The promise of minocycline in neurology

V Wee Yong, Jennifer Wells, Fabrizio Giuliani, Steven Casha, Christopher Power, and Luanne M Metz

The capacity of minocycline to alleviate disease for several neurological disorders in animals is increasingly being recognised. Indeed, that one drug alone can attenuate the severity of disease in stroke, multiple sclerosis, spinal-cord injury, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis is astounding. In this review, we describe the evidence for the efficacy of minocycline in several animal models of neurological disease, discuss the mechanisms by which minocycline affects a range of neurological diseases with diverse causes, and introduce the emerging investigation of minocycline in clinical neurology. The encouraging results of minocycline in experimental neurology bode well for its therapeutic use in human neurological diseases.

Minocycline is a semisynthetic tetracycline that has been in use for over 30 years. It is a small (495 kDa), highly lipophilic molecule capable of crossing the blood–brain barrier. Minocycline penetrates the CSF of human beings better than doxycycline and other tetracyclines.[1,2] Minocycline is readily absorbed from the gut after oral ingestion and, because of its low propensity to produce antibiotic resistance, it is commonly used in the management of chronic conditions such as acne and rosacea. According to registry records in the UK, over 6 million individuals have been treated for acne for an average of 9 months with minocycline. Serious cases of liver toxicity, serum sickness, and systemic lupus erythematosus have been reported,[3] but the frequency of these adverse events is very low, of the order of one in a million individuals treated. Overall, a good safety record for long-term clinical use has been established for minocycline.[4]

In 1998, Yrjanheikki and colleagues[5] showed that minocycline was neuroprotective in an animal model of ischaemia. Since then, there have been numerous reports of the efficacy and neuroprotective effects of minocycline in various models of neurological disease, including haemorrhagic and ischaemic stroke, multiple sclerosis, spinal-cord injury, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS). In this review, we provide an overview of the remarkable results of minocycline in these animal models. A few publications report results to the contrary and these are also reviewed. We discuss possible mechanisms by which minocycline may have an effect on such a wide range of neurological diseases, assess the relevance of animal studies to human diseases, and we provide information on the emerging investigation of minocycline in human neurological diseases.

Minocycline in animal models
Ischaemic and haemorrhagic stroke

The first researchers to report of the efficacy of minocycline in experimental neurology[5] used this drug because of the known expression of several proinflammatory genes in the ischaemic brain and because minocycline had been shown to inhibit the activity and expression of these inflammatory mediators. Thus, Yrjanheikki and colleagues[5] showed that minocycline protected hippocampal neurons from death in a gerbil model of forebrain ischaemia when administered 30 min after brain insult. Shortly thereafter, researchers found that minocycline was also effective in focal brain ischaemia in rats;[6] its injection by the intraperitoneal route reduced cortical infarction volume by 76% when given 12 h before ischaemia and by 63% when given 4 h after the onset of ischaemia. In that study, minocycline had a better neuroprotective effect than doxycycline.[6]

More recently, in rats with focal ischaemic injury induced by embolising a preformed clot into the middle cerebral artery, infarct volume was significantly reduced when minocycline was given from either 1 h or 4 h after embolisation.[7] By comparison, a similar period of delayed hypothermia was not protective.[7] Similarly, in a rat model of neonatal hypoxic-ischaemic brain injury, a single intraperitoneal injection of minocycline given immediately after the insult prevented the damage seen at 1 week in the ipsilateral hemisphere of animals treated with drug vehicle alone.[8] Neuroprotection by minocycline in hypoxic-ischaemic injury in neonatal rats has been reproduced by another group.[9] Finally, intravenously given minocycline, which results in a more predictable plasma concentration and half-life than that achieved by intraperitoneal injection,[10] was neuroprotective after middle-cerebral-artery occlusion in rats at doses lower than those needed by other parenteral routes.[11] In a model of intracerebral haemorrhage in rats, minocycline reduced glial activation, cellular apoptosis and behavioural recovery when given twice daily for the first week starting 1 h after the insult.[12]

Overall, these studies of minocycline in animal models of ischaemic and haemorrhagic stroke hold promise for minocycline as a therapy in patients with stroke.

All authors are at the Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada.

Correspondence: Dr V Wee Yong, Departments of Oncology and Clinical Neurosciences, University of Calgary, 3330 Hospital Drive NW, HMRB 187, Calgary, Alberta T2N 4N1, Canada.
Tel +1 403 220 3544; fax +1 403 283 8731;
email vyong@ucalgary.ca

Multiple sclerosis

Our initial rationale for using minocycline to treat experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, was to target matrix metalloproteinases (MMPs). MMPs are upregulated in multiple sclerosis and EAE, in which they have several detrimental effects including breakdown of the blood–brain barrier, promotion of neuroinflammation, and neurotoxicity.[13] Indeed, one mechanism of action of interferon-beta therapy in multiple sclerosis may be to decrease the production of MMPs by T lymphocytes,[14] thereby attenuating the ability of these leucocytes to penetrate the CNS. As interferon beta affects the synthesis of MMPs but does not influence enzyme activity directly, we reasoned that the combination of interferon beta with a direct inhibitor of MMP enzyme activity might result in a greater reduction of leucocyte influx and a better control of disease activity. We selected minocycline for testing because of reports that it inhibited the enzymatic activity of MMPs.[15–17] Furthermore, the good safety record of minocycline in long-term oral use to treat acne[4] is an important consideration for treatment of a chronic disease such as multiple sclerosis.

