

Commentary & View

Minocycline as a potential therapeutic agent in neurodegenerative disorders characterised by protein misfolding

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Many neurodegenerative disorders share common features including the accumulation of aggregated misfolded proteins, neuroinflammation and the induction of apoptosis. While the contributions of each of these individual elements to neuronal death remain unclear, a commonly used antibiotic, minocycline, has been shown to reduce the progression and severity of disease in several models of neurodegeneration by variously downregulating these molecular pathways. Here we discuss the evidence for the potential of minocycline as a broad-specificity therapeutic agent for those neurodegenerative diseases that are characterized by the presence of misfolded proteins.

Introduction

The neurodegenerative disorders that include transmissible spongiform encephalopathies (TSEs or prion diseases), Alzheimer disease (AD) and related tauopathies, Parkinson's disease (PD), Huntington disease (HD) and motor neuron disease (MND) share many common characteristics despite significant differences in clinical symptoms.¹⁻³ Such commonalities include the aggregation and accumulation of misfolded proteins,¹⁻³ neuroinflammation⁴ and the induction of apoptosis,⁵ all of which likely contribute to extensive neural loss. Therapeutic agents that confer neuroprotection by countering these shared characteristics could therefore have beneficial effects in a wide range of neurodegenerative diseases.

Minocycline hydrochloride (2E, 12aS)-2-[amino(hydroxy)methylidene]-4,7-bis(dimethylamino)-10,11,12a-trihydroxy-4a,5,5a,6-tetrahydro-4H-tetracene-1,3,12-trione hydrochloride (MinocinTM; Wyeth Pharmaceuticals) is a broad spectrum second generation semi synthetic derivative of the bacteriostatic antibiotic tetracycline. Minocycline and tetracycline share a common core of four six-membered rings, with minocycline having modifications at three sites on the conserved structure compared to tetracycline

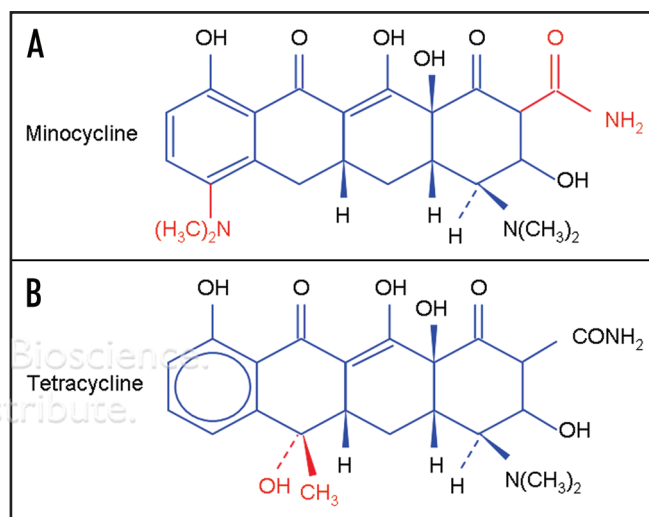


Figure 1. The chemical structure of minocycline and tetracycline. Figure shows the common core of 4x six-membered rings. Modifications at three sites on the conserved tetracycline structure on minocycline are indicated in red.

(Fig. 1). These modifications to the core structure increase its half-life in comparison to first generation tetracyclines and have improved the absorption of minocycline into the central nervous system and its penetration into cerebrospinal fluid.⁶ Minocycline has a low propensity to produce antibiotic resistance and is commonly used in the treatment of acne, rosacea and other infections of the skin and respiratory tract.⁷ In addition to its antibiotic properties, studies over the past 11 years have shown that minocycline has significant neuroprotective effects in pre-clinical studies of neurodegenerative disease.

