Prospects for Minocycline Neuroprotection

Jennifer M. Plane, PhD; Yan Shen, PhD; David E. Pleasure, MD; Wenbin Deng, PhD

M inocycline is a clinically available antibiotic and anti-inflammatory drug that also demonstrates neuroprotective properties in a variety of experimental models of neurological diseases. There have thus far been more than 300 publications on minocycline neuroprotection, including a growing number of human studies. Our objective is to critically review the biological basis and translational potential of this action of minocycline on the nervous system.

The lack of robust treatment options available for neurodegenerative diseases, stroke, and central nervous system (CNS) trauma, all of which often involve both inflammation and apoptotic cell death, has led to an explosion of studies to reexamine the potential of established drugs for treatment of these conditions. Minocycline is a second-generation tetracycline derivative with a proven, safe clinical track record as an antibiotic and anti-inflammatory drug for treating acne and arthritis. Minocycline effectively crosses the blood-brain barrier and has demonstrated neuroprotective qualities in experimental models of CNS trauma, stroke, spinal cord injury, and neurodegenerative diseases including amyotrophic lateral sclerosis, Huntington disease, Parkinson disease, and multiple sclerosis. Thus, it is not surprising that several off-label minocycline clinical trials are under way for a variety of CNS diseases. The emerging success of some of these trials raises optimism that minocycline treatment may soon be translated into clinical practice for CNS diseases, whereas others (eg, in amyotrophic lateral sclerosis) have raised red flags. A thorough understanding of minocycline's modes of action and the targeted cellular mechanisms is warranted, as are guidelines on safe

and effective doses, routes of administration, and establishment of a therapeutic window. Although several lines of evidence point to a convergent action of minocycline in suppression of both apoptosis and CNS inflammation by preventing neural cell death and by inhibiting microglial activation, the exact molecular targets of minocycline have yet to be identified and characterized. This review provides an overview of the established mechanisms of action by which minocycline exerts its neuroprotective effects, summarizes results of animal studies in experimental models of neurological diseases, and discusses results of clinical trials.

MECHANISMS OF ACTION

Several mechanisms of action are thought to be responsible for minocycline's neuroprotective effects; however, the primary target of this drug in the nervous system remains unknown. Recent studies have focused on the anti-inflammatory, anti-apoptotic, and antioxidant properties of minocycline. The main biological effects of minocycline, which are involved in the pathogenesis of several neurological disorders, include inhibition of microglial activation, attenuation of apoptosis, and suppression of reactive oxygen species production.

In experimental disease models, minocycline treatment suppresses microglial pro-

Author Affiliations: Departments of Cell Biology and Human Anatomy (Drs Plane, Shen, and Deng), Pediatrics and Neurology (Dr Pleasure), University of California, Davis, School of Medicine; and the Institute for Pediatric Regenerative Medicine, Shriners Hospital and University of California, Davis, School of Medicine, Sacramento (Drs Deng and Pleasure).
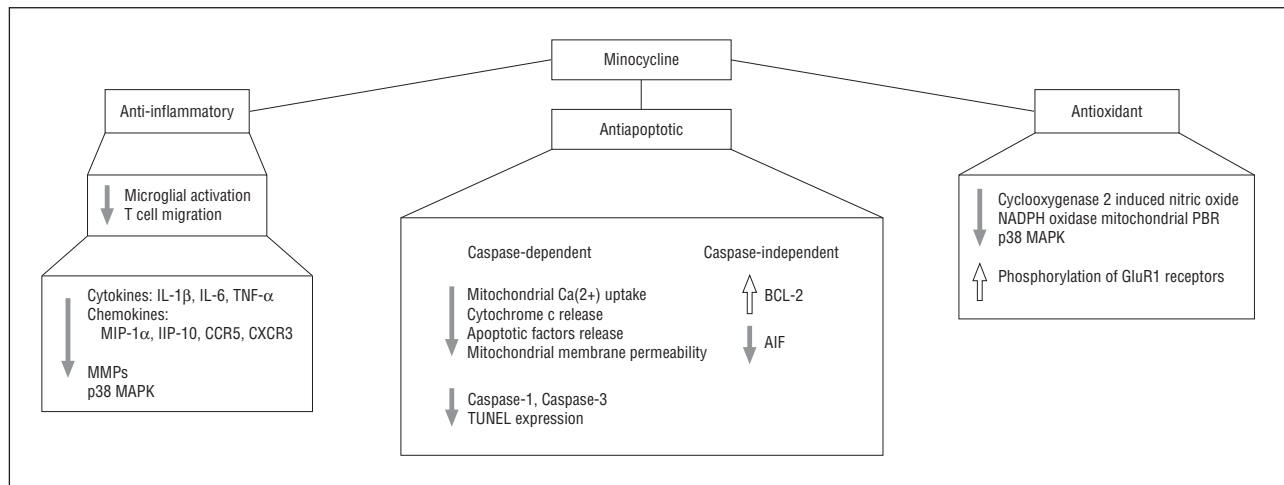
Figure. Signaling mechanisms involved in the neuroprotective actions of minocycline. See the text for detailed descriptions. AIF indicates apoptosis-inducing factor; BCL-2, B-cell leukemia/lymphoma 2; CCR5, chemokine receptor type 5; CXCR3, chemokine (CXC motif) receptor 3; GluR, glutamate receptor; IL, interleukin; IP-10, interferon-inducible protein 10; MAPK, mitogen-activated protein kinase; MIP-1α, macrophage inflammatory protein 1α; MMP, matrix metalloprotease; PBR, peripheral benzodiazepine receptor; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase dUTP (2′-deoxyuridine, 5′-triphosphate)–biotin nick-end labeling.