In EAE, we found that mice treated with minocycline at the time of disease induction with a myelin peptide have delayed onset of clinical symptoms.[18] Animals treated with minocycline eventually succumbed to EAE when the immunisation protocol resulted in severe disease, but if the disease-inducing protocol gave rise to a mild disease, daily minocycline attenuated severity over the entire course of the experiment, even when started at the first appearance of symptoms.[18] Another study, which aimed to prevent microglial activation, reported that minocycline suppressed ongoing disease activity and limited disease progression in chronic relapsing-remitting EAE in rats.[19] Similarly, Nessler and colleagues[20] found that minocycline given intraperitoneally alleviated the severity of adoptive transfer EAE, in which disease was induced through the transfer into naive recipients of activated T cells that were myelin-protein specific. In the same study, however, minocycline given orally did not influence the disease course.[20] These findings suggest that the bioavailability of minocycline varies with the route of administration. As noted earlier, intravenous minocycline resulted in a more predictable serum concentration and a lower dose requirement than intraperitoneal injection,[10,11] and it is possible that even higher oral doses will need to be given to acheive the same serum concentrations as parenteral administration.

Overall, minocycline given at the time of disease induction can delay or abrogate subsequent neuroinflammation and neuropathological changes in EAE, whereas treatment initiated at the onset of clinical signs can still attenuate the disease course.

Spinal-cord injury

Various inflammatory molecules and neurotoxic compounds, including glutamate, free radicals, and MMPs, are upregulated after spinal-cord injury. We reasoned that rather than using a highly selective treatment that targets a specific molecule or pathway, a compound with multifunctional properties that targets several mediators involved in spinal-cord pathology may be more effective. Thus, we selected minocycline for testing in spinal-cord injury in animals given its capacity to inhibit several mediators of damage found in the injured spinal cord. In agreement with our hypothesis, we observed that after spinal-cord compression injury in mice, minocycline treatment, beginning 1 h after injury and then daily for 6 days, provided significant recovery of hind-limb function compared with injured mice that were treated with the drug vehicle alone.[21] Behavioural recovery was accompanied by histological evidence of tissue, neuronal, and axonal preservation at the site of injury (figure 1).[21]

Since this initial report,[21] other groups have reported promising results with minocycline in several models of spinal-cord injury. Rats receiving a weight-drop contusion injury had improved hind-limb motor function and fewer apoptotic cells in the spinal cord if treated with minocycline immediately after spinal-cord injury and then twice more over the next 24 h.[22] Adult rats with a C7–C8 dorsal column transection, with minocycline injected intraperitoneally 30 min, and 8 h after the injury and twice daily thereafter for 2 days, also showed functional improvement as well as a reduction in the number of apoptotic oligodendrocytes at 7 days and 14 days.[23] Furthermore, Teng and colleagues[24] reported the beneficial effect of minocycline in rats with weight-drop spinal-cord injury.

The finding of significant improvement in functional recovery in animals given minocycline within the first 2 days of spinal-cord injury suggests that a critical mechanism of minocycline efficacy involves early events in the pathophysiology of this type of injury. The reports also provide evidence for the efficacy of minocycline in alleviating not only axonal and neuronal loss, but also the destruction of oligodendrocytes and possibly other cell types in the injured area and in projecting fibre tracts.

Finally, in concordance with the spinal-cord injury results, minocycline treatment decreased lesion size and improved rotarod test performance 4 days after traumatic brain injury,[25] thus providing further evidence for the neuroprotective efficacy of minocycline therapy in CNS trauma.

Parkinson's disease

The impressive therapeutic effects of minocycline have been extended to models of Parkinson's disease. Of note is the neuroprotection by minocycline of the nigrostriatal pathway against a very potent neurotoxin, N-methyl-4-phenyl-tetrahydropyridine (MPTP).[26,27] In another model of Parkinson's disease, in which 6-hydroxydopamine was injected into the striatum of mice, minocycline was similarly neuroprotective.[28] In yet another model of damage to the nigrostriatal pathway, in which lipopolysaccharide was injected into the substantia nigra of rats to produce inflammation and damage to the blood–brain barrier, minocycline treatment reduced the severity of these effects.[29] Furthermore, in studies with cultures of mesencephalic and cerebellar granule neurons, minocycline inhibited the lethality mediated by 1-methyl-4-pyridinium ion (MPP+), the active metabolite of MPTP.[26]
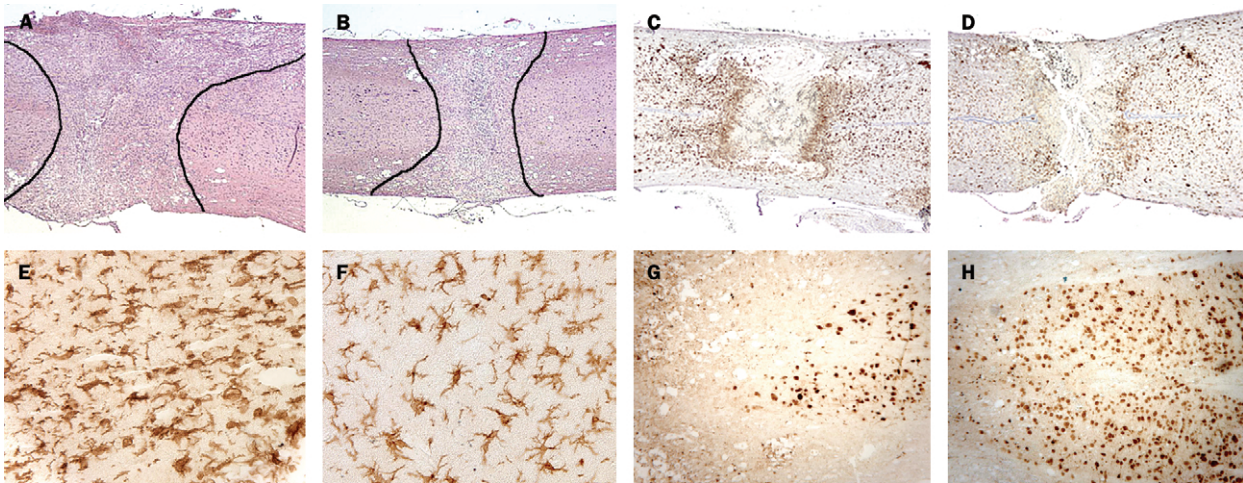
Figure 1. Minocycline reduces the pathology of spinal-cord injury in mice. Mice had a compression injury of the spinal cord and minocycline or saline was injected intraperitoneally from 1 h after injury for 6 days, as described previously.[21] The spinal cord was removed for analyses 28 days after injury. Mice were either treated with saline (the left-hand image of each pair) or with minocycline. In minocycline-treated mice: the lesion volume (haematoxilin-eosin staining, with the area of tissue damage delineated by drawn lines) decreased (A,B), loss of neurons (NeuN) in the area of spinal-cord compression is less (G,H); microglia, detected by Iba1 immunohistochemistry (low [C,D] and high [E,F] magnification of the spinal cord at the area of injury), showed less activity as denoted by the small number and the more ramified and slender nature of the morphology. We previously showed that the integrity of axons throughout the area of injury was relatively preserved in the minocycline-treated mice compared to the saline group, and this was accompanied by significant functional recovery in the minocycline group.[21]

