REVIEW

The therapeutic role of minocycline in Parkinson's disease

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Abstract

Minocycline, a semisynthetic tetracycline-derived antibiotic, has been shown to exert anti-apoptotic, anti-inflammatory, and antioxidant effects. Furthermore, there is rapidly growing evidence suggesting that minocycline may have some neuroprotective activity in various experimental models such as cerebral ischemia, traumatic brain injury, amyotrophic lateral sclerosis, Parkinson's disease (PD), Huntington's disease, and multiple sclerosis. In this perspective review, we summarize the preclinical and clinical findings suggesting the neuroprotective role of minocycline in PD.

Keywords: minocycline, neurodegeneration, neuroprotection, Parkinson's disease.

Introduction

Although the molecular cell death mechanisms involved in neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD), are still not fully understood, progress in the knowledge of neuronal cell death in the common pathways has led to new approaches for neuroprotective therapies. The common pathways may include several pathogenic processes, such as energy (mitochondrial) impairment, secondary glutamate excitotoxicity, oxidative stress, and inflammatory mechanisms.[1] Minocycline has been shown to exert its neuroprotective effect on common pathways in several neurodegenerative diseases, including PD.[2–5] Among the neurodegenerative disorders, PD remains the only one that responds well to symptomatic therapy. Although levodopa treatment has been accepted as the gold standard of treatment, its chronic use is associated with potentially disabling motor complications. Furthermore, although it may improve some of the most disabling symptoms of PD, patients are faced with increased substantial disability over time and a future of dependency. In addition, there is no evidence that levodopa can halt or slow the progressive degeneration of dopaminergic (DA) neurons in the substantia nigra (SN). Moreover, there are controversial findings regarding the toxic actions of levodopa on the remaining dopaminergic neurons. Thus, there is a compelling need to develop treatments that might slow down the motor and nonmotor symptoms such as cognitive impairment in PD. Thus, one of the most important therapeutic needs in PD is the development of effective disease-modifying or neuroprotective therapies.[6–8] In the light of these findings, researchers have spent an enormous effort to develop candidate neuroprotective agents for PD patients for the last 20 years. Recent findings of abnormal protein folding, coupled with oxidative stress and neuroinflammation, provide a scientific rationale for novel therapeutic strategies designed to slow disease progression in PD. In this context, recent research has been focused on the possible neuroprotective effects of anti-inflammatory drugs. So far, numerous studies, including the genetic, neurotoxic animal models, and clinical trials of minocycline in PD, have indicated that it may exert neuroprotective activity through inhibition of microglial activation, neuroinflammation, and neuronal apoptosis. Despite these promising results, no therapy has yet been proven to halt or slow disease progression of PD.

Minocycline has been chosen for this perspective review because of its capacity to decrease oxidative injury and neuroinflammation and so exert a potential neuroprotective activity, which has been proven in various *in vivo* and *in vitro*

animal models. In this respect, our rationale was to evaluate minocycline's neuroprotective effect in PD and to compare relevant studies, including clinical results, pathologic findings, and cognitive outcomes of minocycline. We have included retrospectively recorded results from animal studies, clinical trials, and also reviews in Medline via PubMed, Scopus, and ISI Web of Science. We used 'Minocycline and Parkinson's disease', 'minocycline and neuroprotection', 'minocycline', and 'neurodegeneration' as key search items. From 292 potentially eligible reviews and studies, we selected 92 articles for full-text analysis.

Minocycline

Minocycline is a second-generation, semisynthetic tetracycline analogue, which is a highly lipophilic molecule and can easily penetrate the blood–brain barrier.[9] It shows a better pharmacokinetic profile than the first-generation tetracyclines. However, it is rapidly and completely absorbed, even in elderly populations, with a longer half-life and excellent tissue penetration.[10,11] In addition, it is a highly lipophilic molecule that can easily pass through the blood-brain barrier[12], thus promoting its accumulation in the cerebrospinal fluid (CSF) and central nervous system (CNS)[9,13,14] and enabling its use in the treatment of several CNS diseases.[15–17] In rodents, minocycline readily crosses the blood-brain barrier with a rate of at least fivefold higher than doxycycline, another compound of the same family.[18,19]

Thereby, minocycline has been extensively studied in treating neurodegenerative diseases[17,20,21] and has been reported to exert neuroprotective effects on various experimental models. These studies based on minocycline's ability to inhibit microglia activation, which is a process that has deleterious effects on neurogenesis and neuronal survival could justify its potential effectiveness in the treatment of neuroinflammatory and/or neurodegenerative disorders.