liferation/activation as well as subsequent release of cytokines such as interleukins 1β and 6 and tumor necrosis factor α.[1,2] In addition, it decreases CNS expression of chemokines and their receptors such as macrophage inflammatory protein 1α, interferon-inducible protein 10, chemokine receptor type 5, and chemokine (CXC motif) receptor 3 as well as suppressing the release of lipids and the activity of matrix metalloproteases, which disrupt the blood-brain barrier.[1,2] Furthermore, minocycline inhibits T-cell migration into the CNS. The neuroprotection afforded by minocycline treatment of experimental disease models also appears to be related to p38 mitogen-activated protein kinase inhibition, which both preserves neurons and inhibits microglial activation.[1,3]

The antiapoptotic effects of minocycline are exerted, at least in part, 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, thus resulting in decreased caspase activation and nuclear damage.[4,5] Minocycline also exerts caspase-independent neuroprotective effects including upregulation of antiapoptotic factor BCL-2.[6]

Minocycline possesses antioxidant properties in addition to its antibiotic, antiapoptotic, and antiinflammatory properties. It directly scavenges free radicals and inhibits molecules such as cyclooxygenase 2, induced nitric oxide synthase, and nicotinamide adenine dinucleotide phosphate oxidase. The antioxidant activity of minocycline may be derived from its chemical structure or its modulating effects on enzymes such as nitric oxide synthase and lipoxygenases.

Poly (ADP [adenosine diphosphate]–ribose) polymerase-1 (PARP-1) is activated by DNA damage and is critically involved in excitotoxicity, oxidative stress, and inflammation. Activation of PARP-1 leads to translocation of apoptosis-inducing factor from the mitochondria to the nucleus and finally promotes cell death and inflammation. Alano et al[7] reported that minocycline and other tetracyclines inhibited PARP-1 at nanomolar con-

centrations, and minocycline protected neurons against PARP-1–mediated toxicity. Other minocycline effects include inhibition of mitochondrial peripheral benzodiazepine receptor (recently renamed 18 kDa translocator protein), increased phosphorylation, membranal insertion of glutamate receptor 1, and inhibition of mitogen activated protein kinases.

Minocycline treatment provides neuroprotection against excitotoxic insults in a number of experimental models. The neuroprotective effects of minocycline are evident after glutamate exposure, and oligodendrocyte survival is enhanced by minocycline treatment after lipopolysaccharide-induced inflammation; all likely occur as a result of decreased microglial activation.[8] Overall, although it is still unclear which of these mechanisms plays the pivotal role in eliciting the neuroprotective properties of minocycline, the major targets for minocycline neuroprotection could lie within the complex signaling network linking mitochondria, oxidative stress, excitotoxicity, PARP-1, and apoptosis (**Figure**).