Huntington's disease

The R6/2 mouse has been used to assess the efficacy of minocycline in Huntington's disease. In this model, caused by an expanded polyglutamine repeat in exon 1 of the *huntingtin* gene, mice develop progressive neurological dysfunction and die at about 13 weeks of age. When treated with daily intraperitoneal minocycline beginning at 6 weeks of age, disease progression was delayed and survival was 14% longer than in saline-treated mice;[30,31] by contrast, tetracycline, which does not effectively cross the blood–brain barrier, was ineffective.[30] In a striatal cell model of Huntington's disease, minocycline inhibited the cell death caused by pathways that are caspase dependent or independent.[31] Minocycline, as well as doxycycline and tetracycline, are potent inhibitors of aggregation of huntingtin in a hippocampal slice culture model of Huntington's disease.[32]

By contrast with these favourable reports, the treatment of R6/2 mice with minocycline given orally in the drinking water resulted in no clear differences in behavioural abnormalities or aggregate disease-load post mortem.[32] This negative result may be because of instability of minocycline when kept in drinking water for 7 days,[33] although Hockly and colleagues[34] showed that an aqueous solution of minocycline in 5% sucrose was stable for 1 week at room temperature. Our own experience is that minocycline is an unstable compound in aqueous solution and a standard procedure in our laboratory is to use minocycline solutions that are made fresh daily.

Amyotrophic lateral sclerosis

Mice expressing mutant superoxide dismutase provide an animal model of ALS. These mice show progressive neurological deterioration and destruction of motor neurons in the spinal cord and die prematurely at about age 120 days. Minocycline, started at age 5 weeks, delayed the onset of impaired motor ability on rotarod tests and significantly extended survival of mice from 126 days to 137 days;[35] similar results have been reported by Kriz[36] and Van Den Bosch[37] and their coworkers. Finally, apoptosis induced by exposure of cultured neurons to CSF from patients with motor neuron diseases, including ALS, was ameliorated by minocycline.[38]

Exacerbation of disease by minocycline

By contrast with the numerous reports that minocycline is beneficial in several animal models of neurological diseases (table), there are also data indicating that minocycline worsens CNS disease. Contrary to the reports noted,[26,27] minocycline significantly exacerbated the MPTP-induced damage to dopaminergic neurons in mice.[39] The investigators suggested that this effect could be due to the inhibition of dopamine and MPP+ uptake into striatal vesicles, thereby potentiating the toxicity of the latter, but no data were provided to support this explanation. Species differences did not account for the different effects reported by these studies,[26,27,39] as all three studies used mice. Nevertheless, in cynomolgus monkeys given MPTP, minocycline increased symptoms of parkinsonism and loss of dopaminergic nerve terminals in the putamen when compared with monkeys that received placebo.[40] In the 3-nitropropionic-acid mouse model of Huntington's disease, minocycline treatment aggravated motor scores in behavioural tasks, and produced more neuronal loss in the dorsal striatum than seen in controls.[40]

As noted above, minocycline given to neonatal rats reduced the pathological consequences of a hypoxic-ischaemic brain injury.[8] This result has been reproduced by

Disorders affected by minocycline in animal models	
Model	**Main outcome of minocycline in animals**
Ischaemic stroke	Decreases the size of infarcts
Intracerebral haemorrhage	Reduces lesion volume
Experimental autoimmune encephalomyelitis	Attenuates the extent of neuroinflammation and demyelination
Spinal-cord injury	Produces functional recovery, and decreases axonal, neuronal, and oligodendroglial loss
Traumatic brain injury	Decreases lesion size and improves performance on a rotarod test
Parkinson's disease	Protects the nigrostriatal pathway
Huntington's disease	Delays progression of disease and extends lifespan
ALS	Increases lifespan of mice

Tsuji and colleagues[9] for neonatal rats, but, remarkably, this group also reported that minocycline exacerbated injury in neonatal mice. The mechanism of the contrasting effects of minocycline in mouse and rat neonatal hypoxia-ischaemia was not resolved by the investigators.[9]

In summary, it seems that, in some situations, minocycline may aggravate CNS disease rather than being beneficial. There are no satisfactory explanations thus far for the discrepancy. The possibility that minocycline may be harmful must be borne in mind when considering its use in clinical neurology.

Mechanisms of minocycline's effects

Despite some contradictory reports, the overwhelming majority of studies indicate that minocycline is effective in the treatment of animal models of various neurological disorders. How can one drug have such an impressive effect on many different neurological disorders with seemingly diverse causes? First, the possibility of common mechanisms of neurodegeneration in these diseases must be taken into consideration. Microglial activation and the attendant neurotoxic products generated by persistently activated microglia are common features of these diseases. Apoptotic cell death is also a unifying theme. Thus, that minocycline is a potent inhibitor of microglial activation and of apoptotic pathways is noteworthy.

