

Natural toxins implicated in the development of Parkinson's disease

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Abstract: Experimental models of Parkinson's disease (PD) are of great importance for improving the design of future clinical trials. Various neurotoxic models are available, including 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat and rotenone. However, no model is considered perfect; each has its own limitations. Based on epidemiological data, a new trend of using environmental toxins in PD modeling seems attractive and has dominated public discussions of the disease etiology. A search for new environmental toxin-based models would improve our knowledge of the pathology of the condition. Here, we discuss some toxins of natural origin (e.g. cycad-derived toxins, epoxomicin, *Nocardia asteroides* bacteria, *Streptomyces venezuelae* bacteria, annonacin and DOPAL) that possibly represent a contributory environmental component to PD.

Keywords: animal models, natural toxins, Parkinson's disease, therapeutic testing

Introduction

The clinical motor dysfunction in Parkinson's disease (PD) is primarily linked to the depletion of dopamine in the striatum consecutive to the loss of dopaminergic neurons in the substantia nigra (SNc). Even with the advent of powerful new tools such as genomics, proteomics, brain imaging, gene replacement therapy and knockout animal models, the desired end result of neuroprotection is still beyond our current capability [Arias-Carrión *et al.* 2009]. An integrated approach towards understanding the pathogenesis of this disorder may help in discovering and developing new therapies capable of improving the disease in addition to controlling its clinical manifestations [Obeso *et al.* 2010; Arias-Carrión *et al.* 2007]. In sporadic PD, it is thought that exposure to environmental toxins may be a causative factor, perhaps in individuals rendered susceptible by their genetic profile and/or advancing age [Caldwell *et al.* 2009]. The NIH Committee to Identify Neuroprotective Agents in Parkinson's (CINAP) published a systematic assessment of currently available pharmacologic neuroprotective agents. However, the CINAP members stated that using multiple PD models and testing drugs on a variety of animals would represent a problem in comparing different therapeutic modalities [Emborg, 2004]. Despite a broad

spectrum of models, none is considered a perfect model. This led to an obvious question; what model can be used to verify new neuroprotective drugs? In fact, failure of the available animal models to recapitulate the pathology of Parkinsonism may herald the application of therapeutic approaches in clinical cases [Lohle and Reichmann, 2010; Lane and Dunnett, 2008].

In PD, the primary pathology is the degeneration of dopaminergic neurons in the SNc, resulting in loss of the nigrostriatal pathway and a reduction of dopamine levels in the striatum [Obeso *et al.* 2010; Arias-Carrion *et al.* 2009, 2007]. However, neuronal death also occurs in other brain regions, including the locus coeruleus (LC), dorsal motor nucleus of the vagus (DMN) and nucleus basalis of Meynert (NMB), and this can be more severe than neuronal death in the SNc. At these and other pathological sites, protein-rich structures known as Lewy body inclusions appear in the cytoplasm of some remaining neurons [Dawson *et al.* 2010]. In this context, finding a natural agent that can induce the complete pathological picture of the disease would represent a breakthrough in the field of PD research [Caldwell *et al.* 2009; McNaught *et al.* 2004]. Given the extent of knowledge in the study of PD modeling, it is ambitious to describe all models. However,

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we have reviewed the limitations of available neurotoxic models of PD and some natural toxins implicated as triggering the disorder.

Classic neurotoxin-induced animal models of Parkinson's disease

Whereas recent genetic discoveries have led to a number of different genetic models of PD, they failed to reproduce the broad extranigral pathology and other pathological landmarks of PD such as Lewy bodies [Hardy, 2010; Hisahara and Shimohama, 2010; Gasser, 2009; Lim and Ng, 2009; Meredith *et al.* 2008]. Progress will rely on understanding genetic mutations or susceptibility factors that lead to PD, better translation between preclinical animal models and clinical research, and improving the design of future clinical trials. In this regard, the neurotoxic models remained the cornerstone in simulating PD [Bove *et al.* 2006] and several lines of evidence point to environmental exposure being the contributory factor in the pathogenesis of this disorder.