Yrjanheikki et al.⁸ first described minocycline as a neuroprotective agent in an animal model of ischaemia in which neurons were spared and ischaemia-induced activation of microglia was inhibited upon minocycline treatment. Minocycline has now been shown to reduce disease progression, inhibit neuronal death and increase the life-span of rodents that recapitulate features of neurodegenerative and neurological disorders including PD, HD, MND, multiple sclerosis, spinal cord injury, AD and related tauopathies and central and peripheral prion infections.⁹⁻¹¹

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Pleiotropic effects of minocycline in CNS disorders have been described, including inhibition of poly (ADP-ribose) polymerase-1,¹² downregulation of protein kinases,¹³ suppression of oxidative stress¹⁴ and scavenging of free radicals.¹⁵ In addition, significant reductions in neuroinflammation,¹⁶ modulation of protein aggregation¹⁶ and suppressed induction of apoptosis¹⁷ following minocycline treatment have variously combined to confer protection against neuronal death in cell-based and animal models of neurodegenerative disease. Below, the implications of the diverse effects of minocycline are discussed in relation to the treatment of neurodegenerative disorders characterized by protein misfolding.

The Impact of Minocycline on Misfolded and Aggregated Proteins

Many neurodegenerative disorders exhibit characteristic neuropathological inclusions and/or extracellular deposits of aggregated proteins. It is likely that this aggregation results from a multi-step process that begins with a protein acquiring an abnormal conformation and that goes on to form protein dimers and higher order oligomers. Such oligomeric intermediates then proceed to become protofibrils, often exhibiting beta-sheet secondary structure, and subsequently mature into protofilaments and fibrous structures deposited inside or around neurons. For the purpose of this review the term 'amyloid' will be used to refer to general insoluble fibrous protein aggregates, while 'β-amyloid' or 'Aβ' will be used to describe the peptide formed upon cleavage of the amyloid precursor protein (APP) in AD.

AD is characterized by the aggregation and accumulation of two proteins: amyloid beta-peptide (Aβ) is deposited in extracellular senile plaques and the microtubule-associated protein tau accumulates in a highly phosphorylated form as paired helical filaments that comprise the intraneuronal neurofibrillary tangles (NFTs) and neuropil threads.^{18,19} Similar inclusions are characteristic of other disorders and thus cytoplasmic neuronal inclusions containing aggregated, phosphorylated and C-terminally truncated α-synuclein,²⁰ parkin, DJ-1 and leucine-rich repeat kinase-2 (LRRK2)/dardarin^{21,22} are present in PD brain, polyglutamine-rich aggregates of huntingtin form cytoplasmic inclusions in HD,²³ while in MND, superoxide dismutase (SOD) accumulates in axons and motor neurons.²⁴ Similarly, the primary pathogenic event associated with the onset of TSEs is thought to be a conformational change in soluble prion protein (PrP^c) that results in its transformation into insoluble, protease-resistant species (PrP^{sc}) capable of compromising cell viability.²⁵

One mechanism by which minocycline could exert its neuroprotective action is through its propensity, in common with related tetracycline derivatives, to bind to and to disassemble preformed fibrillogenic structures of amyloid, such as those generated by synthetic peptides of human PrP and Aβ.^{16,26,27} That minocycline targets amyloid proteins common to several diseases may be particularly useful given recent findings of a functional interaction between PrP and Aβ.²⁸ The mechanism involved in the reduction of amyloid fibril formation with minocycline is thought to involve polar interactions and hydrogen bonds formed between tetracyclines and amyloidogenic structures.^{16,26,27} Recent work in our laboratory

has shown that minocycline is effective in reducing the development of abnormal tau species and tau aggregation in htau mice.⁹ The htau transgenic animals express human wild-type tau and progressively develop hyperphosphorylated tau aggregates and NFTs in the hippocampus and cortex.²⁹ We found that minocycline treatment reduced both the amount of tau present in disease-associated conformations as well as the neural load of aggregated tau. Familian et al.¹⁶ demonstrated previously that minocycline also reduced the formation of Aβ fibrils in vitro, thus minocycline has the potential to decrease the development of both plaque and tangle pathology. These results strongly support the use of minocycline as an alternative therapeutic strategy for AD and related disorders in which protein aggregation is a significant factor.