Inhibition of microglial activation

Evidence that minocycline prevents microglial activation was first provided by Yrjanheikki and colleagues[5] in a model of forebrain ischaemia in gerbils. Decreased microglial activation by minocycline has since been reported in models of Parkinson's disease,[27] multiple sclerosis,[19] intracerebral haemorrhage,[12] and spinal-cord injury[23] (figure 1). The expression of several molecules associated with microglial activation, including caspase 1 (interleukin-1β-converting enzyme) and inducible nitric oxide synthase, reduced expression after treatment with minocycline.[5,6] Nevertheless, these reports do not discriminate between a direct effect of minocycline in the inhibition of microglial activation or an indirect effect through reduced neurodegeneration and, consequently, less microglial activation.

Whether minocycline has a direct or an indirect effect on microglial activation was investigated in tissue culture. Minocycline directly inhibited the proliferation and the activation state of cultured microglia.[41,42] Microglial activation in tissue culture contributed to glutamate excitotoxicity, and microglial activation was reduced by minocycline with associated alleviation of excitotoxicity.[43]

That minocycline inhibits microglial activation has been exploited in the area of cell transplants and neurogenesis. A myelin mutation in the spinal cords of Long Evans Shaker rats produces myelin defects that are associated with progressive microglial activation. In this model, the transplantation of oligodendroglial progenitor cells during peak microglial activation did not lead to myelination because the grafted cells died promptly after transplantation. However, pretreatment of these animals with minocycline reduced microglial activation and resulted in cell survival and myelin formation by the implants.[44] Furthermore, hippocampal neurogenesis, a process that continues in the adult brain, was impaired by microglial activation caused by lipopolysaccharide infusion into the brain, and this deficient neurogenesis was restored by the systemic administration of minocycline in association with reduced microglial activation.[45]

Attenuation of apoptosis

Apoptosis and the release of apoptosis-inducing drugs is a common mechanism of neurodegeneration. Minocycline reduces apoptosis of neurons and oligodendrocytes in various neural insults[22–24] and alleviates necrotic cell death.[8] There is increasing evidence to suggest that the antiapoptotic effect is achieved through several mechanisms at the level of the mitochondrion. Minocycline stabilises mitochondria membranes and inhibits the mitochondrial permeability transition-mediated release of cytochrome c into the cytosol,[35,46] which is a potent stimulus for the activation of caspases 9 and 3 and the induction of apoptosis. The stabilisation of mitochondrial membranes also reduces the release into the cytoplasm of other factors that trigger both caspase-dependent and caspase-independent apoptotic pathways, including apoptosis-inducing factor and Smac/Diablo.[31,47] In cell culture, minocycline upregulates the anti-apoptotic factor Bcl-2, which then accumulates in mitochondria to antagonise the pro-apoptotic Bcl-2 family members Bax, Bak, and Bid.[46] Indeed, the downregulation of Bcl-2 by antisense oligonucleotides prevents the protective capacity of minocycline in vitro.[46] Finally, in a cell model of Huntington's disease, minocycline inhibited the generation of the proapoptotic protein, t-Bid.[31]

Suppression of free-radical production

The generation of free radicals, leading to lipid and protein peroxidation and damage to membranes is also a common mechanism across a spectrum of diseases. Minocycline depresses the release of oxygen radicals from various cell types, including leucocytes.[48] As mentioned above, the production of nitric oxide is decreased by minocycline through an effect on nitric oxide synthase.[49]

Inhibition of MMPs

Another mechanism for minocycline action that may account for its effect on various neurological diseases is its inhibition of MMPs. Various MMPs are upregulated in neurological disorders, in which they can contribute to demyelination, neurotoxicity, and neuroinflammation.[13] Thus, it is relevant that minocycline is a direct inhibitor of MMP enzymatic activity[15-17] and can also reduce the production of MMPs by leucocytes.[12,18] An effect on MMPs can also affect the transmigration of leucocytes into the CNS,[13] thereby reducing neuroinflammation further. Various inflammatory cell subsets can disrupt CNS functions and produce toxic effects when present in the CNS in large numbers.

Changes in leucocyte function

Besides inhibiting MMPs, leucocyte migration, and neuroinflammation in the nervous system, minocycline also has a direct effect on the activity of leucocytes. Kloppenburg and colleagues[50,51] noted that minocycline inhibited T-cell proliferation and reduced their production of inflammatory cytokines. By contrast, the addition of minocycline to activated monocytes led to a dose-dependent increase in the production of tumour necrosis factor α and interleukin 6.[51]

Other mechanisms

Other mechanisms may also contribute to the activity of minocycline. The drug is a Ca^{2+} chelator and may sequester excess Ca^{2+} released after injury. The decreasing of Ca^{2+} concentrations may prevent activation of calpains and preserve axonal integrity.[23] Furthermore, minocycline can inhibit the activation of p38 mitogen-activated protein (MAP) kinase[41,52] and thus influence multiple processes that would otherwise reduce cell integrity. The antibiotic activity of minocycline is unlikely to account for its effectiveness in neurological diseases, since infection is not a component of these animal models, and because minocycline derivatives without antimicrobial action can also affect some of the above processes.[16]