6-hydroxydopamine

6-hydroxydopamine (6-OHDA) is a selective catecholaminergic neurotoxin that is widely used to study mechanisms of cell death in dopaminergic neurons [Schober, 2004; Ungerstedt, 1968] since it is thought to be taken up by the dopamine transporter (DAT). When injected into the striatum of rats, 6-OHDA produces a protracted retrograde degeneration of nigrostriatal neurons over several weeks and leads to a stable and permanent depletion of tyrosine hydroxylase (TH)-positive nigral neurons [Schober, 2004]. Systemically administered 6-OHDA fails to cross the blood-brain barrier, so it has to be injected locally using stereotaxic procedures to obtain a unilateral lesion [Betarbet *et al.* 2002; Ungerstedt, 1968]. Usually, there are three sites where injections are made; into the medial forebrain bundle, the SNc or the striatum [Schober, 2004; Betarbet *et al.* 2002]. Bilateral 6-OHDA injections do not represent the most frequently used model, since bilaterally affected animals require intensive care [Schober, 2004; Betarbet *et al.* 2002]. Therefore, it represents a model for end-stage PD, which is considered to be a limiting factor of this model. To overcome this limitation, Richter and colleagues developed a model with multiple low-dose injections to better replace the single-injection model [Richter *et al.* 2008]; however, this model needs multiple injections or special techniques for implanting a pump which carry the risk of

obstruction or failure, the extent of the neuronal loss is moderate and the technical expense limits the utility as a subchronic model [Richter *et al.* 2008; Schober, 2004].

Returning to the standard model of PD, it has many limitations: it is unilateral, does not produce the entire neuropathology of PD and carries the risk of mechanical damage due to the invasive procedure used in its administration [Richter *et al.* 2008; Schober, 2004; Ungerstedt, 1968].

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

The potent neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produces many of PD pathological findings. Humans and many other animal species including non-human primates, guinea pigs, mice and cats are susceptible to this neurotoxin [Haobam *et al.* 2005]. After its systemic administration, MPTP (being highly lipophilic) rapidly crosses the blood-brain barrier. Once in the brain, it is metabolized to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP⁺) by the enzyme monoamine oxidase B (MAO-B) and then (probably by spontaneous oxidation) to 1-methyl-4-phenylpyridinium (MPP⁺), which is considered the active toxin (MPTP → MPDP⁺ → MPP⁺) [Speciale, 2002].

MPP⁺ (which is far less lipophilic than MPTP) depends on specialized carriers to enter into adjacent neurons [Speciale, 2002]. Once in the mitochondria, MPP⁺ inhibits cellular respiration through the blockade of the electron transport enzyme NADH: ubiquinone oxidoreductase (complex I). Moreover, studies have shown that MPP⁺ can also directly inhibit complexes III (ubiquinol: ferrocycytochrome c oxidoreductase) and IV (ferrocycytochrome c: oxygen oxidoreductase or cytochrome c oxidase) of the electron transport chain [Speciale, 2002]. This is not the complete story, as many cofactors seem to contribute to MPTP-induced toxicity, e.g. iron and neuromelanin, vesicular monoamine transporter (VMAT2) levels, reactive oxygen species (ROS) production, apoptosis and changes in pre-mRNA transcripts splicing [Potashkin and Meredith, 2006; Blum *et al.* 2001; Lotharius and O'Malley, 2000].

One interesting feature of the MPTP mouse model of PD is the transient nature of the striatal damage in young mice. In contrast, administration of the drug to older mice results in a

permanent loss of nigrostriatal terminals, as well as cell bodies. This recovery potential in young mice allows for modeling of recovery stages [Jakowec and Petzinger, 2004; Tillerson and Miller, 2003]. Various techniques can be used to modulate the action of MPTP to induce the pathological findings needed to reach the desired PD model. One of these techniques is to give probenecid with MPTP to prolong its persistence in the tissues [Petroske *et al.* 2001].

Several MPTP dosing regimens have been used. The acute regimen consists of multiple systemic administration of MPTP (usually four doses at 2-h intervals per day). The subacute regimen consists of a single systemic administration per day for several consecutive days (usually 5 days) and the chronic regimen through several weeks [Meredith *et al.* 2008]. The comparison of these different models indicated clearly that different schedules of administration of MPTP mimic distinct stages of the disease and might induce different mechanisms of neuronal death [Schober, 2004].

Despite being one of the most popular toxic PD models, MPTP has many limitations, the most important of which is the absence of inclusion bodies in DA neurons. Also MPTP models lack the behavioral deficits apparent on standard motor mouse tests [Kim *et al.* 2010].

Paraquat

The herbicide 1,1-dimethyl-4,4-bipyridium (paraquat, PQ) has been suggested as a possible PD inducing agent in the mid 1980s following the observation that its chemical structure closely resembles that of MPP⁺ (1-methyl-4-phenylpyridinium ion) [McCormack *et al.* 2002]. Exposure of mice to paraquat leads degeneration of dopaminergic nigrostriatal neurons. Moreover, some authors report appearance of inclusion bodies containing α -synuclein in the paraquat model which makes it similar to PD pathology [Peng *et al.* 2005; McCormack *et al.* 2002].