In support of this, a similar effect of minocycline on protein aggregation was observed by Smith et al.³⁰ in organotypic slice cultures derived from the R6/2 transgenic model of HD that expresses mutant huntingtin protein. When minocycline was administered either immediately or three weeks after slice preparation (equivalent to a post-natal age of four weeks in vivo), huntingtin aggregation was decreased up to 10-fold compared with vehicle treatment.³⁰ However, in the same study, administration of minocycline directly to R6/2 mice did not reduce huntingtin aggregation, a finding since confirmed by others.³¹ In contrast, co-administration of minocycline with coenzyme Q10 in R6/2 mice has been shown to attenuate neuronal death and huntingtin aggregation in comparison to separate treatments.³²

Thus, although minocycline can act as an anti-aggregatory agent, the disparity between the results from cells and those obtained from animal studies demonstrates that this is a complex and poorly understood mechanism that could be subject to unidentified modifying factors that are differentially expressed in the various models used. One consideration may be that differences in the concentrations of minocycline used in cell-based assays compared to those used in vivo could be a contributing factor to the observed variation in its effectiveness.^{16,33} It has also been suggested that the inhibitory effect of minocycline on amyloid fibril formation could mediate its anti-inflammatory action.¹⁶ Thus the potential beneficial impact of minocycline may be dependent upon the stage of disease progression, the availability of amyloid fibrils and/or the degree of inflammation present in the diseased brain.

A further consideration is the potential negative impact of disaggregating proteins since this could lead to the release of toxic oligomers that could be detrimental to cell viability. In AD and prion diseases, intermediate oligomers have been shown to be neurotoxic.^{34,35} Furthermore, agents capable of disaggregating Aβ in the CNS have not yet proved to be a viable therapy, with recent trials being halted due to a lack of efficacy, possibly due to the release of toxic Aβ oligomers.^{36,37} Therefore, if the primary action of minocycline is as an anti-aggregatory agent there are likely to be limits to its clinical benefits in CNS disorders.

Minocycline and Neuroinflammation

Reactive microgliosis, astrocytosis, upregulation of pro-inflammatory mediators and activation of inflammation-associated protein kinases are features common to many neurodegenerative

disorders. The effects of minocycline on neuroinflammatory processes are wide-ranging and include suppression of glial activation and immune cell responses. Minocycline has a wide spectrum of inhibitory actions on inflammatory factors such as cyclooxygenase-2,¹⁴ tumor necrosis factor (TNF) α ,¹⁶ interleukins (IL)-2, -6, -1 β and interferon- γ ,^{16,38} inducible nitric oxide synthase,³⁹ matrix metalloproteinases,⁴⁰ and kinases including apoptosis signalling kinase-1 (ASK-1), c-jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase.^{16,41,42} Thus, any potential beneficial effects of minocycline in neurodegenerative diseases might well be exerted through its actions on the molecular pathways involving one or more of these inflammatory factors.

Significant beneficial effects of minocycline on neuroinflammatory processes have been shown in a model of chronic glaucoma in DBA/2J mice,⁴³ 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)⁴⁴ Parkin null⁴⁵ and paraquat-induced⁴⁶ mouse models of PD, G93,⁴⁷ and G37R mutant SOD1,⁴⁸ transgenic models of MND, mice expressing mutant amyloid precursor protein (APP)⁴⁹ and rats injected with A β peptides.¹⁴ Glial fibrillary acidic protein (GFAP) is the predominant intermediate filament in mature astrocytes and, when activated, astrocytes overexpress GFAP. GFAP-positive astrocytes are found in close proximity to amyloid plaques in AD brain and a probable association between GFAP expression and disease-associated changes in tau protein has recently been demonstrated.⁵⁰ Recently, we have noted reduced GFAP levels in the brains of tangle-forming mice treated with minocycline (unpublished data). Furthermore, the protective effect of minocycline against A β -induced neuronal death is absent when astrocytes are removed from primary neuronal cortical cultures,⁵¹ strongly suggesting that the neuroprotective effects of minocycline are mediated, at least partially, by its influence on astrocyte activation. Further investigations are currently underway to determine the nature of this inhibition.