Mechanisms of action of minocycline

Parkinsonian symptoms are caused by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the consequent loss of their projecting nerve fibers in the striatum.[22] Neuroinflammation and microglial activation, a hallmark of neuroinflammation that has been generally considered as an integral component of the progressive neurodegenerative process, has been increasingly implicated in the PD pathogenesis.[23] It is noteworthy that minocycline is a potent inhibitor of microglial activation and of apoptotic pathways. Considering all of this evidence, it is not unreasonable to assume that minocycline exerts neuroprotective activity on animal models with PD *via* protecting the nigrostriatal pathway. Modes of actions of minocycline are summarized in Figure 1 and Box 1.

The anti-inflammatory effect of minocycline

Until now, studies have already indicated that neuroinflammation is mediated by microglia and astrocytes, which produce inflammatory cytokines, reactive oxygen species, and other toxic materials in the CNS.[25] Here, reactive microgliosis, astrocytosis, an increase in proinflammatory mediators, and activation of inflammation-associated protein kinases are features common to many neurodegenerative disorders. For instance, a chronic release of proinflammatory cytokines by activating astrocytes and microglia leads to the exacerbation of DA neurons degeneration in the SNpc. These findings together suggest that microglia are one of the major cell types that are involved in the inflammatory response in the CNS. In parallel with that, it has been proposed that activated microglia may be beneficial to the host, at least in the early phase of the neurodegeneration process.[26–28]

The above-mentioned chronic neuroinflammation is also one of the hallmarks of PD pathophysiology. Beyond preclinical studies suggesting that there is a very similar chronic neuroinflammation process in PD, clinical data indicating the role of neuroinflammation are also rapidly replicating in PD.

Postmortem analyses of human PD patients indicate that the activation of glial cells and increases in proinflammatory factor levels are common features of the PD brain. However, long-term overactivation of microglia in the PD brain significantly upregulates the expression of a large group of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1 beta (β), and interferon gamma (IFN-γ), which contribute to the acceleration of nigral DA neuron degeneration.[29–31]

The main biological effects of minocycline, which are involved in the pathogenesis of several neurodegenerative disorders, include inhibition of microglial activation, attenuation of apoptosis, and suppression of reactive oxygen species (ROS) production. These effects appear completely different from its antimicrobial action.[32] Minocycline exerts its neuroinflammatory actions by modulating microglia, immune cell activation, and subsequent release of cytokines, chemokines, lipid mediators of inflammation, MMPs, and NO release.[33] Proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are produced by microglial cells, astrocytes, neutrophils, and macrophages and augment both inflammation and following immune responses.[33] Thus, any potential beneficial effects of minocycline in neurodegenerative diseases might well be exerted through its actions on the molecular pathways involving the modulation of one or more of these above-mentioned inflammatory factors.