Despite the similarity with MPTP, there are certainly toxicokinetic and toxicodynamic differences which give paraquat a unique pattern of neurotoxicity [Prasad *et al.* 2009]. An improvement in modeling of paraquat induced PD can be achieved through adding maneb (another neurotoxic herbicide) [Uversky, 2004]. However, paraquat has a higher systemic toxicity profile which

may lead to higher mortalities in the experimental animals [Prasad *et al.* 2009].

Rotenone

Rotenone is a naturally occurring pesticide derived from the roots of *Derris elliptica* species and it is known to be an inhibitor of mitochondrial complex I. Chronic systemic exposure to rotenone reproduces many features of PD, including nigrostriatal dopaminergic degeneration and the formation in nigral DA neurons of cytoplasmic inclusions. Rats show bradykinesia, postural instability, unsteady gait and some evidence of tremors [Shimohama *et al.* 2003]. However, Höglinger and colleagues collected several arguments against suitability of the rotenone model, e.g. lack of recapitulation of PD symptoms, degeneration of striatal neurons, accumulation of tau protein which is more abundant than α -synuclein aggregation and the high mortality rate of the examined animals with subsequent less accurate results obtained [Höglinger *et al.* 2006]. In Table 1 we list the classical toxins used for inducing PD in animals.

PD models – where do we stand?

Emborg [2004] stated that no single model is perfect for PD. The same conclusion was obtained by Shimohama and colleagues suggesting further work to reach 'the optimal PD model' [Shimohama *et al.* 2003]. Manning-Bog and Langston advised using a *model fusion* to overcome the single model limitations through combining genetic with toxic models [Manning-Bog and Langston, 2007]. There is no question that using a combined toxic-genetic model is a promising strategy. However, choosing the perfect toxic substance which mimics 'natural' conditions is mandatory. Here we have tried to draw together the possible candidate toxins that can be used for this purpose.

Natural PD inducing toxins

While use of pesticides has been suggested to be partially responsible for PD in rural areas, this is not correlated to disease prevalence, as the odds ratio for farming itself cannot be accounted for by pesticide exposure alone [Caldwell *et al.* 2009]. Associated with rural living, humans have a strong relation with the surrounding environment, and both individual exposure (e.g. occupation) or group exposure (e.g. drinking well water) are present. As such, exposure to environmental agents may contribute to the onset or progression of PD [Pereira and Garrett, 2010].

Table 1. Characteristics and limitations of available toxic Parkinson's disease (PD) models.

Toxic model	Characteristics	Limitations
6-OHDA	<ul style="list-style-type: none"> - Leads to a stable and permanent depletion of tyrosine hydroxylase (TH)-positive nigral neurons. - Depending on the injection site, frequency of injections and amount of neurotoxin, the animals present different models of PD with polymorphic manifestations. 	<ul style="list-style-type: none"> - Unilateral. - Does not produce the entire neuropathology of PD. - Carries the risk of mechanical damage due to the invasive procedure. - Bilaterally affected animals require intensive nursing care.
MPTP	<ul style="list-style-type: none"> - Humans and many other animal species including nonhuman primates, guinea pigs, mice and cats are susceptible to this neurotoxin. - Transient nature of the striatal damage in young mice. - Different schedules of administration of MPTP mimic distinct stages of the disease and might induce different mechanisms of neuronal death. 	<ul style="list-style-type: none"> - Inability to induce behavioral deficits apparent on standard motor mouse tests. - Does not recapitulate the whole pathology of PD. - Different strains susceptibility.
Rotenone	<ul style="list-style-type: none"> - Chronic systemic exposure to rotenone reproduces many features of PD, including nigrostriatal dopaminergic degeneration and the formation in nigral DA neurons of cytoplasmic inclusions. 	<ul style="list-style-type: none"> - High mortality rate. - Inconsistency of results. - No inclusion bodies.
Paraquat	<ul style="list-style-type: none"> - Capable of inducing dopaminergic neuron damage. 	<ul style="list-style-type: none"> - Severe systemic toxicity.

6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

The success of new environmental toxins to develop mitochondrial complex I inhibition and degeneration of dopaminergic neurons *in vitro* [Abdulwahid Arif and Ahmad Khan, 2010], coupled with epidemiological data suggesting natural environmental toxin involvement in Parkinsonism pathogenesis would invite us to think of these natural toxins as new candidates for developing models [Tanner, 2010].

The choice of certain natural toxins for PD modeling needs some guidelines as supposed by Shaw and Höglinger [2008]. Based on many works dealing with natural toxins some properties must be fulfilled to make them suitable candidates for toxic models. These characteristics include:

1. The agent must be of natural origin.
2. The agent must be available worldwide to contribute to the wide prevalence of PD in the whole world.
3. The agent must recapitulate PD pathology in experimental animals.