STUDIES IN EXPERIMENTAL MODELS OF HUMAN NEUROLOGICAL DISEASES

Ischemic Injury

Yrjanheikki et al[9] first published the neuroprotective effects of minocycline on cerebral ischemia, prompting numerous subsequent evaluations of minocycline on experimental models of stroke, neurodegenerative diseases, and spinal cord injury (**Table 1**). Some of these studies indicated substantial neuroprotective effects, while in others, minocycline was ineffective or even deleterious. These studies varied widely in dosage, route of administration, timing of drug treatment, species, and experimental model used. Several studies that tested multiple doses of minocycline treatment after ischemia reported neuroprotection with relatively low doses but observed toxicity with high-dose minocycline treatment.[6,10] Interestingly, minocycline treatment exacerbated injury in neo-

Table 1. Results of Experimental Studies on Minocycline Treatment for Neurological Diseases

Animal Model	Dose and Route	Timing	Outcome	Source
Stroke				
MCAO	45 mg/kg IP on d 1, then 22.5 mg/kg for 2 d	12 h prior to MCAO, twice daily for 3 d	Reduced infarct volume, inhibited microglial activation, reduced COX-2 expression	Yrjänheikki et al[9]
MCAO	20 or 100 mg/kg IV	60 min after stroke	Low dose improved behavioral function, reduced infarct volume, decreased apoptosis, upregulated BCL-2; high dose increased behavioral deficit, increased infarct volume, increased apoptosis, and suppressed BCL-2 expression	Matsukawa et al[6]
OGD	0.001-100 μM in vitro	1.5-12 h	Low dose increased neuron viability, decreased neuron death, increased BCL-2 expression; high dose decreased neuron and astrocyte viability, increased astrocyte death	Matsukawa et al[6]
Hypoxia-ischemia	45 mg/kg, IP or SC	12 h prior to hypoxia	Decreased infarct volume in rats, increased infarct volume in mice	Tsuji et al[10]
Hypoxia-ischemia	45 mg/kg, SC, for 1 d, then 22.5 mg/kg SC for 2 d	Twice per d starting 12 h prior to hypoxia	Increased infarct volume in mice	Tsuji et al[10]
Hypoxia-ischemia	135 mg/kg SC for 1 d, then 68 mg/kg for 2 d	Twice per d starting 12 h prior to hypoxia	Increased infarct volume in mice	Tsuji et al[10]
Hypoxia-ischemia	45 mg/kg on d 1, then 22.5 mg/kg or 22.5 mg/kg on d 1, then 10 mg/kg IP	1 week, starting 2 h after hypoxia	Decreased white matter injury, decreased microglial activation	Carty et al[11]
Hypoxia-ischemia	50 mg/kg IP	Every 12 h for 4 d, starting immediately after hypoxia	Reduced white matter injury, reduced microglial activation	Lechpammer et al[12]
Parkinson Disease				
MPTP	60, 90, or 120 mg/kg, oral gavage	24 h after MPTP	Reduced neuron death, reduced microglial activation	Du et al[13]
Rotenone	1, 5, 10 μM in vitro	6 d	Decreased neuron death	Radad et al[14]
MPTP in monkey	200 mg/kg, oral	2 weeks during MPTP	Increased neuron death, worsened motor symptoms	Diguet et al[15]
MPTP	45 or 60 mg/kg IP	Twice per d for 3 d	Increased neuron death, inhibited microglial activation	Yang et al[16]
Weaver mutant	60 mg/kg, oral gavage	P10-P24	Decreased neuron death	Peng et al[17]
Huntington Disease				
3-NP	45 mg/kg IP	5 d during 3-NP	Worsened behavioral function, increased neuron death	Diguet et al[15]
R6/2 mice	5 mg/kg IP	Daily	Delayed disease progression, inhibited caspase expression, delayed mortality	Chen et al[18]
R6/2 mice	1 or 10 mg/mL in drinking water	Daily	No change in behavioral function, no change in aggregate formation	Smith et al[19]
R6/2 mice	5 mg/kg IP with CoQ10	Daily	Improved behavioral function, decreased neuron damage, decreased aggregate formation	Stack et al[20]
Quinolinic acid injection	50 mg/kg on d 1, 25 mg/kg after, IP	7 or 21 d after injection	Decreased neuron death, decreased microglial activation	Ryu et al[21]
Alzheimer Disease				
Tg-SwDI mice	50 mg/kg IP	Every other day for 28 d at 12 mo of age	Decreased microglial activation, decreased IL-6, improved behavioral function	Fan et al[22]
APP-tg mice	55 mg/kg in chow	Daily for 3 mo	Improved cognitive performance, decreased microglial activation	Seabrook et al[23]
Multiple Sclerosis				
EAE	45 mg/kg IP	For 14 d at symptom onset	Decreased clinical scores, decreased T-cell infiltration	Nikodemova et al[24]
Amyotropic Lateral Sclerosis				
SOD1 mice	10 mg/kg IP	Daily starting at 5 wk of age	Delayed disease onset, prolonged survival	Zhu et al[25]