Figure 1. Signaling mechanism involved in the neuroprotective actions of minocycline.
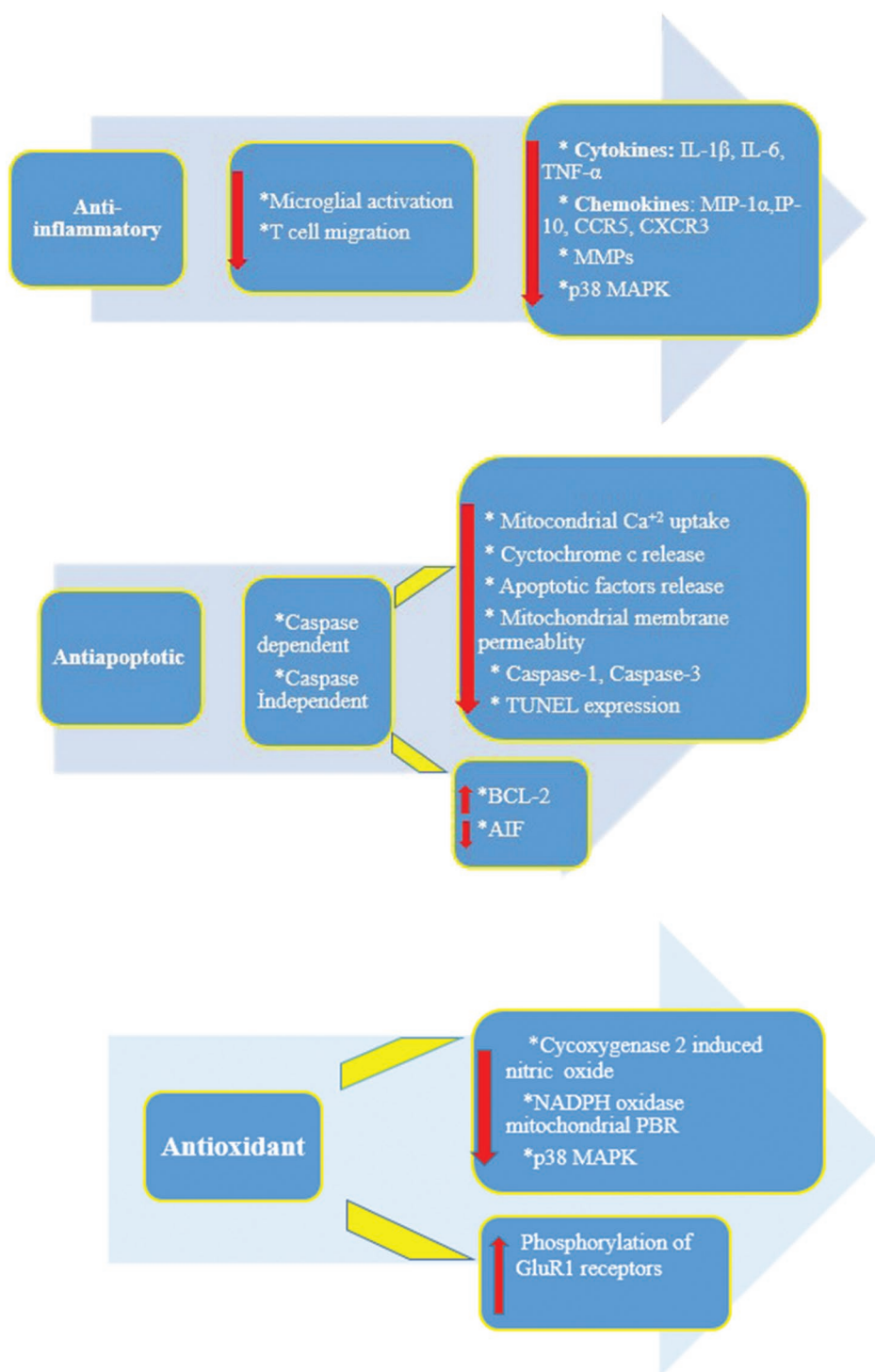
AIF, apoptosis-inducing factor; BCL-2, B-cell leukemia/lymphoma 2; CCR-5, chemokine receptor type 5; CXCR3, chemokine (CXC motif) receptor; GluR, glutamate receptor; IP-10, interferon-inducible protein; MAPK, mitogen-activated protein kinase; MIP-1α, macrophage inflammatory protein 1α; MMP, matrix metalloprotease; NADPH, nicotinamide-adenine dinucleotide phosphate; PBR, peripheral benzodiazepine receptor; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase dUTP (2´-deoxyuridine, 5´-triphosphate).

Box 1. Modes of action of minocycline.[24]

A. Anti-inflammatory actions

➢ Modulation of microglia:

- To reduce the proliferation/activation of resting microglial cells, subsequently decreasing the release of cytokines, chemokines, lipid mediators of inflammation, MMPs, and nitric oxide (NO) release

➢ Alteration of immune cell activation:

- To inhibit transmigration of T lymphocytes

B. Anti-apoptotic actions

➢ Caspase-dependent anti-apoptotic actions:

- Inhibition of cytochrome c release from mitochondria by attenuating mPT

- Inhibition of caspase-1 and -3 expression

➢ Caspase-independent anti-apoptotic actions:

- Increase in the expression of Bcl-2

- Inhibition of AIF release from mitochondria

C. Involvement in the signaling pathways

Inhibition of p38 MAPK activation in microglia, thereby attenuating the production of IL-8, superoxide generation, and neutrophil chemotaxis

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AIF, apoptosis-inducing factor; Bcl-2, B-cell leukemia/lymphoma 2; IL-8, interleukin-8; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloprotease; mPT, mitochondrial permeability transition.

Signaling pathways involved in minocycline's neuroprotective actions

Signaling mechanisms are also involved in the neuroprotective actions of minocycline. Minocycline shows its neuroprotective actions on p38 mitogen-activated protein kinase (MAPK)-dependent mechanism in the signaling pathway.

P38 MAPKs are serine–threonine kinases that play significant roles in signaling for a vast number of cellular functions including cell migration, proliferation, and differentiation. It is initially identified as a stress-activated phosphorylated in response to inflammatory cytokines.[34] The reduction effect of minocycline in p38 MAPK activities has inspired studies for validating minocycline's neuroprotective effect.

It has been shown that minocycline increased neuronal survival in mixed spinal cord cultures, reduced NO-induced death of rats cerebellar granule neurons,[35,36] and attenuated cell death by 50% in hypoxic-ischemic injury-induced death of a motor neuron cell line[37] by reducing microglia activation through p38 MAPK-dependent mechanisms.