The following natural agents represent promising candidates for application in experimental PD

models. Although they do not show the criteria of an ideal PD model, we must put in consideration the fact that they need further research regarding the dosing regimen and species vulnerability before comparing their value to the classical toxins of PD models.

Cycads

The Cycadaceae (cycad) family contains about 45 primitive species of seed-bearing plants that probably dominated the world's vegetation during the Jurassic and early Cretaceous periods [Barceloux, 2009]. Cycads, like all plants, produce a variety of secondary substances. The most important of such allelochemicals are, in addition to dimeric flavones, the nitrogen-containing methylazoglucosides cycasin, macrozamin and several neocycasins. Moreover, there is a minor cycad toxin which is an unusual, nonprotein amino acid, a derivative of alanine (beta-methylamino alanine [BMAA]), which in higher concentrations has been found to be neurotoxic to mammals and chickens [Schneider *et al.* 2002].

The linkage between cycads and neurodegenerative diseases began in 1945. In that year Zimmerman reported cases of motor neuron

disease among Chamorros, the local citizens of Guam. Later, it was confirmed that the disease was clinically and pathologically similar to amyotrophic lateral sclerosis (ALS), although it was familial and confined to local Chamorros (it was 100 times more prevalent than elsewhere in the world) [Spencer *et al.* 2009].

Another atypical Parkinson-like syndrome was identified with onset in middle life and accompanied by mental slowness. This syndrome was noticed in the same families who developed the ALS syndrome. In 1961, Hirano and colleagues introduced the term Parkinsonism–dementia complex (PDC) of Guam, and they also noted the widespread presence of Alzheimer-type neurofibrillary tangles (NFTs) in those with the syndrome. It seemed that the ALS and PDC syndromes represented the spectrum of a single disease. This hypothesis was based on the observation of NFTs in both clinical entities [Konagaya *et al.* 2003].

Two additional links between the Guam syndrome and other neurodegenerative diseases appeared. The first is the transactive response (TAR) DNA-binding protein 43 (TDP-43) and the second is the leucine-rich repeat kinase 2 (Lrrk2). TDP-43 functions as a transcriptional repressor and splicing regulator where it is present in ubiquitin-positive, tau and α -synuclein negative inclusions in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) and in ALS. Moreover, it has been shown to occur in some cases of Lewy body disorders alone or in association with AD. Although the role of TDP-43 in these proteinopathies is unknown, it may provide a common pathologic link [Hasegawa *et al.* 2007].

LRRK-2 (which is strongly linked to autosomal dominant Parkinson disease) may be central to the pathogenesis of several major neurodegenerative disorders associated with Parkinsonism [Wood-Kaczmar *et al.* 2006]. Pathologic studies of both ALS/PDC and nonsymptomatic Chamorro cases reveal that they are more prone to NFT development. Immunohistochemical studies reveal that the tau isoform distribution is similar to AD; however, the cortical laminar distribution is similar to progressive supranuclear palsy (PSP). In these lesions, immunostaining with antibodies to TDP-43 and LRRK-2 shows a commonality with PD, ALS, and some other

neurodegenerative disorders [Spencer *et al.* 2009].

Failure to find any genetic abnormalities in Guam cases led scientists to think about an environmental component and more specifically *Cycas micronesica* which is the source of flour of fadang, a traditional food in this locality [Spencer *et al.* 2005].

The first incriminated toxic component of *C. micronesica* was the nitrogen-containing methylazoglucosides cycasin. In rat neuronal cultures, the aglycone of cycasin methylazoxymethanol (MAM) can damage neuronal DNA, disrupt glutamate transmission, and promote the accumulation of tau protein and neuronal degeneration [Morris *et al.* 2001]. However, failure to induce neuropathology in experimental animals using cycasin excluded it from being the causative toxin [Schneider *et al.* 2002].

Another attractive toxic compound was BMAA. The possible role of BMAA in ALS/PDC replaced the old cycasin theory; however, it was not before 1987 when Spencer and colleagues managed to show the capacity of BMAA to induce pathological changes in primates' brain. The interest toward BMAA as a putative toxic agent damaging DAergic cells has grown considerably due to the discovery that this toxin is not only present in the Guamanian islands but appears to be ubiquitous throughout the world [Jonasson *et al.* 2010; Murch *et al.* 2004; Cox *et al.* 2003]. This natural amino acid deposits in human tissue proteins. This protein binding represents a source of chronic exposure through protein catabolism [Murch *et al.* 2004]. BMAA is distributed worldwide with the chance of frequent repetitive exposures.