In summary, among several activities, minocycline impairs microglial activation, neuroinflammation, and apoptosis (figure 2), which are all common to many neurological diseases. The early appearance of these mediators of injury may help account for the apparent transient need for giving minocycline in the first few days after damage, such as in spinal-cord injury. Although many of the benefits of minocycline are likely to be derived from
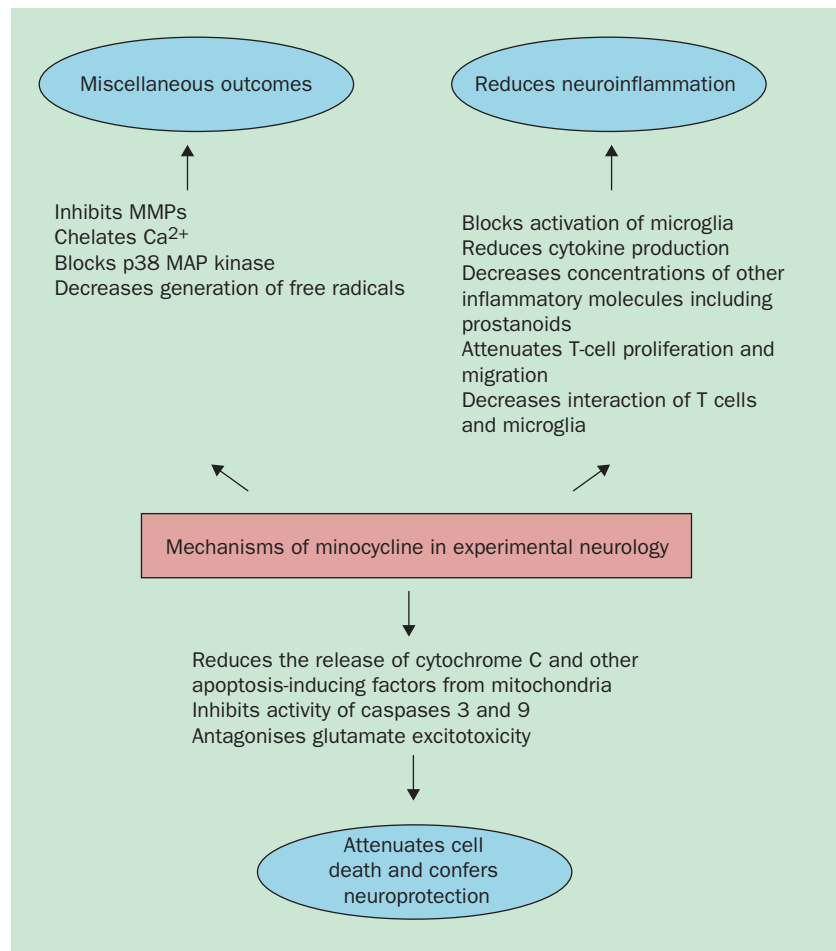

Figure 2. Multiple mechanisms of minocycline account for its beneficial effects in experimental neurology.

its action in the CNS, its effects on T cells[18,50,51] and other leucocyte subsets could also happen in the periphery (figure 3).

Relation of animal data to human diseases

Most reports in animals have used systemic injections of minocycline with doses ranging from 10 to 100 mg/kg (mean 50 mg/kg). If translated directly to a 70 kg average human being, this equates to a mean of 3·5 g per individual, which is more than ten times higher than the average of 200 mg minocycline daily taken by human patients for other indications. At a first glance, this implies that animals have been exposed to much higher doses than human beings. However, animals often require higher doses of drugs (per kg bodyweight) than human beings, because of the higher rate of liver metabolism in small animals, particularly rodents. Indeed, it has been found that the half-life of minocycline in rodents is about 2–3 h,[53,54] whereas in human beings the half-life is about 15 h.[55] Thus, many animal studies using a single daily administration of minocycline have probably underdosed animals despite achieving significant results.[21,23]

Another pharmacokinetic consideration is the use of intraperitoneal administration in animals in many studies,
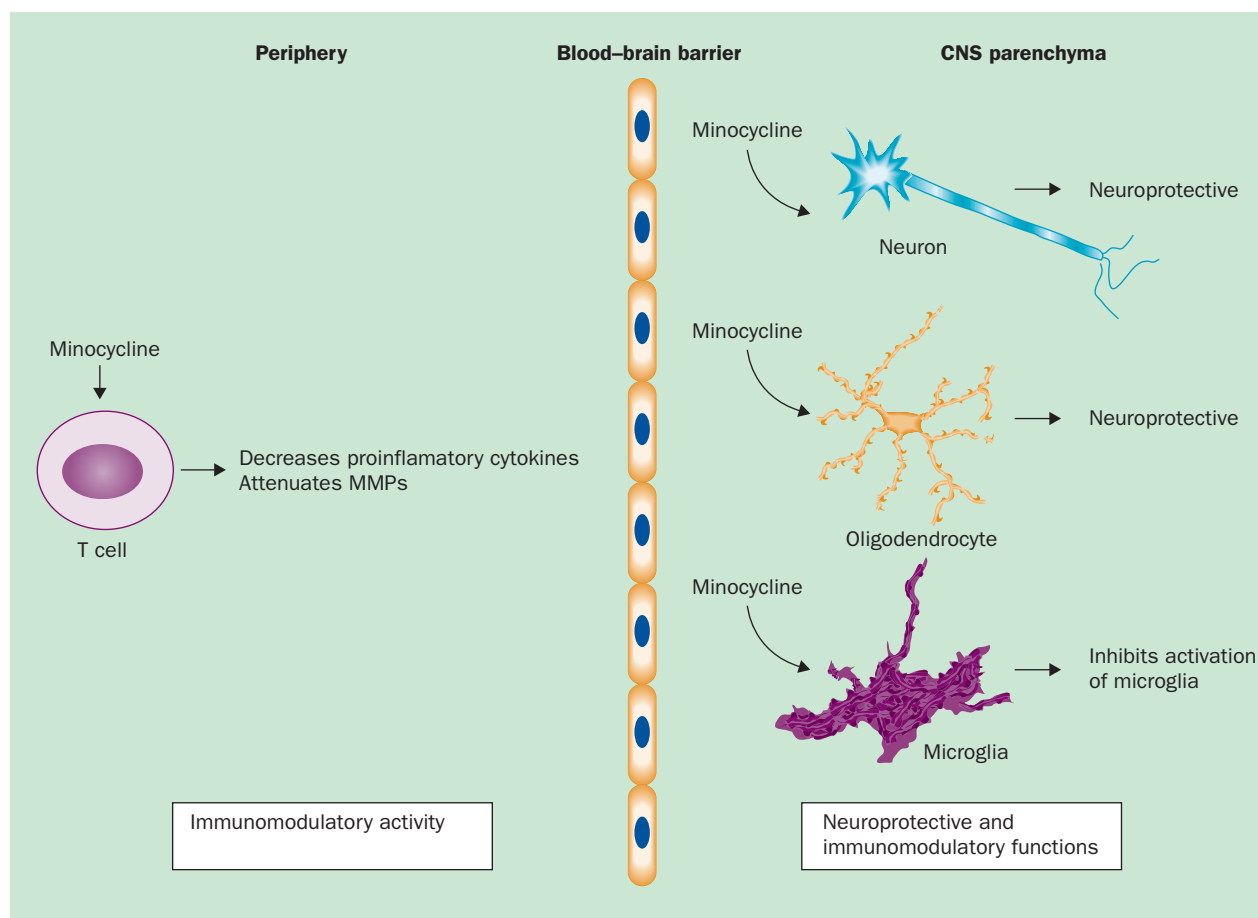
Figure 3. Peripheral and central functions of minocycline. Minocycline has immunomodulatory activity in the periphery and both immunomodulatory and neuroprotective capacity within the CNS. The likelihood of a direct action at the level of the blood–brain barrier has not been addressed thoroughly. MMPs=matrix metalloproteinases.

