Minocycline for Short-Term Neuroprotection

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Minocycline is a widely used tetracycline antibiotic. For decades, it has been used to treat various gram-positive and gram-negative infections. Minocycline was recently shown to have neuroprotective properties in animal models of acute neurologic injury. As a neuroprotective agent, the drug appears more effective than other treatment options. In addition to its high penetration of the blood-brain barrier, minocycline is a safe compound commonly used to treat chronic infections. Its several mechanisms of action in neuroprotection—antiinflammatory and antiapoptotic effects, and protease inhibition—make it a desirable candidate as therapy for acute neurologic injury, such as ischemic stroke. Minocycline is ready for clinical trials of acute neurologic injury.

Key Words: minocycline, stroke, neuroprotection.
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OUTLINE

Numerous studies have been conducted to develop an effective neuroprotective agent for acute brain injury, particularly ischemic stroke. To date, no pharmacologic agent has been effective in a context of short-term intervention. Agents such as calcium channel blockers and glutamate antagonists do not provide a benefit in acute brain injury for several reasons, including poor penetration of the blood-brain barrier, dose-limiting toxicity of the agent,[1–4] and a time window of effectiveness that is too short for clinical use.[3–6] After carefully reviewing studies of failed agents, we clearly found a need to investigate new approaches to acute brain injury.

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Background

Minocycline is a widely used semisynthetic tetracycline antibiotic[7] with known antiinflammatory, antiapoptotic, and glutamate-antagonist properties in several models of brain injury. The drug has been used for decades to treat infections caused by a variety of gram-negative and gram-positive organisms. Minocycline is indicated for the treatment of several diseases including acne vulgaris,[8] central nervous system and urinary tract infections, gonorrhea, meningitis, shigellosis, conjunctivitis, psittacosis, Q fever, relapsing fever, and syphilis. Minocycline is a generic drug, available in oral or intravenous formulations in humans, and subgingival sustained-release microspheres are used in adults with periodontitis.[9]

Like other tetracycline compounds, minocycline interferes with bacterial protein synthesis by binding to the 30S ribosomal subunit, inhibiting messenger RNA–transfer RNA interaction and

Table 1. Efficacy of Minocycline in Animal Models of Acute Neurologic Injury

Animal Model and Type of Injury	Minocycline Dosage	Therapeutic Window	Outcome
Rat focal ischemia, TMCAo (90 min)[14]	45 mg/kg i.p. b.i.d. on day 1, then 22 mg/kg i.p. b.i.d. for 2 days	Pretreatment: 2–4 hrs after insult:	76% infarct reduction (72 hrs) 63% reduction
Rat focal ischemia, embolic clot[15]	45 mg/kg i.p. b.i.d. on day 1, then 22.5 mg/kg i.p. b.i.d. on day 2	1 hr after insult	42% infarct reduction (48 hrs)
Mouse focal ischemia, PMCAo[16]	90 mg/kg i.p.	Pretreat 60 min before or 30 min after insult	Reduced infarction and brain swelling
Rat focal ischemia, TMCAo (90 min)[17]	3 and 10 mg/kg i.v.	4 hrs (3 mg/kg), 5 hrs (10 mg/kg)	40–50% infarct reduction (24 hrs)
Gerbil global ischemia[18]	45 mg/kg i.p. x 1, then 90 mg/kg i.p. b.i.d. on day 1, then 45 mg/kg after 36 hrs	Pretreatment: 30 min after insult:	Increased survival of CA1 neurons from 10% to 77% Increased survival to 71%
Rat neonatal hypoxia-ischemia[19, a]	45 or 22.5 mg/kg i.p.	Immediately before or after insult	Robust protection before and at 30 min, but not at 3 hrs
Rat spinal cord injury[20]	50 mg/kg i.p. b.i.d. for 2 days	30 min after insult	Improved function
Rat spinal cord injury[21]	90 mg/kg i.p. x 1, then 45 mg/kg i.p. for 5 days	1 hr after insult	Enhanced long-term hind-limb locomotion, coordinated motor function, and hind-limb reflex recovery
Mouse spinal cord injury[22, b]	50 mg/kg	1 hr after insult	Improved hind-limb function and strength, axonal sparing, superior to methylprednisolone
Mouse traumatic brain injury[23]	90 mg/kg i.p. x 1, then 45 mg/kg i.p. b.i.d. until sacrifice	Pretreatment or 30 min after insult	Improved function (Rotorod test), decreased lesion size
Rate intracerebral hemorrhage[24, c]	45 mg/kg i.p. b.i.d. on day 1, then 22.5 mg/kg i.p. x 1	1 hr after insult	Improved function