For this reason, recent studies properly quantified the concentration of BMAA in postmortem brain tissues of patients [Pablo *et al.* 2009]. The deleterious mechanisms of the toxin could underlie neurological conditions such as ALS–PDC and, potentially, idiopathic PD. Some studies have investigated the effects of BMAA on nigrostriatal DAergic neurons either indirectly (by measuring the ability of these cells to uptake or release dopamine) or directly (through studying the effects of BMAA on single DAergic neurons of the SNpc in an *in vitro* slice preparation). It was found that brief BMAA applications, at a concentration similar to that found in ALS–

PDC brains [Pablo *et al.* 2009; Murch *et al.* 2004], induced reversible and reproducible membrane depolarizations/inward currents, firing increase, and Ca^{2+} accumulation. In contrast, prolonged exposures (12 min) caused functional impairment, cellular shrinkage, calcium overload, cyt-c release, and ROS production that did not return to control values for up to 20 min or several hours after BMAA washout.

Cucchiaroni and colleagues found that the action of BMAA may be due to activation of TRPC-like channels, through mGluR1. Interestingly, GABAergic SNpc cells respond to BMAA with an AMPA-mediated inward current that is not associated with an intracellular calcium increase [Cucchiaroni *et al.* 2010]. BMAA has been previously reported to activate mGluRs in hippocampal, striatal, and cerebellar neurons [Lobner *et al.* 2007]. Alternatively, it predominantly activates NMDA receptors in cortical neurons and AMPA/kainate receptors in spinal motoneurons [Rao *et al.* 2006].

The BMAA hypothesis, however, faced criticism because of the high doses used in the experiment which cannot be true to real life. This criticism led to fading of the BMAA theory for a while. The enthusiasm towards BMAA was regained with recent findings of the possible exposure to high doses (in contrast to what was expected before) through the bound fraction of the toxin rather than the free amino acid, the biomagnification of BMAA exposure through eating animals previously fed on the cycad plants, e.g. flying fox, and the transfer of this cyanotoxin through aquatic ecosystems suggesting further human exposure [Jonasson *et al.* 2010; Chen *et al.* 2002; Cox and Sacks, 2002].

BMAA as an inducer of PD faces a lot of debate; first, there is no detected animal model for such a toxin; second, it lacks specificity towards SN. In other words, BMAA cannot be considered a specific PD-inducing toxin [Karamyan and Speth, 2008]. The previous research points out that cycads are not an attractive research tool in the field of PD modeling. However, a recent finding of the possible role of cycads was based on another constituent (sterol glucosides).

Cycad's sterol glucosides

Recently a new direction rose proposing a new component in cycads as the agent responsible

for neurodegeneration. These sterol b-D-glucosides have been isolated by Schulz and colleagues, who found that these glucosides induce the excitotoxic release of glutamate [Schulz *et al.* 2006].

Based on this hypothesis, Shen and colleagues used washed cycad seeds to make flour pellets (the washed seeds are devoid of BMAA) and fed them to outbred Sprague-Dawley rats [Shen *et al.* 2010]. The experiments were performed with the intention of developing a model of ALS-PDC. However, the rats showed Parkinsonism pathology without ALS syndrome. Also the cycad-fed rats showed the gradual development of multiple PD motor abnormalities after 2–3 months of feeding, including spontaneous unilateral rotation, shuffling gait, and stereotypy. Histological and biochemical examination of brains from cycad-fed rats revealed an initial decrease in the levels of dopamine and its metabolites in the striatum, followed by neurodegeneration of dopaminergic cell bodies in the SNc. Alpha-synuclein and ubiquitin aggregates were found in the DAergic neurons of the SNc and neurites in the striatum (STR). In addition, α -synuclein aggregates were found in the neurons of the locus coeruleus and cingulate cortex. In an organotypic slice culture of the rat SN and the striatum, an organic extract of cycad causes a selective loss of dopamine neurons and α -synuclein aggregates in the SN.

Unlike genetic models or toxicant-based end-stage models of Parkinsonism that may have a great deal of phenotypic similarity among animals, there is considerable variability in the response of individual animals to cycad ingestion. However, one of the strengths of this cycad-fed rat model is that it recapitulates the variability and progression of symptoms and neuropathology seen in human Parkinsonism. Moreover, this model also allows examination of the early stages of the disorder based on the chronic progressive nature of the induced disease.

This new trend of using the seeds without attempting to extract a single toxic component may prove useful. In fact, it seems that this approach is more representative of real life exposure. Despite the debate about which toxic component in cycads is responsible for neurodegeneration, they represent a possible new substance for PD modeling with proven capacity to induce progressive and chronic

neurodegeneration in a fashion similar to reality [Bradley and Mash, 2009]. To sum up, we can see that cycad offers a possible natural toxin candidate. It has been linked epidemiologically to a neurodegenerative condition with pathological similarity to PD: cycads are available worldwide and their use in *in vivo* studies could recapitulate the pathological features of PD [Shen *et al.* 2010].