and the fact that minocycline is a weakly acidic solution. Indeed, it has been proposed that intraperitoneal minocycline (pH 5) produces abdominal irritation that leads to a stress response and the release of endogenous corticosteroids,[20] which may ultimately account for its beneficial properties. We have addressed these issues and have shown that the intraperitoneal injection of saline at pH 5, or of corticosteroid, cannot reproduce the beneficial effects of minocycline in spinal-cord injury in mice.[21]

In summary, higher doses of minocycline are necessary in animals than in patients particularly because of its very short half-life in rodents. In short-term experiments (days), animals may have been underdosed despite an impressive functional outcome; this observation invites optimism in the outcome of minocycline use in patients with neurological diseases.

Combination treatment involving minocycline

Few studies have explored the effects of combining minocycline with other drugs to achieve a better response in animals. In a murine model of ALS, in which either minocycline or creatine treatment results in improvement in motor performance and extended survival, combination of these two drugs provided additional neuroprotection.[56] In

another study involving a mouse model of ALS, the three-drug combination of minocycline, riluzole (a glutamate receptor antagonist), and nimodipine (a voltage-gated calcium-channel blocker) delayed the onset of disease, slowed the loss of muscle strength, and increased the average longevity of mice by 6 weeks,[57] but this was not compared with minocycline alone.

In our own studies, the combination of minocycline with glatiramer acetate, an immunomodulator currently used to treat patients with multiple sclerosis, provided superior control of disease activity and neuropathology compared with that provided by either drug alone.[58] The combination of minocycline with interferon beta, another therapy for multiple sclerosis, may also lead to improved outcome, because interferon beta is degraded by MMPs, the activity of which is lowered by minocycline.[59] In addition, a mechanism of action of interferon beta may be attributed to the decreased production of MMPs by T lymphocytes,[14] thereby attenuating the ability of these leucocytes to use proteolytic activity to penetrate the CNS. As interferon beta affected the synthesis of MMPs but does not affect enzyme activity directly, the combination of interferon beta with a direct inhibitor of MMP enzyme activity (eg, minocycline) might result in a greater

reduction of leucocyte influx and a better control of disease activity in multiple sclerosis.

Clinical trials of minocycline in neurology

Reports are emerging of clinical trials of minocycline in neurological diseases. Two open-label phase I trials of minocycline in Huntington's disease have been published. Bonelli and colleagues[60] reported that minocycline (100 mg/day for 6 months) was well tolerated and safe in 14 patients. Thomas and colleagues[61] similarly reported that minocycline was well tolerated when given to 30 patients with Huntington's disease for 6 months. In a randomised, double-blind, placebo-controlled study of 60 patients with Huntington's disease, minocycline given for 8 weeks at daily doses of 100 mg and 200 mg was tolerated well and the frequency of adverse events was similar between treatment and placebo groups.[62]

In ALS, two double-blind, randomised, placebo-controlled feasibility trials of minocycline have been reported. In the first trial, 19 patients received 200 mg daily or placebo for 6 months, and no significant differences in adverse events were reported.[63] In the second trial, 23 patients received up to 400 mg daily in an 8 month cross-over trial. The mean tolerated dose was 387 mg daily; with higher doses, there was a trend toward more gastrointestinal adverse events and an increase in blood urea nitrogen and liver enzymes.[63]

On the basis of the encouraging results of minocycline that we observed in EAE,[18] we initiated a cross-over trial of minocycline in relapsing-remitting multiple sclerosis. After a 3-month run-in period, ten patients received minocycline (100 mg twice daily). They had monthly MRI scans and quarterly clinical assessment. The primary endpoint was reduction of gadolinium-enhancing lesions during the first 6 months of treatment compared with the run-in phase. Secondary measures included monitoring of tolerance. Five patients did not have enhancing lesions for the 3 months before treatment and they remained negative during the 6 months of treatment, which is reassuring as it indicates that minocycline did not precipitate MRI lesions.[64] The other five patients had enhancing lesions before treatment on 19 of 20 scans, whereas no enhancements occurred after 2–6 months of therapy.[64] No significant toxic effects were observed. Patients continue to be followed clinically and with annual MRI scans.