TMCAo = temporary occlusion of the middle cerebral artery; i.p. = intraperitoneally; PMCAo = permanent occlusion of the middle cerebral artery.
[a]Carotid occlusion plus hypoxia.
[b]Extradural compression with aneurysm clip.
[c]Collagenase.

protein translation.[10] In addition to the antibacterial properties, evidence supports antiinflammatory actions of tetracyclines.[11, 12] Because of its anticollagenase, immunosuppressive, and immunomodulating effects, minocycline hydrochloride has been used to manage rheumatoid arthritis.[13]

Neuroprotective Properties

Over the past 5 years, numerous reports have demonstrated the efficacy of minocycline in a variety of animal models of acute neurologic injury (Table 1).[14–24] The drug had a broad neuroprotective effect unrivaled by those of other agents. Minocycline was effective in animal models of global cerebral ischemia,[18, 19] focal cerebral ischemia,[14, 17, 25] traumatic brain injury,[23] spinal cord injury,[20–22] and intracerebral hemorrhage.[24] All of these injuries share common pathophysiologic mechanisms and the need for early (probably within 3–6 hrs of onset) interventions and treatment. Minocycline not only reduced tissue injury but also improved functional recovery.

Minocycline is likely to be more successful than other studied neuroprotective compounds in that it avoids the common pitfalls stated above. Minocycline has superior penetration of the blood-brain barrier,[26] and it was protective for longer than 3 hours in the studied animals.[14, 17, 24] In addition, because it is a safe compound, minocycline is particularly well suited for a clinical trial. The drug appears to be an ideal candidate as a therapy that will overcome the issues identified in neuroprotective trials of failed agents.

Mechanisms of Action as a Neuroprotective Agent

Antiinflammatory Effects

The antiinflammatory actions of tetracyclines

have been demonstrated in both acute and chronic brain injury. Minocycline has anti-inflammatory effects on neutrophils, monocytes, microglial cells, and neurons. It inhibits neutrophil-mediated tissue injury by inhibiting neutrophilic migration and degranulation and by suppressing the formation of oxygen radicals.[27] In a model of focal cerebral ischemia, minocycline inhibited enzymes that contribute to inflammation, such as the inducible form of nitric oxide synthase and interleukin-1β converting enzyme,[18] it suppressed apoptosis, and it reduced microglial activation.[14, 18] Minocycline inhibited nitric oxide release (likely by suppressing the expression of nitric oxide synthase) from monocytic cells induced by lipopolysaccharide or interferon-γ expression.[28, 29]

In an acute toxin model of Parkinson's disease, minocycline protected neurons (induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in an oxygen radical–based mechanism of injury), where inflammation prominently contributed to neuronal injury.[30] In this model, minocycline prevented microglial activation and expression of interleukin-1 and the inducible form of nitric oxide synthase.

Minocycline was also studied in a rat model of immune-inflammatory encephalitis in which microglia, monocytes, and T-cell activation mediated neuronal injury by means of several inflammatory mediators.[31] The drug delayed and reduced the progression of disease (including demyelination) as well as the infiltration of inflammatory cells.

At nanomolar concentrations, minocycline inhibited glutamate excitotoxic effects in mixed neuron–glial cell cultures in correlation with the inhibition of p38 phosphorylation and interleukin-1 release.[32] The drug protected rat neurons (cerebellar granules) from excitotoxic injury induced by reactive oxygen species and nitric oxide. Minocycline neuroprotection in vitro was associated with the inhibition of inflammatory signaling kinases, such as p38.[33] In a model of immune-inflammatory encephalitis, minocycline reduced the release of tumor necrosis factor from activated oligodendrocytes while enhancing the release of interleukin-10, an antiinflammatory cytokine.[31]

The antiinflammatory effects of minocycline have also been demonstrated in humans. At doses commonly used for indications other than neuroprotection, minocycline provided antiinflammatory benefits in rheumatoid arthritis that was not treated with other disease-modifying agents.[34, 35] In a small pilot clinical trial of multiple sclerosis, minocycline 200 mg/day reduced the number of gadolinium-enhancing lesions on magnetic resonance imaging,[36] demonstrating its ability to decrease the inflammatory damage associated with the disease.