The anti-apoptotic effect of minocycline

A characteristic of many neurodegenerative diseases is neuronal cell death. Cell death occurs by necrosis or apoptosis.[38] Although there were controversial data on the special cell death form of PD,[39] recent studies support that apoptotic rather than the necrotic degeneration of dopaminergic cells in the SN plays a critical role in the pathogenesis of PD. Consistently, apoptosis has been demonstrated in the SN of postmortem brains of PD

patients.[39–41] Tetracycline and its derivatives, especially minocycline, are a group of semisynthetic antibiotic agents that, besides their anti-inflammatory and antimicrobial properties, interfere with the activation of enzymes in the apoptotic pathway.[42] Minocycline decreased apoptotic neuronal cell death observed under various experimental models of neurodegenerative diseases such as ischemia, HD, PD, AD, and ALS.[43,44] This apoptotic effect is mainly being exerted on mitochondria. By reducing mitochondrial calcium overloading, minocycline stabilizes the mitochondrial membrane and inhibits release of cytochrome c and other apoptotic factors into the cytoplasm, which finally results with decreased caspase activation and nuclear damage[45,46] that play a critical role in the apoptotic cell death cascade.

Furthermore, Caspase 8 activates a series of other caspases that finally lead to a series of lethal cleavages resulting in apoptosis (Figure 2).[47] It has been revealed that B-cell leukemia/lymphoma 2 (Bcl-2) exerts an anti-apoptotic effect through stabilizing the mitochondrial membrane. From this point of view, minocycline treatment also exerts a neuroprotective effect through shifting the Bcl-2 family proteins toward the anti-apoptotic ratio in neuron/microglia co-cultures.[48] This is consistent with the anti-apoptotic action of minocycline that is associated with its modulating effect on the expression of Bcl-2 proteins in various models.[49–52]

In gerbils with global brain ischemia, minocycline increased the survival of hippocampal neurons and this protection was associated with reduced caspase-1 expression.[53] Minocycline inhibits both caspase-dependent (cytochrome c and Smac/DIABLO) and caspase-independent anti-inflammatory

Figure 2.   Schematic representation of apoptosis and proposed sites of inhibition. 1. Free oxygen radicals, lipid peroxidation, and increased cytosolic calcium cause mitochondrial depolarization and an opening of the mitochondrial permeability transition pore on the inner membrane and an outer membrane pore in comprising products of the Bcl-2 family member, Bax. This causes the release of cytochrome c into the cytosol. 2. Cytochrome c combines with Apaf-1 forming a complex called the apoptosome. Procaspase 9 is eventually cleaved on the apoptosome forming active caspase 9 (initiator caspase). 3. Caspase 9 activates the executioner caspase 3. Caspase 3 causes DNA damage, PPAR-1 activation and finally apoptosis. 4. Caspase 8 is activated by the DISC, which is attached to the death receptor (belongs to the family of TNF receptors). 5. Minocycline inhibits cytosolic calcium overload, binds with free oxygen radicals (ROS), inhibits Bax, and activates Bcl-2 (antiapoptotic gene) 6. It also inhibits caspase 1, and activation and reactivation of caspase 3. 7. It stabilizes the mitochondrial membrane. 'Activation' is shown by black arrows and 'inhibition' is shown by red arrows. H^+
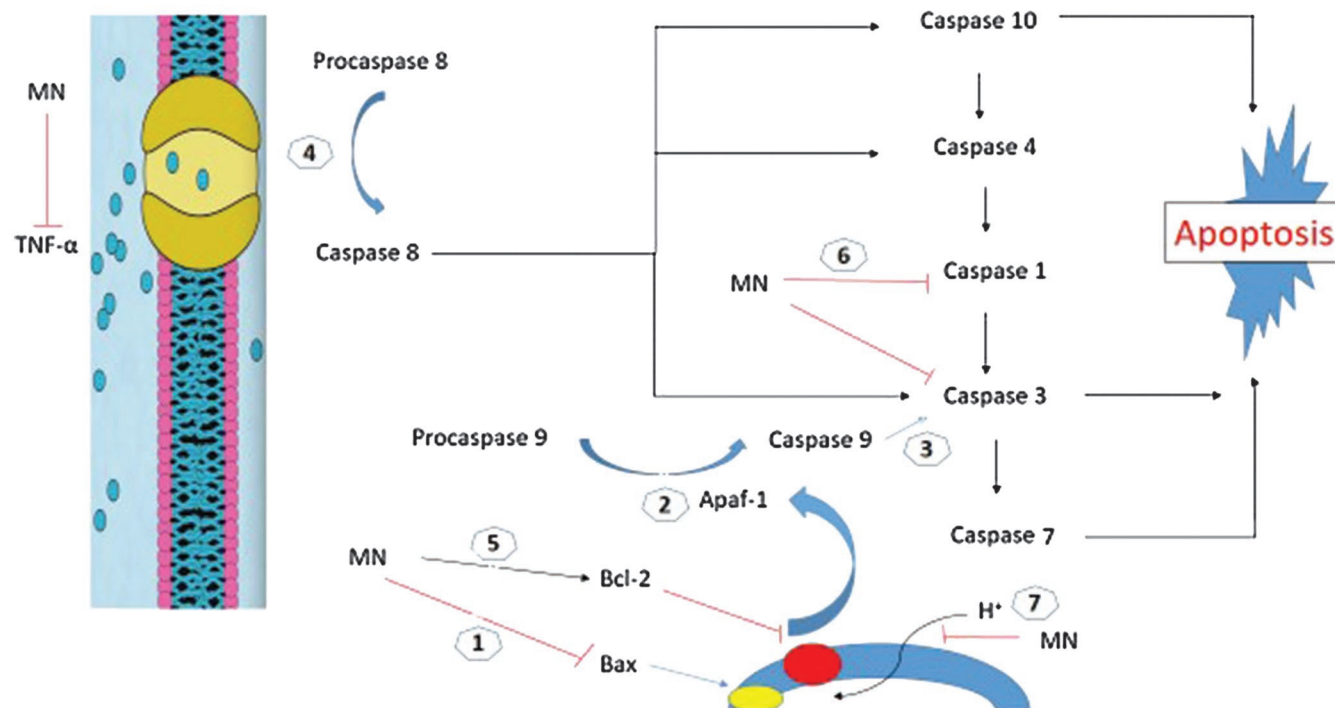
Apaf-1, apoptotic protease activating factor; Bax, Bcl-2-associated X-apoptosis regulator; Bcl-2, B-cell leukemia/lymphoma 2; DISC, death-inducing signaling complex; DNA, deoxyribonucleic acid; PPAR-1, peroxisome proliferator-activated receptors-1.