Although cycad seeds have been linked to ALS/PDC syndrome, the recent findings of Shen and colleagues introduce them as promising tool for modeling PD experimentally [Shen *et al.* 2010].

Epoximicin

Epoximicin is a natural proteasome inhibitor (PI). It is a product of actinomycetes bacteria which live in moist soil. When the bacteria release this toxic metabolite it can pass into the underground water store providing a route for human exposure [Ensign *et al.* 1993].

PIs are small, bioavailable molecules that attack the 20S proteasomal subunit, the primary site responsible for the cleavage of peptide bonds. By covalently binding to the 20S subunit, PIs successfully block proteolysis and degradation of proteins. Defects in the ubiquitin–proteasome system (UPS) and degradation of unwanted proteins are considered a common feature in PD. Moreover, in hereditary forms of PD, mutations in the UPS (parkin and ubiquitin C-terminal hydrolase L1) are responsible for the development of the disease. Mayer used the Cre-recombinase/loxP genetic approach to ablate the proteasomal Psmc1 ATPase gene and deplete 26S proteasomes in neurons in different regions of the brain to mimic neurodegeneration [Mayer, 2003]. This approach generates dopaminergic neurodegeneration accompanied by Lewy pathology. Based on the previous data, proteasome inhibition can be used to induce PD [Bedford *et al.* 2009].

The original idea of using epoximicin as a systemic PI in PD modeling was presented by McNaught and colleagues who showed that systemic exposure of rats to epoximicin can induce a model of PD. After a latency of 1–2 weeks, treated animals develop a gradually progressive, L-dopa/apomorphine-responsive, Parkinsonian syndrome with a decrease in striatal 11C-CFT binding on positron emission tomography

(PET). Pathologically, there is neurodegeneration in the SNc, as well as in the locus coeruleus (LC), dorsal motor nucleus of the vagus (DMN), and nucleus basalis of Meynert (NMB), a pattern of neuronal loss similar to that found in PD. Furthermore, neurodegeneration is accompanied by intracytoplasmic Lewy body-like inclusions that stain positively for α -synuclein, ubiquitin, and other proteins [McNaught *et al.* 2004]. Thus, PIs can induce a Parkinsonian syndrome in rats that more closely recapitulates the features of the illness in humans than other models.

The same model, however, has not been reproducible in many trials in rodents in spite of using the same doses as used initially. This raised concerns about the possible application of epoximicin in animal models of PD [Bove *et al.* 2006; Kordower *et al.* 2006]. Matsui and colleagues, however, demonstrated that the administration of this natural PI to medaka fish via the cerebrospinal fluid (CSF) induces PD-like symptoms in the form of reduced spontaneous movement. Fish treated with epoximicin developed inclusion bodies similar to Lewy bodies throughout the central nervous system, which are associated with PD. Treatment with epoximicin also induced selective loss of dopaminergic and noradrenergic neurons. So, in this model epoximicin replicated the cardinal signs of PD in the form of locomotor dysfunction, selective dopaminergic cell loss, and inclusion body formation. The reason for this controversy between models is unknown, but in medaka the TH-positive neurons have relatively large cell bodies with well-developed fibers. These large neurons may require more optimal conditions for survival and this may explain the increased vulnerability of these cells. The problems with animal models of epoximicin can be solved by increasing the dose to overcome the lesser vulnerability of animal neurons compared with the medaka fish [Matsui *et al.* 2010].

These findings demonstrate that epoximicin can be considered to be an effective dopaminergic neurotoxin that may offer a good model for PD, as it is widely distributed, linked epidemiologically to PD, and can re-induce the pathological features of PD experimentally.

Nocardia asteroides

The epidemic of postencephalic Parkinsonism was first described by von Economo in 1917.

This gave rise to the belief that PD might be associated with an infectious agent such as a virus, more infectious agents such as *Borrelia burgdorferi*, *Mycoplasma pneumoniae* and *Helicobacter pylori* have been postulated as possible inducing agents for Parkinsonism [Tam *et al.* 2002].

Currently, there is a well-documented animal model for PD in mice, using the GUH-2 strain of *Nocardia asteroides*. *N. asteroides* is a bacterial strain found worldwide that is commonly isolated from soil and aquatic sources. It is a Gram-positive aerobic bacterium that primarily affects the lung and skin. Pathogenic strains can cause a variety of pulmonary, skin, systemic and brain lesions in immunocompromised or normal humans. *N. asteroides* (GUH-2) is neuroinvasive after tail vein injection into mice [Barry and Beaman, 2007].