In summary, compelling evidence from the last few years suggests that minocycline modulates inflammation and that this drug might be a novel therapeutic approach to diseases characterized by the stimulation of inflammatory cascades, such as acute ischemic brain injury.

Antiapoptotic Effects

Apoptosis, or program cell death, is thought to play a role in both acute and chronic brain injury. Minocycline prevented apoptosis and the release of cytochrome c from mitochondria in both in vitro and in vivo models. Minocycline delayed the progression of amyotrophic lateral sclerosis–like syndrome in superoxide dismutase-1 mutant mice and inhibited mitochondrial release of cytochrome c in vitro and in vivo.[37] The drug inhibited mitochondrial cell death, both caspase dependent (cytochrome c and Smac/Diablo release) and caspase independent (apoptosis inducing factor release), in a Huntington striatal-cell model.[38] Minocycline also protected the renal proximal tubule cells from apoptosis on exposure to azide, hypoxia, staurosporine, and cisplatinum.[39]

Furthermore, minocycline induced the upregulation of the antiapoptotic protein bcl-2 at the messenger-RNA level. In fact, the antiapoptotic effects of minocycline were lost when cells were pretreated with bcl-2 antisense; this finding suggested that the antiapoptotic action of minocycline depended on bcl-2. Moreover, bcl-2 was upregulated in neurons in vitro when they were incubated with equivalent doses of clinically therapeutic concentrations of minocycline.[40] In cardiomyocytes exposed to anoxia and reoxygenation, minocycline inhibited the release of cytochrome c and Smac/Diablo from mitochondria and inhibited both caspase activation and apoptosis.[41]

Inhibition of Matrix Metalloproteinases

Tetracyclines are known to inhibit matrix metalloproteinases.[42] Low-dose doxycycline, the first matrix-metalloproteinase inhibitor the United States Food and Drug Administration approved, is used in periodontal disease.[43]

In a rat model of adjuvant arthritis, doxycycline and tetracycline (two close analogs of minocycline) reduced joint swelling and inflammation and improved radiologic evidence of damage when they were given with a standard nonsteroidal antiinflammatory agent. In this model, the arthritic syndrome was associated with the suppression of matrix metalloproteinase-2 (gelatinase) activation in the inflamed joints.[44]

Minocycline also reduced levels of matrix metalloproteinase-9 in a model of immune-inflammatory encephalitis.[31] In a collagenase-induced model of intracerebral hemorrhage, minocycline reduced MMP-12 and improved functional outcomes.[24] In addition, minocycline reduced renal microvascular leakage in a rat model of ischemic renal injury. This action was probably due to diminishing the activity of matrix metalloproteinases.[45]

Matrix metalloproteinases are increasingly associated with diseases that involve degeneration of extracellular proteins and matrix in the brain.[46] For this reason, the inhibition of these proteases with minocycline seems to be an attractive experimental therapy.

Summary

Minocycline has several mechanisms of action, including antiinflammatory, matrix- metalloproteinase inhibitory, and antiapoptotic effects, that make it an attractive neuroprotective agent. It has demonstrated activity in many acute and chronic animal models of neurologic disease, and it is one of few agents that has been shown to be efficacious in animal models of spinal cord injury, traumatic brain injury, intracerebral hemorrhage, or global or focal cerebral ischemia.

These observations point to a key element that distinguishes minocycline from other neuroprotective agents, namely, the diversity of cellular mechanisms affected. Minocycline may likely act by means of vascular mechanisms as well. These mechanisms have been correlated with the intensity of the inflammatory response to injury and with the severity of damage to the brain parenchyma.[47] Finally, in humans, minocycline appears to exert antiinflammatory properties with the same dosage regimens as those clinically used for antibacterial treatment. Therefore, minocycline is a likely candidate as a drug for neuroprotection in acute brain injury in its present human formulation and dosage.

Pharmacokinetic Issues

In acute brain injury, the ability to rapidly deliver a potential neuroprotective agent to the systemic circulation is a necessity. In this setting, intravenous administration is most often required.

Because minocycline has been used for decades, its clinical pharmacokinetics are well described in humans. After a 200-mg intravenous dose, the mean peak concentration is 4.0 mg/L,[48] and steady-state concentrations after a dose of 100 mg given orally twice/day for 3 days are 1.4–1.8 mg/L.[26] Minocycline is the most lipophilic of the commonly used tetracycline antibiotics, and its concentration in the cerebrospinal fluid is 11–56% of plasma concentrations.[26] Therefore, concentrations in cerebrospinal fluid after long-term dosing are expected to be approximately 0.5 mg/L. In addition, urinary excretion is lower with minocycline than with other tetracyclines; therefore, minocycline is safer than the other tetracyclines for patients with renal insufficiency.