activity in mitochondrial death pathways[16] that is in line with its inhibitory effect on cytochrome c release, caspase-3 activation, and inflammation as shown by *in vivo* and *in vitro* models of HD and ALS.[16,54]

Many studies have revealed that PD, cerebral ischemia, ALS, and HD share common pathophysiological pathways (i.e., oxidative injury, neuroinflammation) during the neurodegenerative process. As specifically mentioned earlier for the neuroprotective actions of minocycline, it has become a candidate for slowing or halting the degenerative process in the CNS.

Minocycline and neuroprotection

Yrjanheikki and colleagues first described minocycline as a neuroprotective agent that inhibits the ischemia-induced

activation of microglia in an animal model of ischaemia.[53] The neuroprotective role of minocycline was confirmed with further studies, which showed that minocycline inhibited neuronal death and reduced neurodegenerative disease progression including in different animal models such as PD, HD, MND, AD, and multiple sclerosis. For instance, in AD models, minocycline inhibited Aβ fibril formation,[55] attenuated amyloid-induced microglial activation,[56] and reduced the inflammatory events that were associated with the prevention of cognitive deficits.[57] In line with this, minocycline also reduced the *N*-methyl D-aspartic acid (NMDA) toxicity in animal models of ischemic stroke[35,58] and slowed the neurodegenerative progression in a mice model of HD.[3,16,59] Unfortunately, despite promising results in experimental models of ALS,[60,61] patients on minocycline declined more

quickly than those on a placebo in clinical trials (phase III stage).[62]

It should also be noted that a recent study has revealed that treatment with minocycline improved spatial memory that was mediated through reduced the levels of Aβ and microglial activation in a mice model of AD.[63] In addition, a clinical study has suggested that minocycline reduced the risk of conversion of clinically isolated syndrome to multiple sclerosis (MS) in a randomized, placebo-controlled trial of early multiple sclerosis patients at 6 months.[64] The results of this clinical study were confirmed with a recent experimental study suggesting that minocycline enhanced the self-renewal capability of neural stem cells into the oligodendrocytes that were independent of its inhibitor effect on microglial activation in an autoimmune encephalomyelitis that is an animal model for multiple sclerosis.[65] The neuroprotective and cognitive effects of minocycline in animal and human studies in PD have been summarized in Table 1.