Abbreviations: 3-NP, nitropropionic acid; CoQ10, coenzyme Q10; COX-2, cyclooxygenase-2; EAE, experimental autoimmune encephalomyelitis; IL, interleukin; IP, intraperitoneal; IV, intravenous; MCAO, middle cerebral artery occlusion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OGD, oxygen glucose deprivation; P, postnatal day; SC, subcutaneously; SOD1, superoxide dismutase 1.

natal mouse models of stroke, whereas it attenuated injury in rats using a similar model and dosing paradigm.[10,11] Furthermore, minocycline exerts different effects on different types of cells. One recent study found that, while low-dose minocycline was protective to neurons in both in vivo and in vitro experimental models of stroke, it provided no protection to astrocytes in the same models.[6] Yet, other studies describe decreased ischemia-induced astrogliosis and reduced white matter damage after is-chemic injury following minocycline treatment.[12,26] In addition, though most of these publications addressed only the acute effects of minocycline treatment on ischemic injury, it will be important, from a translational point of view, to determine whether minocycline treatment confers long-term neuroprotection or simply delays the onset of cell death. One promising observation, in this respect, is that ischemia-induced behavioral deficits lessened after minocycline treatment.[27]

Table 2. Summary of Human Trials of Minocycline Treatment for Neurological Diseases

Disease	Dosage, mg/d	Route	Duration	Effect	Source
Acute stroke	200	Oral	5 d	Lower NIHSS, lower mRS, increased BI	Lampl et al[29]
Spinal cord injury	400 or 800	Intravenous	7 d	Improved ASIA, FIM, SCIM	Casha et al[30]
Multiple sclerosis	200, with glatiramer acetate	Oral	9 mo	Reduced gadolinium-enhancing lesions	Metz et al[31]
Parkinson disease	200	Oral	18 mo	Passed the safety and efficacy trial	NINDS-NET-PD Investigators[32]
Huntington disease	200	Oral	24 mo	Ameliorated psychiatric symptoms	Bonelli et al[33]
Huntington disease	200	Oral	6 mo	No significant differences in UHDRS, AIMS, and MMSE	Thomas et al[34]
ALS	200	Oral	9 mo	Declined ALSFRS-R	Gordon et al[35]

Abbreviations: AIMS, Abnormal Involuntary Movement Scale; ALS, amyotropic lateral sclerosis; ALSFRS-R, ALS functional rating scale; ASIA, American Spinal Injury Association; BI, Barthel Index; FIM, Functional Independence Measure; MMSE, Mini-Mental State Examination; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; SCIM, Spinal Cord Independence Measure; UHDRS, Unified Huntington Disease Rating Scale.

Parkinson Disease

The effects of minocycline treatment on neurodegeneration in chemically induced Parkinson disease models have varied. While most studies found reduced microglial activation and neuronal cell death,[13,14] others reported decreased microglial activation and enhanced neuron toxicity.[15,16] In one genetic model, the Weaver mouse, minocycline treatment resulted in nigrostriatal neuroprotection.[17]

Huntington Disease

In a genetic model of Huntington disease (HD), the R6/2 mouse, one group reported that minocycline provided neuroprotection,[15] another observed no clinical evidences of therapeutic efficacy,[18] and a third reported that minocycline treatment was detrimental.[19] Several studies investigated the effects of minocycline treatment combined with other therapies. Treatment with minocycline and pyruvate or coenzyme Q10 afforded significant neuroprotection in mice with HD,[20,21] suggesting that minocycline may best be used as a combinatorial therapy.