In addition, various intermediate oligomeric forms of human PrP and A β have been shown to bind complement factors.⁵² C1q, a subunit of the C1 enzyme complex that activates the serum complement system, together with serum amyloid P component (SAP), a universal constituent of amyloid deposits, both accumulate in A β -containing plaques in AD. Interestingly, these factors are required for the accumulation of activated microglia around plaques.⁵³ Familian et al.¹⁶ have shown that minocycline inhibits SAP and C1q-enhanced A β fibril formation, microglial activation and the release of pro-inflammatory mediators in human microglial cell cultures. In addition, Seabrook et al.⁴⁹ demonstrated that minocycline significantly reduces cytokine production including that of IL-1 β , IL-6 and TNF α , in cultured glial cells treated with A β . However, when J20 transgenic mice expressing mutant APP were treated with minocycline the A β burden was actually increased in young (eight months old) mice, most likely as a result of minocycline suppressing the microglial degradation or phagocytosis of accumulated A β . This presumed deleterious effect appeared to be transient since older (12 months old) mice did not show an increased A β plaque load.⁴⁹ Thus, this study demonstrates that inhibiting microglial activation using minocycline could have disease-enhancing, rather than suppressing, effects by blocking beneficial glial responses. It remains unclear whether the

anti-inflammatory impact of minocycline is through its direct action, from a secondary event resulting from inhibition of neuronal cell death or amyloid aggregation, or by a combination of these mechanisms.

Minocycline and Apoptotic Cell Death

Induction of the apoptotic cell death pathway is common to many neurodegenerative conditions including those exhibiting protein misfolding and this process may represent one of the more potent molecular actions of minocycline in these disorders. Apoptosis is a tightly regulated and highly efficient cell death program of which caspases are the central initiators and executioners. Intrinsic apoptosis pathways are mediated by the activation of caspase-9 upon release of cytochrome *c* from mitochondria, while extrinsic apoptosis is dependent upon the activation of specific cell surface 'death' receptors with subsequent signalling mediated by the cytoplasmic part of the receptors, the death domain (DD). Minocycline is reported to target both extrinsic and intrinsic apoptotic pathways.

Caspase activation and/or expression is elevated in post-mortem human brain tissue from AD, PD, MND, HD and TSEs,^{54,55} suggesting that apoptotic pathways may be elevated in these conditions. A β and prion protein fragments have been found to trigger activation of caspases 3, 6 and 8, with resulting neuronal cell death.⁵⁶ Furthermore, in TSEs, spontaneous neurodegeneration of transgenic mice expressing mutant PrP is mediated by the binding and co-aggregation of PrP with Bcl-2 family members, of which Bax and Bak induce the release of cytochrome *c* and other proapoptotic factors from the mitochondria into the cytosol.⁵⁷

In addition, caspase activation has been implicated in disease pathogenesis in HD and MND^{58,59} and the detrimental effects of the dominant mis-sense mutations in LRRK2, the most common genetic cause of Parkinson disease (PD), are believed to be mediated by caspase-8 signalling.⁶⁰ Further evidence for the involvement of apoptotic pathways in PD comes from the finding that protein kinase C δ is proteolytically activated by caspase-3 and this induces apoptosis,⁶¹ while inhibiting cleavage of this kinase prevents neuronal death in dopaminergic neurons.⁶¹ Taken together, it seems likely that, for at least some of the major neurodegenerative disorders, apoptotic mechanisms may have a major part to play in neuronal death, either as a causal event or subsequent to another initiating factor.

Several anti-apoptotic actions of minocycline are likely to contribute towards its neuroprotective effects. Minocycline has been shown to stabilise the mitochondrial membrane and to inhibit the release of cytochrome *c* into the cytosol, thereby preventing activation of caspase-3 and caspase-9 and subsequent apoptosis in mice with MND.⁶² Minocycline suppresses the Fas-triggered mitochondrial apoptotic pathway⁶³ and through its action in stabilising the mitochondrial membrane, inhibits activation of caspase-independent apoptotic pathways in retinal neurodegeneration.⁶⁴ Minocycline upregulates expression of Bcl-2 in vitro and also reduces the cleavage, and therefore the activation, of Bid, a pro-apoptotic protein of the Bcl-2 family.⁶⁵ Thus, minocycline targets a number of key events during apoptotic neuronal death