Neuroprotective effects of minocycline in animal models of Parkinson's disease

Oxidative stress, mitocondrial dysfunction, inflammation, excitotoxicity, and abnormal processing of mutant proteins have played a critical role of the cell death of PD.[42] Although the underlying mechanisms of the neuroprotective effect of minocycline are unclear, it is reasonable to assume that minocycline might exert its effect through an interactive signaling network including the mitochondria, oxidative stress, excitotoxicity, PARP-1, and apoptosis.[42]

To reveal minocycline's protective effects in PD, animal models with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are widely used. MPTP reproduces some of the basic PD features in primates and rodents. Thereby, it is regarded as a well-established model.[66] In one MPTP model, minocycline treatment blocked dopamine depletion in the nucleus accumbens after MPTP administration.[5] This

Table 1. Results of experimental studies on minocycline treatment for Parkinson's disease.

Study	Results
Du et al. (2001)[5]	↓MPTP-induced degeneration of DA neurons in the SNpc preventing loss of striatal DA and its metabolites ↓MPP+ mediated inducible NO synthase expression *in vivo* and blocked NO-induced neurotoxicity *in vitro*
Yi et al. (2001)[80]	↓Microglial activation and protection to tyrosine hydroxylase positive cells at 14 days after 6-OHDA injection.
Lin et al. (2001)[36]	↓p38 MAP kinase phosphorylation at 3 h by NO
Lin et al. (2003)[105]	↓6-OHDA-induced neuronal death in rat CGN
Yang et al. (2003)[74]	↑ Neuron death, ↓ microglial activation after MPTP in DA neurons *in vitro* and *in vivo*
Diguet et al. (2004)[75]	↑Severe/rapid parkinsonism, behavior deficits, and greater loss of nerve endings after MPTP in monkeys
Quintero et al (2006)[106]	↓The loss of tyrosine hydroxylase-immunoreactive cells in 6-OHDA-lesioned nigras in rats
Peng et al. (2006)[107]	↓ Nigrostriatal dopaminergic neurodegeneration in murine mutant *weaver* mouse *in vivo*
McCoy et al. (2006)[30]	↓ 50% the retrograde nigral degeneration with neutralization of soluble TNF induced by a striatal injection of 6-OHDA *in vivo*
Faust et al. (2009)[108]	↑ DA neuroprotection in a *Drosophila* model of PD and after rotenone toxicity in rodents
Radad et al. (2010)[69]	↓Neuron death after rotenone toxicity in embryonic mice
Bao-Ping Jiang et al. (2014)[67]	↓ICAD degradation and the NF-κB activation induced by 6-OHDA in pheochromocytoma (PC12) cells.
Cronin et al. (2017)[70]	↓Neuronal loss in a Zebrafish 6-OHDA model
Clinical Trials	
Study	**Results**
The NINDS NET-PD Investigators (2006)[93,94]	Minocycline and creatine are futile in slowing down the progression of disability in PD at 12 months and 18 months.

6-OHDA, 6-hydroxydopamine; CGN, cerebellar granule eurons; DA, dopaminergic neuron; ICAD, inhibitor of caspase-activated DNase; MAPK, mitogen-activated protein kinase; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NET-PD, neuroprotective exploratory trials in Parkinson disease; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NINDS, National Institute of Neurological Disorders and Stroke; NO, nitric oxide; PD, Parkinson's disease; SNpc, substantia nigra pars compacta.

Table 2. Neuroprotective effects of minocycline on pathological features of Parkinson's disease.[81]

Outcome	Animal	Outcome	Animal	Human
↑ Dopaminergic cell survival[82]	+	Mitochondrial dysfunction and oxidative stress[5,83]	↓cytochrome c release and blocks apoptosis ↓caspase 1 expression	–
↓ Alpha-synuclein expression[84]	+	Neuroinflammation[22]	Inhibits MPTP/6-OHDA and blocks microglial activation	–
↑ GRP78 expression[85]	+	↓ MPP+-induced apoptosis[86]	+	–

6-OHDA, 6-hydroxy-dopamine; GRP78, glucose-regulated protein; MPP+, 1-methyl-4-phenylpyridinium, MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

neuroprotective effect of minocycline was associated with marked reductions in inducible NO synthase (iNOS), caspase 1, mature interleukin-1, and the activation of NADPH–oxidase that are microglial-derived cytotoxic mediator expression.[5] The same group has also shown that mutant iNOS-deficient mice treated with minocycline are more resistant to MPTP than iNOS-deficient mice not treated with minocycline.[22] They commented the microglial-related inflammatory events' crucial role in the MPTP neurotoxic process and suggested that minocycline may be a valuable neuroprotective agent for the treatment of PD.[22] The neuroprotective effect of minocycline was also shown against the NO-induced phosphorylation of p38 mitogen-activated protein (MAP) kinase[36] as well as the 6-hydroxydopamine (6-OHDA)-induced cell toxicity.[67] Accordingly, the treatment of minocycline stimulated the differentiation of newly generated neuroblasts into mature striatal oligodendrocyte by increasing the activity of adult stem cells in a mice model of PD that suggested the possible role of oligodendrogenesis responsible for the behavioral improvements in the PD symptoms due to the increased stability and efficiency of axonal function.[68]