Since 1999, most reported studies of the neuroprotective effects of minocycline in rodent models of brain injury used large intraperitoneal doses of 10–90 mg/kg.[14, 16, 17, 19, 23–25, 39, 49, 50] Even in stroke models, in which timely cerebral neuroprotection is important, intraperitoneal administration was used.[14, 19, 24, 39]

Because the pharmacokinetics of large intraperitoneal doses of minocycline in rodents were unknown but necessary to extrapolate experimental results to humans, we studied this issue. We found that the intraperitoneal route resulted in widely variable serum concentrations of minocycline and that it delayed absorption in the systemic circulation, with peak concentrations achieved at a mean of 2.5 hours after injection. Compared with intravenous administration, intraperitoneal administration resulted in a bioavailability of 10–80%, which was probably due to the frank deposition of the drug in the peritoneal cavity.[51] The intraperitoneal route of administration probably accounts for the wide range of high doses reported in the literature. Intravenous dosing was needed to determine the true therapeutic window and the dose-response relationship in focal cerebral ischemia.[51] We determined that peak serum concentrations above 3.5 mg/L and trough concentration above 2 mg/L were neuroprotective in temporary focal cerebral ischemia in rats.[17] Low doses are being studied.

When intravenous administration was used to overcome the absorption problems of both oral and intraperitoneal administration, the volume of distribution of minocycline was similar in rats and in humans when adjusted by weight.[51] In other words, the intravenous administration of 3 mg/kg in humans and in rats is expected to achieve peak concentrations of the same magnitude (3–5 mg/L). The main difference in the pharmacokinetic parameters between the species is the half-life, which is approximately 17 hours in humans[26] and only 3 hours in rats.[51, 52]

In summary, intravenous doses of minocycline commonly used in humans should achieve serum and cerebrospinal fluid concentrations that were neuroprotective in animal models.

Adverse Effects

Most available information on the tolerability of minocycline was obtained after long-term oral administration. In studies of ambulatory patients taking minocycline long term, increasing the dosage above 100 mg twice/day was problematic because of the common adverse effect of dizziness (affecting 26–78% of patients).[8, 53] More recently, most adverse effects of oral minocycline therapy in patients with amyotrophic lateral sclerosis were gastrointestinal.[54] The mean tolerated dose was 387 mg/day, and all patients could tolerate at least 300 mg/day. No patient had dizziness, but elevated concentrations of blood urea nitrogen and liver enzymes were reported over the 6-month treatment period.

Doses of up to 400 mg given intravenously have safely been used to treat serious infections in humans. In a case series of 119 patients who received intravenous minocycline 200–400 mg for 2–24 days to treat an infectious disease, 21 (18%) had adverse effects, 50% of which were gastrointestinal.[55] Only one patient discontinued therapy prematurely. This patient developed azotemia, but a chronic urinary tract infection complicated its attribution.

In a search for adverse effects associated with intravenous minocycline that have been reported to the World Health Organization Collaborating Center for International Drug Monitoring (Uppsala, Sweden) since 1975, we found 122 case reports of adverse drug reactions. No assessment of causality was given, and the reports do not represent the opinion of the World Health Organization. The most common event was abnormal hepatic function (19 reports). Thrombocytopenia was reported 11 times, and injection-site reaction was reported once. The data of the World Health Organization were limited because no denominator could be ascertained and because the dosage and duration of intravenous minocycline treatment were unknown.

The dose of minocycline that is neuroprotective and tolerable in humans is still unknown. In addition, the feasibility of rapidly administering intravenous doses of minocycline to patients and the preliminary evidence of the activity of the compound should be determined before minocycline is further developed as a treatment for acute neuroprotection. The question of optimal duration should be addressed by assessing a biomarker of inflammation and by measuring serum levels of minocycline in the patient. In addition, further translational studies in animals will contribute to our understanding of the optimal duration of minocycline treatment for neuroprotection.

Conclusion

Minocycline is already in clinical trials for the chronic brain injury of amyotrophic lateral sclerosis and multiple sclerosis and has a strong potential for treating brain diseases that require acute intervention, such as stroke. Minocycline has long been established as a safe drug for clinical use, it has several mechanisms of action, and it had a delayed therapeutic window in experimental models. Minocycline is ready for clinical trials as a short-term neuroprotectant.

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