Alzheimer Disease

While minocycline treatment produced mixed results in HD and Parkinson disease studies, it appears to be beneficial in experimental models of Alzheimer disease. Both in vivo and in vitro studies report suppressed microglial activation in Alzheimer disease models along with attenuated neuronal cell death, astrogliosis, and improved behavioral performance.[22,23] Further studies are necessary to confirm that these beneficial effects lead to long-term neuroprotection.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron death in the spinal cord, brainstem, and motor cortex. Mutations in superoxide dismutase 1 account for a proportion of cases of familial ALS, and mutant superoxide dismutase 1 trans-genic mice provide a useful tool for treatment-testing paradigms. Minocycline treatment improved the symptoms of ALS in these mice via inhibition of cytochrome c release and caspase activation, thus delaying the onset of degeneration and muscle strength decline.[25] Owing to the success of minocycline in these experimental trials, it quickly advanced to clinical trials but these yielded unexpectedly poor results (see "Clinical Studies" section).

Experimental Autoimmune Encephalomyelitis

Minocycline suppresses T-cell proliferation, migration, and cytokine production in an autoimmune animal model of multiple sclerosis, experimental autoimmune encephalomyelitis, and delays the onset and diminishes the severity of clinical neurological disability.[24,28]

Spinal Cord Injury

In animal models of spinal cord injury, minocycline treatment reduced microglial activation and astrogliosis and enhanced oligodendrocyte survival but reports of functional recovery were conflicting.[2]

CLINICAL STUDIES

The use of minocycline as an antibiotic as well as its excellent safety profile and penetration into the CNS spurred interest in using this drug to treat neurological diseases. Clinical trials are under way for the use of minocycline to treat several neurological diseases such as stroke, multiple sclerosis, spinal cord injury, Huntington disease, and Parkinson disease. Trials already completed have revealed both positive and negative effects (**Table 2**).

Brain Ischemia

A number of completed clinical studies reported beneficial effects of minocycline treatment without increased incidence of severe adverse events. In an open-label, evaluator-blinded study, oral administration of 200 mg/d of minocycline for 5 days 6 to 24 hours after acute CNS infarction caused no major complications, significantly de-

creased scores on the National Institutes of Health Stroke Scale and modified Rankin Scale, and increased Barthel Index scores in treated patients at days 7, 30, and 90 of follow-up. These observations suggest that the treatment of acute stroke with minocycline at this stage is associated with better clinical outcome.[29] There are, however, several limitations to this study including the open-label design and small sample size. Future studies of minocycline administration after CNS infarction should include double-blind, controlled trials that use a broader window of treatment time (especially earlier) as well as oral and intravenous dosages.

Spinal Cord Injury

A small, phase 1/2 pilot study is under way to assess minocycline treatment in human patients after spinal cord injury. In this randomized, double-blind trial, patients receive 200 or 400 mg of minocycline intravenously twice daily for 7 days and are evaluated for 1 year. Results obtained thus far indicate that minocycline treatment enhances recovery, as shown by improved scores on the American Spinal Injury Association examination, Functional Independence Measure, Spinal Cord Independence Measure, London Handicap Score, and Short Form–36. These improvements were limited to patients with cervical spinal cord injury, whereas those with thoracic complete spinal cord injury showed no treatment effect.[30] The sample size in this study is small, so conclusions of neuroprotection are preliminary.

Multiple Sclerosis

Several small clinical trials found minocycline treatment to be effective in reducing lesion size and occurrence rate in relapsing-remitting multiple sclerosis.[36,37] More recently, a double-blind, placebo-controlled study was completed to evaluate the effects of minocycline combined with glatiramer acetate treatment in patients with relapsing-remitting multiple sclerosis. In this study, patients received 100 mg of minocycline twice daily combined with glatiramer acetate or glatiramer acetate/placebo for 9 months. The combination of drugs reduced the total number of gadolinium-enhancing lesions, the total number of new and enlarging lesions, and the total disease burden compared with glatiramer acetate/placebo treatment.[31] Although the number of patients in this study was relatively small, it provides support for the use of minocycline as a combinatorial therapy for multiple sclerosis.