The first experimental trial of *N. asteroides* was conducted by Kohbata and Beaman who through multiple injections of this pathogen were able to induce a PD-like condition in mice which was later confirmed by postmortem pathological examination [Kohbata and Beaman, 1991]. They found that after administration of a non-lethal dose of log phase cells of GUH-2 (<200 colony forming units reaching the brain), Nocardiae invaded through the capillary endothelium, crossed the basal lamina, and then grew perivascularly within the brain parenchyma without associated inflammatory responses. These bacteria grew primarily within neurons, along axons, and in astroglia. Approximately 10 days after infection, mice developed one or more features of neurological impairment. Later on, the headshaking, stooped posture, and bradykinesia tended to persist for the life of the mouse. However, administration of levodopa (20 mg/kg i.p.) caused transient reversal of head-shaking, tremors, and hypoactivity. These abnormal movements were confirmed by histopathological analysis of the brains from mice. Histopathological findings showed decrease of neurons in the substantia nigra, a loss of Nissl substance in the remaining neurons, and diminished immunostaining for tyrosine hydroxylase in the substantia nigra pars compacta. The previous data suggest a possible dopaminergic involvement in the mice inoculated with GUH-2. Another interesting finding in this model was the selective reduction in neostriatal DA concentrations at 1 week after GUH-2 inoculation,

without a change in NE or 5HT concentrations. Moreover, the selective changes for neostriatal metabolism at 1 week suggest that GUH-2 has specificity for nigral neurons engaged in dopamine synthesis. A significant finding in the case of idiopathic PD is the Lewy body, although no animal model of Parkinsonism has led to their generation. In the case of GUH-2-induced models, hyaline-like intraneuronal inclusions were found in the murine brain, which is considered a major strength of this type of model with regards to its relation to the progression of disease [Hyland *et al.* 2000].

Based on many studies, we can say that *Nocardia* induce neurodegeneration *in vivo* and *in vitro* similar to that seen with MPTP, supporting this model for the study of Parkinsonism [Kohbata and Beaman, 1991; Hyland *et al.* 2000]. Moreover, another study used *N. otitidiscaviarum* (GAM-5) which is isolated from a patient with an actinomycetoma. GAM-5 produced signs similar to PD following intravenous administration to NMRI mice. Motor signs included head tremor, akinesia/bradykinesia, flexed posture, deviation of the head, trunk flexion, and postural instability in a manner similar to *N. asteroides*. In both models (GAM-5 and GUH-2), the loss of bacteria in the brain coincided with the appearance of the motor impairments described above [Diaz-Corrales *et al.* 2004].

Why the mice developed signs of Parkinsonism after the apparent elimination of the bacteria from the brain is still unknown. A possible explanation could be the release of neurotoxic substances from the nocardial cells as they were killed and cleared from the brain [Beaman and Tam, 2008].

A final strength supporting the use of these bacteria in PD models is the intracytoplasmic hyaline-like inclusion bodies in neurons, mostly in the substantia nigra, that resemble Lewy bodies. Whether or not the chemical composition of these intracytoplasmic hyaline-like inclusion bodies is the same as that found in PD remains to be determined. Nevertheless, *Nocardia*-induced PD animal models represents a new, different, and promising approach based on the previous findings [Barry and Beaman, 2007].

Other natural agents

Many other agents have been suggested as possible substitutes to the classical PD-inducing

agents. Some of them are of bacterial origin, e.g. *Streptomyces venezuelae*, and others are of plant origin, e.g. annonacin [Höllerhage *et al.* 2009].

On Guadeloupe island, the consumption of *Annona muricata* has been linked to sporadic tauopathy. The main element in *A. muricata* (annonacin) can easily cross biological membranes, owing to its high lipophilicity, so is capable of entering nerve cells. After chronic exposure in rats, neuronal cell loss and gliosis were observed in the brain stem and basal ganglia [Caparros-Lefebvre and Lees, 2005].

The aggregation of the microtubule-associated protein (tau protein) characterizes several neurodegenerative diseases known collectively as tauopathies [Trojanowski and Lee, 2005]. While PD, the most frequent cause of Parkinsonism, is the best known of the α -synucleinopathies, tau pathology is also seen in many PD cases. Staining of some Lewy bodies with multiple tau antibodies has been reported, suggesting cellular colocalization of these two pathologies [Ding and Johnson, 2008]. Höllerhage and colleagues found that lipophilic complex I inhibitors of natural or

synthetic origin can reproduce *in vitro* tauopathies. Exposure to annonacin led to phosphorylation and somatodendritic redistribution of tau in a manner similar to MPTP effects. Epidemiological and bioanalytical studies should be undertaken to verify this hypothesis. Moreover, *in vivo* experiments are needed to combine these findings with the data on absorption, distribution, metabolism, and elimination (ADME) which are still lacking [Höllerhage *et al.* 2009].

