated with rotenone showed a significant decrease in the number of choline acetyltransferase immunoreactive cells. These results are in line with the evidence that a remarkably reduced complex I activity has been found in PSP cybrids, responsible for decreased oxygen consumption and cellular ATP levels [74].

Together, these findings indicate two peculiar pathogenic processes occurring in PSP: i) an early mitochondrial impairment; ii) a selective involvement of the cholinergic systems, in particular the striatal cholinergic transmission.

MULTIPLE SYSTEM ATROPHY

Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder characterised by a complex symptomatology including parkinsonism, autonomic failure, pyramidal signs and cerebellar ataxia [82]. Depending on the predominance of either parkinsonian or cerebellar motor symptoms, MSA can be distinguished in two subtypes: MSA-P and MSA-C, showing 80% and 20% incidence, respectively. Differential diagnosis of MSA-P and PD may be difficult in the early stages, due to overlapping symptoms

and to a transient improvement of motor symptoms in response to L-DOPA therapy in a percentage of MSA-P patients. In MSA several brain structures show cell loss and oligodendroglial cytoplasmic inclusions immunoreactive for α -synuclein [71].

The neuropathological findings in MSA-P consist in a striato-nigral degeneration, though a widespread, less severe pattern of degeneration is also observed [37]. The GABAergic neurons in the caudal/dorsolateral putamen and the dopaminergic cells in the substantia nigra pars compacta are primarily affected. A possible pathogenic role of environmental toxins has been considered, though a clear correlation was not demonstrated [82]. Yet, differently from PD and PSP, neither neuropathological findings, nor experimental evidence supports a mitochondrial dysfunction in the pathogenesis of MSA. The neuropathology of striato-nigral degeneration has been mimicked in a double lesion rat model, by sequential unilateral 6-OHDA lesion of the medial forebrain bundle followed by quinolinic acid injection into the lateral motor striatum [65,81]. In this model, as in human MSA-P, both the lack of antiparkinsonian effect and the induction of dyskinesias after L-DOPA treatment were observed [72].

PERSPECTIVES FOR DRUG DEVELOPMENT

Several exogenous toxins are known to induce basal ganglia degeneration. Experimental use of these toxins allowed to develop useful animal models of human neurodegenerative diseases. These disease models are undoubtedly imperfect, in that they do not reproduce the human clinical syndrome. However, during the past twenty years, our knowledge on the pathogenic mechanisms underlying PD and HD has largely improved. A major advance in our understanding has been the discovery of a clear involvement of a mitochondrial impairment in most basal ganglia disorders. This progress has prompted much effort in the field of drug discovery.

In developing therapeutic strategies for PD, pilot trials with antioxidants did not provide unequivocal results, and as yet, have not provided the expected results [40,69]. However, a phase II trial of high-doses of coenzyme Q10 in early diagnosed PD patients, appeared to slow disease progression [69].

NMDA glutamate receptor antagonists have gathered much attention in the last years [31]. Yet, much interest arose from studies on metabotropic glutamate receptors. Indeed, both antagonists at group I and agonists at group II metabotropic glutamate receptors have proven useful in ameliorating parkinsonian symptoms in animal models [6,7,47,54]. A promising field of interest prompted by animal model studies is represented by trophic factors, as glial cell line-derived neurotrophic factor (GDNF) showed encouraging effects in advanced PD patients [29]. Another field of therapeutic advance is represented by L-DOPA-induced dyskinesias (LIDs). Indeed, LIDs represent a frequent, disabling side-effect in course of dopamine replacement therapy. Current knowledge of the pathogenic mechanisms underlying LIDs, and the possibility to replicate these LIDs in animal models, allowed to test a number of

potential drugs capable of reducing or preventing LIDs, with encouraging results in humans (for review, see ref. 8).

As potential targets in HD therapy, either ionotropic or metabotropic glutamate receptors have been considered [3,66]. Yet, in attempt to develop new therapeutic strategies, antioxidant agents, caspase inhibitors, classical anticonvulsants and trophic factors have been tested on animal models.

Regarding PSP and MSA, the current pharmacological trials have been disappointing. In PSP, any attempt to replace cholinergic deficits by cholinomimetic drugs and cholinesterase inhibitors did not provide the expected results. Similarly, a significant benefit is not expected from nicotine treatment.

Apart from replacement therapies, interest has been focused on putative etiologic treatments.

However, given the complexity of mechanisms that are likely taking place in neurodegenerative diseases, a single therapeutic strategy will not be sufficient to cope with these disabling pathologies [17].

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