Final perspectives

The initial report of the efficacy of minocycline in ischaemic stroke[5] is now supported by many studies describing the effectiveness of minocycline in various animal models of neurological disease. Where comparisons have been made, minocycline has tended to be more effective than doxycycline and tetracycline,[6,30] which is probably related to

its better penetration of the blood–brain barrier.[1,2] The effectiveness of minocycline in various neurological diseases is likely to relate to its antagonism of the multiple mechanisms that lead to injury in many nervous system diseases, including the alleviation of apoptosis and neuroinflammation. A few reports caution that minocycline exacerbates disease in animal models of neurological disorders,[9,39,40] and this has to be noted when considering the risks versus the benefits of minocycline in specific diseases. Nevertheless, it is encouraging that phase I trials of minocycline in Huntington's disease,[60–62] ALS,[63] and multiple sclerosis[64] show that minocycline is well tolerated in these patients. The promising results of minocycline in a clinical trial of multiple sclerosis, in which brain lesional activity detected through gadolinium-enhancing MRI lesions was reduced within the first 2 months of starting treatment,[64] invite optimism that the efficacy of minocycline in animals can be extended to human beings. Finally, its widespread availability, oral formulation, and low cost bode well for the combined use of minocycline with existing therapies to improve the prognosis of several neurological disorders.

References

1 Carney S, Butcher RA, Dawborn JK, Pattison G. Minocycline excretion and distribution in relation to renal function in man. *Clin Exp Pharmacol Physiol* 1974; **1:** 299–308.
2 MacDonald H, Kelly RG, Allen ES, Noble JF, Kanegis LA. Pharmacokinetic studies on minocycline in man. *Clin Pharmacol Ther* 1973; **14:** 852–61.
3 Elkayam O, Yaron M, Caspi D. Minocycline-induced autoimmune syndromes: an overview. *Semin Arthritis Rheum* 1999; **28:** 392–97.
4 Seukeran DC, Eady EA, Cunliffe WJ. Benefit-risk assessment of acne therapies. *Lancet* 1997; **349:** 1251–52.
5 Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci USA* 1998; **95:** 15769–74.
6 Yrjanheikki J, Tikka T, Keinanen 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 USA* 1999; **96:** 13496–500.

7   Wang CX, Yang T, Noor R, Shuaib A. Delayed minocycline but not delayed mild hypothermia protects against embolic stroke. *BMC Neurol* 2002; **2**: 2.

8   Arvin KL, Han BH, Du Y, Lin SZ, Paul SM, Holtzman DM. Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. *Ann Neurol* 2002; **52**: 54–61.

9   Tsuji M, Wilson MA, Lange MS, Johnston MV. Minocycline worsens hypoxic-ischemic brain injury in a neonatal mouse model. *Exp Neurol* 2004; **189**: 58–65.

10   Fagan SC, Edwards DJ, Borlongan CV, et al. Optimal delivery of minocycline to the brain: implication for human studies of acute neuroprotection. *Exp Neurol* 2004; **186**: 248–51.

11   Xu L, Fagan SC, Waller JL, et al. Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. *BMC Neurol* 2004; **4**: 7.

12   Power C, Henry S, Del Bigio MR, et al. Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. *Ann Neurol* 2003; **53**: 731–42.

13   Yong VW, Power C, Forsyth P, Edwards DR. Metalloproteinases in biology and pathology of the nervous system. *Nat Rev Neurosci* 2001; **2**: 502–11.

14   Stuve O, Dooley NP, Uhm JH, et al. Interferon beta-1b decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase-9. *Ann Neurol* 1996; **40**: 853–63.

15   Golub LM, Ramamurthy N, McNamara TF, et al. Tetracyclines inhibit tissue collagenase activity. A new mechanism in the treatment of periodontal disease. *J Periodontal Res* 1984; **19**: 651–55.

16   Paemen L, Martens E, Norga K, et al. The gelatinase inhibitory activity of tetracyclines and chemically modified tetracycline analogues as measured by a novel microtiter assay for inhibitors. *Biochem Pharmacol* 1996; **52**: 105–11.

17   Ryan ME, Usman A, Ramamurthy NS, Golub LM, Greenwald RA. Excessive matrix metalloproteinase activity in diabetes: inhibition by tetracycline analogues with zinc reactivity. *Curr Med Chem* 2001; **8**: 305–16.

18   Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* 2002; **125**: 1297–308.

19   Popovic N, Schubart A, Goetz BD, Zhang SC, Linington C, Duncan ID. Inhibition of autoimmune encephalomyelitis by a tetracycline. *Ann Neurol* 2002; **51**: 215–23.

20   Nessler S, Dodel R, Bittner A, et al. Effect of minocycline in experimental autoimmune ncephalomyelitis. *Ann Neurol* 2002; **52**: 689–90.

21   Wells J, Hurlbert J, Fehlings M, Yong VW. Neuroprotection by minocycline facilitates significant recovery from spinal cord injury. *Brain* 2003; **126**: 1628–37.

22   Lee SM, Yune TY, Kim SJ, et al. Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat. *J Neurotrauma* 2003; **20**: 1017–27.

23   Stirling DP, Khodarahmi K, Liu J, et al. Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *J Neurosci* 2004; **24**: 2182–90.

24   Teng YD, Choi H, Onario RC, et al. Minocycline inhibits contusion-triggered mitochondrial cytochrome c release and mitigates functional deficits after spinal cord injury. *Proc Natl Acad Sci USA* 2004; **101**: 3071–76.

25   Sanchez Mejia RO, Ona VO, Li M, Friedlander RM. Minocycline reduces traumatic brain injury-mediated caspase-1 activation, tissue damage, and neurological dysfunction. *Neurosurgery* 2001; **48**: 1393–99.

26   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 USA* 2001; **98**: 14669–74.

27   Wu DC, Jackson-Lewis V, Vila M, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* 2002; **22**: 1763–71.

28   He Y, Appel S, Le W. Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. *Brain Res* 2001; **909**: 187–93.

29   Tomas-Camardiel M, Rite I, Herrera AJ, et al. Minocycline reduces the lipopolysaccharide-induced inflammatory reaction, peroxynitrite-mediated nitration of proteins, disruption of the blood–brain barrier, and damage in the nigral dopaminergic system. *Neurobiol Dis* 2004; **16**: 190–201.