Additionally, minocycline was found to produce significant neuroprotection in primary dopaminergic cultures[69] and zebrafish 6-OHDA model that was associated with the prevention and reversion of the locomotor deficits related to 6-OHDA.[70]

Minocycline's protective effect on striatal dopamine loss was suggested with a different study model of SIV/macaque model of HIV-associated CNS disease showing that minocycline prevented striatal dopamine loss via elevated monoamine oxidase activity and antioxidative effect.[71] In line with this, Eunju and colleagues have recently shown that minocycline also inhibited Prothrombin Kringle-2 (pKr-2) driven microglial activation in vivo. Similar antioxidant effects of minocycline have been also defined in oxidative impairment due to cypermethrin.[72] The neuroprotective effects of minocycline were suggested following transgenic and MPTP studies including the Parkin null mutation[23] and paraquat-induced mouse models.[73] Despite these promising results, there are controversial preclinical data regarding the neuroprotective effect of minocycline.

For instance, although minocycline inhibited microglial activation, minocycline significantly exacerbated MPTP-induced damage to DA neurons[74] that is due to the inhibition of DA and 1-methyl-4-phenylpyridinium (MPP+) uptake into striatal vesicles. Additionally, Diguet and colleagues have shown the deleterious effect of minocycline on a mean motor score that was associated with impaired histopathologic outcomes in an MPTP mouse model of PD.[75] It is difficult to estimate the cause of these inconsistent preclinical results; however, it can be hypothesized that different neurodegeneration and neuroinflammatory pathways could be responsible for failed positive minocycline study results in the literature. Despite these negative trials, minocycline has been proposed as a potential therapeutic agent for the treatment of neurodegenerative diseases[17,76] due to its wide range of protective effects in different animal models of brain diseases.[2,3,5,22,43,53,54,77] In line with this, the significant beneficial effects of minocycline on neuroinflammatory processes have been shown in PD (Table 2) as well as stroke animal models and in other neurodegenerative disease model.[4,2,35,78–80]

Procognitive effects of minocycline in animal models of Parkinson's disease

To the best of our knowledge, no study in the literature has evaluated cognitive outcomes in a PD animal model. As neuroinflammatory processes are implicated in neurodegenerative disorders characterized by prominent cognitive impairment, minocycline could be a promising candidate. Animal data would give us important clues regarding the possible procognitive effect of minocycline that is probably related to its anti-inflammatory effect through modulating the lipopolysaccharide (LPS).

It has been already revealed that LPS, a bacterial endotoxin, a commonly used inducer of neuroinflammation, can lead to cognitive impairment.[87] In this context, it is interesting to note that minocycline attenuates LPS-induced cognitive impairments in mice are associated with its action to suppress the neuroinflammation. From this point of view, it can be

hypothesized that minocycline may normalize the activation of microglia astrocytes and brain-derived neurotrophic factor expression.[87]

Additionally, in experimental models of LPS-induced neuroinflammation, minocycline reversed the nigral dopaminergic neuronal cell death and the loss of reactive astrocytes that were associated with improved cognitive outcomes.[88,89]

Consistently, minocycline has been also shown to improve neurobehavioral deficits and cognitive decline in AD animal models that were related to the inhibition of neuroinflammatory markers, Aβ fibril formation, and NMDA toxicity.[35,55,56,90] Agreeably, neuroinflammation, including reactive astrocytes and activated microglia, correlates with a serious cognitive decline and brain atrophy in AD.[91,92]

Neuroprotective effects of minocycline in clinical trials of Parkinson's disease and Parkinson plus syndromes

Several mechanisms of action have been proposed to be responsible for the neuroprotective effects of minocycline, which include the attenuation of microglial activation, apoptosis, and ROS production.