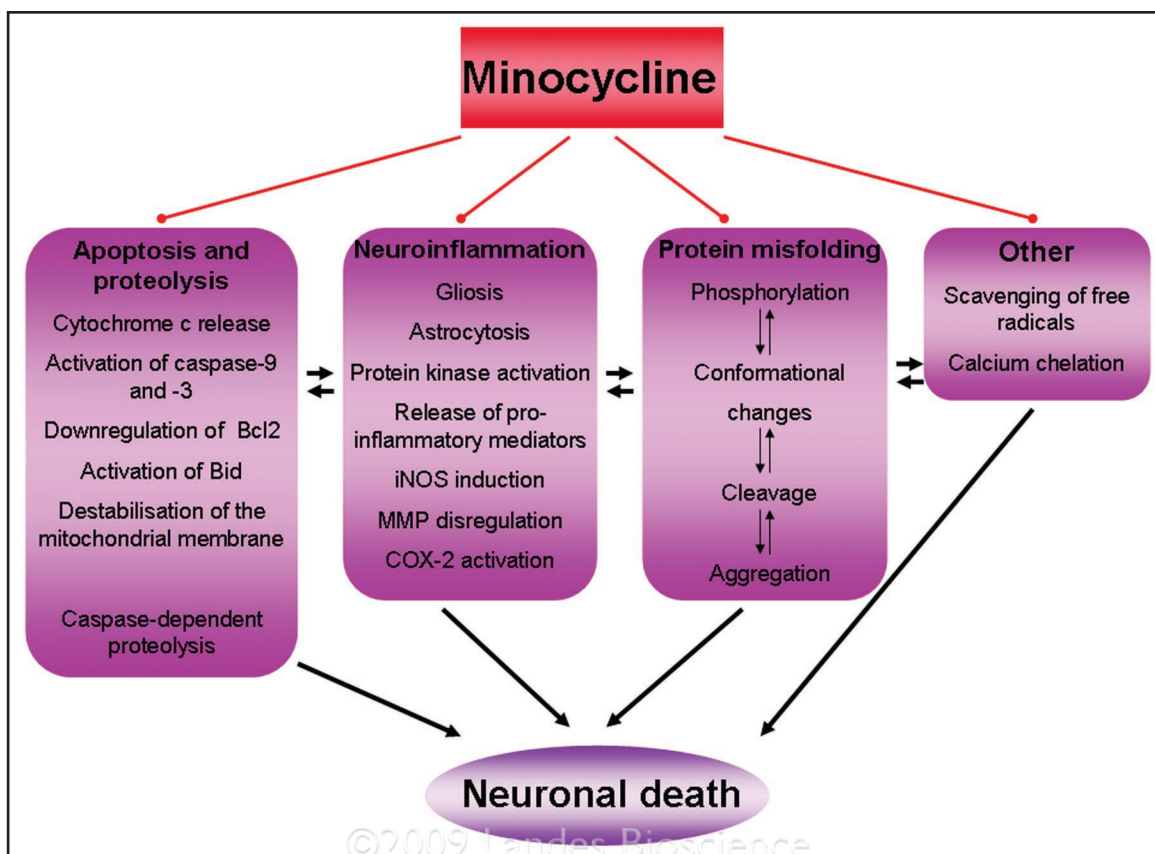


Figure 2. Summary of some of the molecular pathways targeted by minocycline that are common to many neurodegenerative disorders. Common disease-associated events known to be targeted by minocycline include (1) neuroinflammation with the release of pro-inflammatory mediators, (2) apoptosis, caspase activation and caspase-mediated protein proteolysis and (3) protein misfolding and aggregation. Abbreviations: inducible nitrogen oxide synthase (iNOS); matrix metalloproteases (MMP); cyclooxygenase-2 (COX-2).

and appears to be effective at reducing neurodegeneration in a large number of disease systems through suppression of apoptosis and/or apoptotic stimuli.