Parkinson Disease

In pilot clinical trials using minocycline treatment for Parkinson disease, 200 mg/d of minocycline was given for 18 months and patients were assessed for changes in the total Unified Parkinson Disease Rating Scale and a number of other measures to identify disability and symptoms.[32] The patients treated with minocycline showed neither a decline in symptoms nor any increase in adverse events.

Huntington Disease

An initial open-label study found no obvious toxicity and observed improvements in motor function in minocycline-treated patients with HD based on their Unified HD Rating Scale and Mini-Mental State Examination after 6 months of treatment.[33] In a 24-month follow-up, these authors reported stabilization of motor function and amelioration of psychiatric symptoms.[38] The study included 14 patients, with only 11 included in the final analyses, making it difficult to draw firm conclusions. An 8-week, double-blind, randomized, placebo-controlled study by the Huntington Study Group in which patients with HD were treated with the same dose (100 or 200 mg/d) as the Bonelli group indicated that, although minocycline was well tolerated, it had no effect on Unified HD Rating Scale scores and actually worsened scores on the Stroop Interference test.[39] Another small pilot study reported similar findings of good tolerability but lack of motor improvement on the Unified HD Rating Scale after 6 months of minocycline treatment.[34]

Amyotrophic Lateral Sclerosis

In a completed phase III clinical trial, patients with ALS received minocycline at up to 400 mg/d for 9 months; they were evaluated on the revised ALS functional rating scale and for forced vital capacity, manual muscle testing, quality of life, survival, and drug safety. Patients treated with minocycline showed faster deterioration than the placebo group according to the ALS functional rating scale and trended toward a faster decline in forced vital capacity and manual muscle testing scores. There was also a nonsignificant increase in mortality in the minocycline treatment group as well as gastrointestinal and neurological adverse events. Despite the negative effect of minocycline treatment on disease progression and adverse events, minocycline-treated patients described similar quality-of-life scores to the placebo patients.[35] The authors of this study concluded that minocycline is not a viable treatment for ALS and warned against its use to treat other neurodegenerative diseases. Among possible reasons for this disappointing result are (1) that an inadequately low dosage schedule was used and (2) that the dosage of minocycline, by increasing glutamate receptor 1 phosphorylation and membrane insertion, promoted glutamate toxicity and motor neuron loss.[40]

SUMMARY OF AVAILABLE CLINICAL TRIALS DATA

While the clinical trials mentioned reveal both beneficial and deleterious results of minocycline treatment, all hope is not lost regarding its use as a future therapeutic. More studies are needed to confirm the appropriate timing and dosage, which may vary by disease. Some researchers speculate that doses of 100 to 200 mg/d, typically used in current clinical trials, may be ideal for humans to alleviate symptoms without toxic consequences. Others believe that this dose is too low, given that the typical dose for animal studies ranges from 10

to 100 mg/kg/d, which would theoretically equal approximately 3 to 7 g/d for humans. The dose of 200 mg/d provided improvement for acute stroke, yet had no effect on Parkinson disease or patients with HD. Thus, the possibility remains that increasing the dose of minocycline would improve its efficacy. In addition, minocycline treatment could be combined with other drug treatments in hopes of enhancing the beneficial effects without increasing the risk of harmful adverse effects seen with high doses of minocycline treatment. This therapeutic strategy has already proved useful in preliminary studies of multiple sclerosis and may be advantageous in a number of other diseases as well.

CONCLUSIONS

The practical utility of the neuroprotective effects of minocycline, though based on a substantial body of promising in vitro and animal studies, continues to be a matter of intense debate, with contradictory evidence ranging from neuroprotection to the exacerbation of toxicity in various experimental models and human trials. Studies to date have clearly established that minocycline exerts neuroprotective effects but have also shown that the actions of this drug are complex, and that its administration can, under certain circumstances, also have neurotoxic consequences. More studies, both clinical and preclinical, and both combinatorial and sequential strategies, should be carried out to ascertain the therapeutic window and the indications for which minocycline could be useful, effective, and safe.

Correspondence: Wenbin Deng, PhD, Department of Cell Biology and Human Anatomy, University of California, Davis, School of Medicine, 2425 Stockton Blvd, Sacramento, CA 95817 (wbdeng@ucdavis.edu).