30   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**: 797–801.

31   Wang X, Zhu S, Drozda M, et al. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci USA* 2003; **100**: 10483–87.

32   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**: 186–96.

33   Hersch S, Fink K, Vonsattel JP, Friedlander RM. Minocycline is protective in a mouse model of Huntington's disease. *Ann Neurol* 2003; **54**: 841.

34   Hockly E, Beunard JL, Lowden PA, Bates GP. Minocycline is protective in a mouse model of Huntington's disease: author reply. *Ann Neurol* 2003; **54**: 842–43.

35   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**: 74–78.

36   Kriz J, Nguyen MD, Julien JP. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 2002; **10**: 268–78.

37   Van den Bosch L, Tilkin P, Lemmens G, Robberecht W. Minocycline delays disease onset and mortality in a transgenic model of ALS. *Neuroreport* 2002; **13**: 1067–70.

38   Tikka TM, Vartiainen NE, Goldsteins G, et al. Minocycline prevents neurotoxicity induced by cerebrospinal fluid from patients with motor neurone disease. *Brain* 2002; **125**: 722–31.

39   Yang L, Sugama S, Chirichigno JW, et al. Minocycline enhances MPTP toxicity to dopaminergic neurons. *J Neurosci Res* 2003; **74**: 278–85.

40   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**: 3266–76.

41   Tikka T, Fiebich BL, Goldsteins G, Keinanen R, Koistinaho J. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 2001; **21**: 2580–88.

42   Kim SS, Kong PJ, Kim BS, Sheen DH, Nam SY, Chun W. Inhibitory action of minocycline on lipopolysaccharide-induced release of nitric oxide and prostaglandin E2 in BV2 microglial cells. *Arch Pharm Res* 2004; **27**: 314–18.

43   Tikka TM, Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* 2001; **166**: 7527–33.

44   Zhang SC, Goetz BD, Duncan ID. Suppression of activated microglia promotes survival and function of transplanted oligodendroglial progenitors. *Glia* 2003; **41**: 191–98.

45   Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O. Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci USA* 2003; **100**: 13632–37.

46   Wang J, Wei Q, Wang CY, Hill WD, Hess DC, Dong Z. Minocycline up-regulates Bcl-2 and protects against cell death in mitochondria. *J Biol Chem* 2004; **279**: 19948–54.

47   Scarabelli TM, Stephanou A, Pasini E, et al. Minocycline inhibits caspase activation and reactivation, increases the ratio of XIAP to smac/DIABLO, and reduces the mitochondrial leakage of cytochrome C and smac/DIABLO. *J Am Coll Cardiol* 2004; **43**: 865–74.

48   Gabler WL, Smith J, Tsukuda N. Comparison of doxycycline and a chemically modified tetracycline inhibition of leukocyte functions. *Res Commun Chem Pathol Pharmacol* 1992; **78**: 151–60.

49   Amin AR, Attur MG, Thakker GD, et al. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. *Proc Natl Acad Sci USA* 1996; **93**: 14014–19.

50   Kloppenburg M, Verweij CL, Miltenburg AM, et al. The influence of tetracyclines on T cell activation. *Clin Exp Immunol* 1995; **102**: 635–41.

51   Kloppenburg M, Brinkman BM, de Rooij-Dijk HH, et al. The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. *Antimicrob Agents Chemother* 1996; **40**: 934–40.

52   Lin S, Zhang Y, Dodel R, Farlow MR, Paul SM, Du Y. Minocycline blocks nitric oxide-induced neurotoxicity by inhibition p38 MAP kinase in rat cerebellar granule neurons. *Neurosci Lett* 2001; **315**: 61–64.

53   Andes D, Craig WA. Animal model pharmacokinetics and pharmacodynamics: a critical review. *Int J Antimicrob Agents* 2002; **19**: 261–68.

54   Colovic M, Caccia S. Liquid chromatographic determination of minocycline in brain-to-plasma distribution studies in the rat. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003; **791**: 337–43.

55   Saivin S, Houin G. Clinical pharmacokinetics of doxycycline and minocycline. *Clin Pharmacokinet* 1988; **15**: 355–66.

56   Zhang W, Narayanan M, Friedlander RM. Additive neuroprotective effects of minocycline with creatine in a mouse model of ALS. *Ann Neurol* 2003; **53**: 267–70.

57   Kriz J, Gowing G, Julien JP. Efficient three-drug cocktail for disease induced by mutant superoxide dismutase. *Ann Neurol* 2003; **53**: 429–36.

58   Giuliani F, Metz L, Bar-Or A, Yong VW. Synergistic activity of minocycline and glatiramer acetate in an animal model of multiple sclerosis. *Mult Scler* 2004; **9** (suppl 1): S76.

59   Nelissen I, Martens E, Van den Steen PE, Proost P, Ronsse I, Opdenakker G. Gelatinase B/matrix metalloproteinase-9 cleaves interferon-beta and is a target for immunotherapy. *Brain* 2003; **126**: 1371–81.

60   Bonelli RM, Heuberger C, Reisecker F. Minocycline for Huntington's disease: an open label study. *Neurology* 2003; **60**: 883–84.

61   Thomas M, Ashizawa T, Jankovic J. Minocycline in Huntington's disease: a pilot study. *Mov Disord* 2004; **19**: 692–95.

62   Huntington Study Group. Minocycline safety and tolerability in Huntington disease. *Neurology* 2004; **63**: 547–53.

63   Gordon PH, Moore DH, Gelinas DF, et al. Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis. *Neurology* 2004; **62**: 1845–47.

64   Metz LM, Zhang Y, Yeung M, et al. Minocycline reduces gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. *Ann Neurol* 2004; **55**: 756.