Regarding the neuroprotective properties of minocycline, the National Institute of Neurological Disorders and Stroke (NINDS) has aimed to identify potential agents to modify disease progression in PD. Through the Neuroprotective Exploratory Trials in Parkinson's Disease (NET-PD) program, creatine and minocycline were used for the first study over 12 months (The NET-PD Futility Study 1, NET-PD FS-1).[93] Although there was evidence that both agents can protect against the dopaminergic neuronal loss associated with MPTP administration in a mouse model of PD, this study has failed to confirm the clinical neuroprotective effect of minocycline. An additional 6 months of follow up aimed to assess the safety and potential interactions of the study interventions with anti-Parkinsonian therapy (NET-PD FS-2). At the end of following-up for 18 months, this phase II clinical study suggested that there was neither a beneficial effect nor an adverse event from using either creatine or minocycline in the treatment of PD. The authors commented that there was a need for a larger, phase III efficacy trial.[94]

NET-PD investigators have also conducted a recent cohort-analysis to measure the disease progression in early PD through analyses an evaluation of progression-sensitive scales and questionnaires. Evaluating the effect of minocycline, creatine, Coenzyme Q10 and GPI-1485, a product candidate that belongs to a class of small molecule compounds called neuroimmunophilin ligands, and placebo in different control and patient groups, they have found no difference, neither in

the minocycline group nor in the others regarding the primary indicator of disease progression that was defined as the time for starting a symptomatic pharmaceutical treatment.[95] The results of that study were in accordance with another study assessing the efficacy of minocycline in patients with Multiple System Atrophy-Parkinsonian subtype (MSA-P) that also revealed no symptomatic beneficial effects of minocycline in MSA-P.[96] Nowadays, there is still no randomized clinical minocycline therapy trial conducted on a massive scale.

Possible procognitive effects of minocycline in clinical trials of Parkinson's disease and Alzheimer's disease

Cognitive dysfunction is a common and significant nonmotor symptom of PD. PD mild cognitive impairment (PD-MCI) was defined in 2012 by the International Parkinson and Movement Disorder Society,[97] and it has shown that approximately one-third of people have PD-MCI.[98,99]

Longitudinal cohort studies have demonstrated that approximately half of the patients with PD for 10 years develop PD dementia (PDD),[100] while the point prevalence of dementia among those with PD is roughly 30%.[101] Although the underlying mechanisms of cognitive decline or dementia associated with PD remain unclear, it is believed that the presence of Lewy pathology within the limbic system and neocortex play a critical role in the impairment of cognition in PD. Lewy bodies are abnormal alpha-synuclein (α-syn) proteins, which are greatly responsible for the neurotoxicity via different mechanisms, including impairment of axonal transport, oxidative stress, mitochondrial changes, and synaptic dysfunction.[102]

Despite increased understanding of pathology, neurotransmitter, and genetic drivers, there are no proven pharmacological treatments or protective agents for PD-MCI. Moreover, studies until now have shown that there is an only modest benefit for antidementia drugs that are licensed for PDD. These findings together suggest that there is an emerging need for a mechanism-based clinical treatment strategy for cognitive impairment in PD.[103]

In this respect, it is worth to notice, that a new study, The minocycline in Alzheimer's Disease Efficacy (MADE) study, is a multicenter, randomized, controlled trial in patients with very mild AD.[104] Recruitment began in January 2014 and the trial ended in December 2017. It aimed to determine whether minocycline is effective in reducing the rate of cognitive and functional decline over a 2-year period and assess the safety and tolerability of minocycline. This multicenter, randomized double-blind placebo-controlled semifactorial (2×1) designed phase II clinical trial tested the efficacy and tolerability of 400 and 200 mg minocycline in disease modification of AD. There were 480 patients with very mild AD patients recruited

in England and Scotland. The patients were assessed with Standardized Mini-Mental State Examination and the Bristol Activities of Daily Living Scale.[104] The results of the study have not been published yet.

Conclusions

Minocycline is a tetracycline that exerts antimicrobial, anti-inflammatory, anti-apoptotic, and antioxidant effects on the CNS.[106] There is rapidly growing evidence showing that neurodegeneration and inflammation are fundamental aspects of many neurological diseases.[109] In this respect, much attention has been focused on the potential use of currently available anti-inflammatory drugs to prevent neurodegeneration. In our perspective review, we have summarized various studies in animals and humans, which have confirmed beneficial effects and safety of minocycline, alone or combined with other drugs in PD.

Numerous *in vitro* and *in vivo* experimental studies have pointed toward minocycline as a promising agent in the protection of PD; however, clinical studies have failed to confirm the neuroprotective effect of minocycline in PD.

Considering all of these promising preclinical data, it may be reasonable to assume that minocycline is a neuroprotective candidate with its well-known effect on different pathological pathways in neurodegenerative diseases.

PD affects about 1 million Americans[110] with annual direct medical care costs of more than $US10,000 per patient.[111] If clinical efficacy were ever proven, then minocycline would be a very cheap therapeutic option. Although we consider our perspective review findings to be significant the confirmation of preclinical results from clinical studies are still lacking. In this respect, further well-designed clinical studies with a larger number of patients with Parkinson's disease would be the next logical step in this field of clinical neuroprotection research.

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