REFERENCES

1. Stirling DP, Koochesfahani KM, Steeves JD, Tetzlaff W. Minocycline as a neuroprotective agent. *Neuroscientist.* 2005;11(4):308-322.
2. Kim HS, Suh YH. Minocycline and neurodegenerative diseases. *Behav Brain Res.* 2009;196(2):168-179.
3. Guo G, Bhat NR. p38alpha MAP kinase mediates hypoxia-induced motor neuron cell death: a potential target of minocycline's neuroprotective action. *Neurochem Res.* 2007;32(12):2160-2166.
4. Garcia-Martinez EM, Sanz-Blasco S, Karachitos A, et al. Mitochondria and calcium flux as targets of neuroprotection caused by minocycline in cerebellar granule cells. *Biochem Pharmacol.* 2010;79(2):239-250.
5. Antonenko YN, Rokitskaya TI, Cooper AJ, Krasnikov BF. Minocycline chelates Ca2+, binds to membranes, and depolarizes mitochondria by formation of Ca2+-dependent ion channels. *J Bioenerg Biomembr.* 2010;42(2):151-163.
6. Matsukawa N, Yasuhara T, Hara K, et al. Therapeutic targets and limits of minocycline neuroprotection in experimental ischemic stroke. *BMC Neurosci.* 2009; 10:126.
7. Alano CC, Kauppinen TM, Valls AV, Swanson RA. Minocycline inhibits poly(ADP-ribose) polymerase-1 at nanomolar concentrations. *Proc Natl Acad Sci U S A.* 2006;103(25):9685-9690.
8. Guimarães JS, Freire MA, Lima RR, Picanço-Diniz CW, Pereira A, Gomes-Leal W. Minocycline treatment reduces white matter damage after excitotoxic striatal injury. *Brain Res.* 2010;1329:182-193.
9. Yrjänheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A.* 1999;96(23):13496-13500.
10. Tsuji M, Wilson MA, Lange MS, Johnston MV. Minocycline worsens hypoxic-ischemic brain injury in a neonatal mouse model. *Exp Neurol.* 2004;189(1): 58-65.
11. Carty ML, Wixey JA, Colditz PB, Buller KM. Post-insult minocycline treatment attenuates hypoxia-ischemia-induced neuroinflammation and white matter injury in the neonatal rat: a comparison of two different dose regimens. *Int J Dev Neurosci.* 2008;26(5):477-485.
12. Lechpammer M, Manning SM, Samonte F, et al. Minocycline treatment following hypoxic/ischaemic injury attenuates white matter injury in a rodent model of periventricular leucomalacia. *Neuropathol Appl Neurobiol.* 2008;34(4):379-393.
13. Du Y, Ma Z, Lin S, et al. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci U S A.* 2001;98(25):14669-14674.
14. Radad K, Moldzio R, Rausch WD. Minocycline protects dopaminergic neurons against long-term rotenone toxicity. *Can J Neurol Sci.* 2010;37(1):81-85.
15. Diguet E, Fernagut PO, Wei X, et al. Deleterious effects of minocycline in animal models of Parkinson's disease and Huntington's disease. *Eur J Neurosci.* 2004; 19(12):3266-3276.
16. Yang L, Sugama S, Chirichigno JW, et al. Minocycline enhances MPTP toxicity to dopaminergic neurons. *J Neurosci Res.* 2003;74(2):278-285.
17. Peng J, Xie L, Stevenson FF, Melov S, Di Monte DA, Andersen JK. Nigrostriatal dopaminergic neurodegeneration in the weaver mouse is mediated via neuroinflammation and alleviated by minocycline administration. *J Neurosci.* 2006; 26(45):11644-11651.
18. Chen M, Ona VO, Li M, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med.* 2000;6(7):797-801.
19. Smith DL, Woodman B, Mahal A, et al. Minocycline and doxycycline are not beneficial in a model of Huntington's disease. *Ann Neurol.* 2003;54(2):186-196.
20. Stack EC, Smith KM, Ryu H, et al. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. *Biochim Biophys Acta.* 2006;1762(3):373-380.
21. Ryu JK, Choi HB, McLarnon JG. Combined minocycline plus pyruvate treatment enhances effects of each agent to inhibit inflammation, oxidative damage, and neuronal loss in an excitotoxic animal model of Huntington's disease. *Neuroscience.* 2006;141(4):1835-1848.
22. Fan R, Xu F, Previti ML, et al. Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid. *J Neurosci.* 2007;27(12):3057-3063.
23. Seabrook TJ, Jiang L, Maier M, Lemere CA. Minocycline affects microglia activation, Abeta deposition, and behavior in APP-tg mice. *Glia.* 2006;53(7):776-782.
24. Nikodemova M, Lee J, Fabry Z, Duncan ID. Minocycline attenuates experimental autoimmune encephalomyelitis in rats by reducing T cell infiltration into the spinal cord. *J Neuroimmunol.* 2010;219(1-2):33-37.