Table 2. Natural toxins that can be used in Parkinson's disease models.

Agent	Source
Sterol glucosides	Cycads
Epoximicin	Actinomycetes bacteria
<i>N. asteroides</i>	<i>Nocardia asteroides</i> bacteria
<i>S. venezuelae</i>	<i>Streptomyces venezuelae</i> bacteria
Annonacin	Annonacea plant
DOPAL	Dopamine metabolite

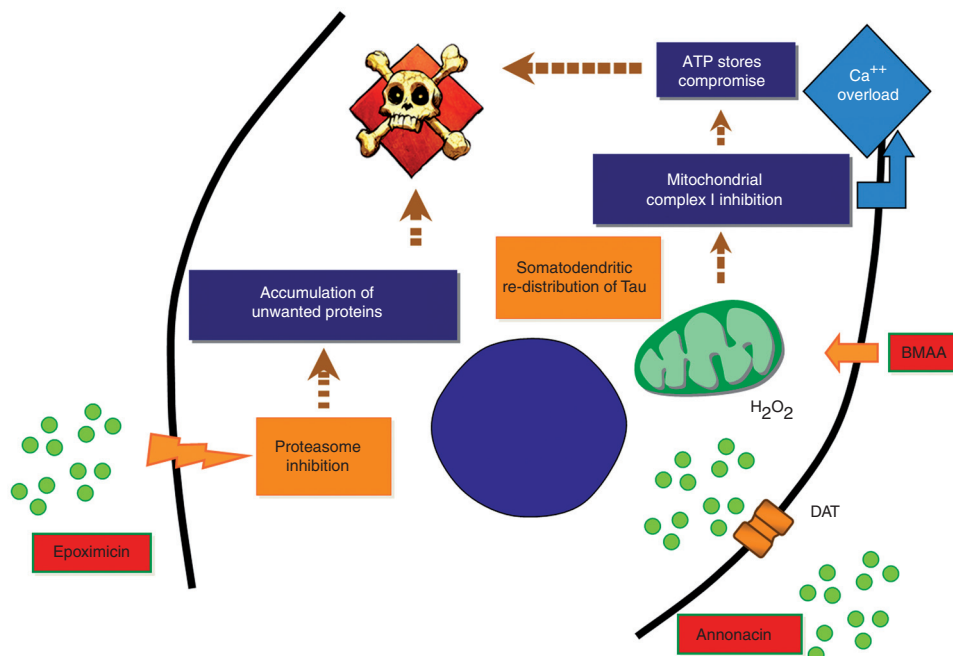


Figure 1. A diagram showing different pathways of some natural agents that can induce PD experimentally. Epoximicin through proteasome inhibition leads to the accumulation of unwanted scaffold proteins which will further lead to dopaminergic degeneration and inclusion body formation. Beta-methylamino alanine (BMAA) through an increase in reactive oxygen species (ROS) and increasing calcium intake will lead to cellular degeneration. Annonacin is working as a mitochondrial complex I inhibitor which affects oxidative process and aids in dopaminergic degeneration.

Another agent suggested by Panneton and colleagues was the dopamine metabolite DOPAL. They demonstrated that injections of DOPAL selectively kill SN DA neurons and trigger a behavior consistent with other PD animal models. This study supports the ‘catecholaldehyde hypothesis’ as an important link for the etiology of sporadic PD [Panneton *et al.* 2010]. However, future validation of DOPAL in PD modeling is needed to justify its use in experimental studies.

Table 2 shows a list of suggested natural agents that can be used in toxic PD models, while Figure 1 represents different mechanisms exerted by some natural toxins to induce PD.

Conclusion

Owing to the limitations of the available PD models, a search for new modeling techniques seems mandatory to improve the PD research and evaluation of new promising therapies. Recently, natural toxins have seemed more appropriate to be used in PD models, depending on the epidemiological data that suggest the role of exposure to such natural agents in inducing PD. In our review we have explored some natural agents that can be used as targets for future research to replace classical toxic PD models. Based on previous research, some of the natural agents induce a chronic progressive PD model in rats with pathological findings recapitulating the pathology of this disease (cycad seeds, *N. asteroides*). Other agents prove efficient in inducing dopaminergic degeneration in cell cultures (annonacin) or medaka fish brains (epoximicin). However, most of the suggested natural agents need further optimization both on the levels of exposure dose and the precise pathological findings predicted from such natural toxins. We consider this review an invitation to conduct more research to validate possible natural agents inducing PD models. Only after appropriate design of PD models based on natural toxins can new therapeutic modalities be challenged more confidently for neuroprotection against PD.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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