In addition to inducing cell death directly, proteolytic cleavage of proteins by caspases is thought to contribute to neurodegeneration. In AD, caspases cleave APP, presenilin, actin, fodrin and tau, with caspase-3-mediated C-terminal truncation of tau appearing to be an early event in AD pathogenesis.⁶⁶ Tau fragments generated by cleavage have a propensity to aggregate and often display toxic properties⁶⁷ leading several groups to suggest that caspase-cleavage of tau could be a key factor in AD. Similarly, the proteolytic cleavage of huntingtin by caspases and calpains which results in its nuclear translocation is an important event in HD. The resulting N-terminal fragments of huntingtin are more toxic than the full-length protein and these are incompletely degraded by the proteasome,⁶⁸ leading to their accumulation in characteristic inclusions. In PD, parkin is susceptible to proteolysis by caspase-3, compromising parkin function, lowering the cellular stress threshold and leading to further caspase activation and neuronal cell death.⁶⁸ We have shown recently that minocycline reduces the caspase-3 activation observed in primary neurons treated with A β , and in tau transgenic mice. In both systems, the suppression of caspase-3 activity reduced caspase-cleavage of tau

and generation of toxic, potentially pro-aggregatory, tau fragments.⁹ Thus, minocycline not only directly targets key elements in the apoptotic cascade, but it also impacts on disease-associated mechanisms that are downstream of caspase activation, potentially increasing its neuroprotective impact.

Summary and Further Considerations

The evidence we present herein reveals that the wide variety of targets of minocycline in protein misfolding disorders, including AD, PD, HD, MND and TSEs, make this compound an attractive potential therapeutic agent (Fig. 2). Indeed, the results of these pre-clinical studies have elicited several clinical trials for minocycline in diseases including MND, HD, PD, multiple sclerosis and multiple system atrophy, with some trials in MND and HD having entered Phase III (www.clinicaltrials.gov).

However, reports from some recently completed clinical trials of minocycline for PD, HD and MND have yet to show any positive disease-modifying effects. This may be related to some clinical trials having been criticised for the high dose of minocycline used (double the established well-tolerated dose) and the suitability of the selected functional tests, particularly in patients susceptible to fatigue, such as those suffering from MND.⁷⁰ Several pre-clinical studies, using a wide variation of treatment

regimens including those previously reported as effective, have also failed to determine any impact, or have even found deleterious effects, of minocycline.^{30,71-74} It is possible that different targets of minocycline may be specifically affected depending upon the dosing method in laboratory animals (oral gavage, intraperitoneal, intracerebroventricular) and/or the concentration of minocycline used, since the latter can vary between 10–100 mg/kg/day. Alternatively, the upregulation of specific pathways during different stages of disease progression might also influence therapeutic efficacy. Nevertheless, these reports indicate that a fuller understanding of the mechanisms involved in the action of minocycline in vivo is required before its therapeutic potential can be accurately assessed.

Although comparing laboratory and clinical studies is a difficult task, not least due to the differing measurable outcomes of neuropathological, biochemical and behavioural assays in rodents, compared to qualitative ‘quality of life’ scoring in humans, there are some important differences apparent when considering minocycline. There is significant variation in dosage regimens (for example, an average of daily 3.5 g systemic injections of minocycline given to rodents compared with 100–400 mg oral daily dosing in humans), the half-life of minocycline (2–3 hours in rodents compared to 15 hours in humans), and/or the time-course of administration (often weeks in rodents compared to months in humans). These are factors which should perhaps be considered by the pharmaceutical industry should they seek to develop derivatives of minocycline with improved neuroprotective properties.

In summary, minocycline is a relatively inexpensive, commonly prescribed drug, with few reported adverse side effects even after prolonged use. Recent studies describe potent neuroprotection of this tetracycline derivative that may be mediated through targeting apoptotic, neuroinflammatory, or protein aggregatory pathways, or more likely through a combination of these disparate but common mechanisms involved in neurodegenerative disease. Although it is apparent that future studies are required to unravel the molecular mechanisms involved in its action, it seems likely that minocycline, or further chemically modified derivatives, will have potential as a therapeutic approach for treating CNS disorders that are characterized by protein misfolding.

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