25. Zhu S, Stavrovskaya IG, Drozda M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature*. 2002;417(6884):74-78.

26. Cai ZY, Yan Y, Chen R. Minocycline reduces astrocytic reactivation and neuro-inflammation in the hippocampus of a vascular cognitive impairment rat model. *Neurosci Bull*. 2010;26(1):28-36.

27. Liu Z, Fan Y, Won SJ, et al. Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. *Stroke*. 2007;38(1):146-152.

28. Zemke D, Majid A. The potential of minocycline for neuroprotection in human neurologic disease. *Clin Neuropharmacol*. 2004;27(6):293-298.

29. Lampl Y, Boaz M, Gilad R, et al. Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. *Neurology*. 2007;69(14):1404-1410.

30. Casha S, Zygun D, McGowan D, Yong VW, Hurlbert JR. Neuroprotection with minocycline after spinal cord injury: results of a double blind, randomized, controlled pilot study. *Neurosurgery*. 2009;65(2):410-411. doi:10.1227/01.neu.0000358700.03703.a5.

31. Metz LM, Li D, Traboulsee A, et al; GA/minocycline study investigators. Glatiramer acetate in combination with minocycline in patients with relapsing-remitting multiple sclerosis: results of a Canadian, multicenter, double-blind, placebo-controlled trial. *Mult Scler*. 2009;15(10):1183-1194.

32. NINDS NET-PD Investigators. A pilot clinical trial of creatine and minocycline in early Parkinson disease: 18-month results. *Clin Neuropharmacol*. 2008;31 (3):141-150.

33. Bonelli RM, Heuberger C, Reisecker F. Minocycline for Huntington's disease: an open label study. *Neurology*. 2003;60(5):883-884.

34. Thomas M, Ashizawa T, Jankovic J. Minocycline in Huntington's disease: a pilot study. *Mov Disord*. 2004;19(6):692-695.

35. Gordon PH, Moore DH, Miller RG, et al; Western ALS Study Group. Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. *Lancet Neurol*. 2007;6(12):1045-1053.

36. Zabad RK, Metz LM, Todoruk TR, et al. The clinical response to minocycline in multiple sclerosis is accompanied by beneficial immune changes: a pilot study. *Mult Scler*. 2007;13(4):517-526.

37. Zhang Y, Metz LM, Yong VW, et al. Pilot study of minocycline in relapsing-remitting multiple sclerosis. *Can J Neurol Sci*. 2008;35(2):185-191.

38. Bonelli RM, Hödl AK, Hofmann P, Kapfhammer HP. Neuroprotection in Huntington's disease: a 2-year study on minocycline. *Int Clin Psychopharmacol*. 2004; 19(6):337-342.

39. Huntington Study Group. Minocycline safety and tolerability in Huntington disease. *Neurology*. 2004;63(3):547-549.

40. Manev H, Manev R. Interactions with GluR1 AMPA receptors could influence the therapeutic usefulness of minocycline in ALS. *Amyotroph Lateral Scler*. 2009; 10(5-6):416-417.

Announcement

Research Letters. The Research Letter is intended to provide a means to communicate short original research in a highly focused manner. Important, fast-breaking research that lends itself to a short communication and that can be reviewed rapidly is our objective. Papers should not exceed 600 words of text and should have fewer than 6 references. A single table or figure may be included. In general, Research Letters should be divided into the following sections: an introduction (with no heading), Methods, Results, and Comment. Research Letters should be double spaced and a word count should be provided with each letter.