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Minocycline Development for Acute Ischemic Stroke

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Abstract

Minocycline, a tetracycline antibiotic, has shown anti-inflammatory, anti-apoptotic, and neuroprotective effects in many models of cerebral ischemia and neurodegenerative disease. Its high penetration of the blood–brain barrier, good safety profile, and delayed therapeutic window make it an ideal candidate for use in stroke. In animal models, minocycline reduced infarct size and improved neurologic outcome when administered acutely, with similar neuroprotective benefits seen following delayed administration. To date, two early phase clinical trials have shown minocycline to be safe and potentially effective in acute ischemic stroke, alone or in combination with tissue plasminogen activator. A large efficacy clinical trial is now needed to confirm previous studies, allow for subgroup analysis, and pinpoint the potential place for minocycline in acute stroke therapy.

Keywords

Minocycline; Drug development; Acute stroke; Vascular protection

Introduction

Despite the physical and financial burdens caused by stroke, intravenous tissue plasminogen activator (tPA) is the only United States Food and Drug Administration (FDA)-approved agent for acute ischemic stroke. This agent is frequently underutilized due to its limited therapeutic window, risk of intracerebral hemorrhage, and difficult administration methods. Only 2% of patients benefit from tPA administration, with an estimated 40% of ischemic stroke patients left to endure significant functional disability or death following stroke [1, 2]. Stroke researchers are now pursuing additional agents to combine with tPA administration

to enhance efficacy and improve outcomes associated with stroke. Agents with ease of administration and even modest efficacy in reducing tissue damage during ischemic stroke would drastically decrease the burden of stroke on society, improving patient outcomes and quality of life.

Minocycline

Minocycline is an anti-infective agent of the tetracycline family that has been used for decades for the treatment of infections caused by a wide range of Gram-negative and Gram-positive organisms. Of the tetracyclines, minocycline is distinguished by its lipophilicity, leading to superior blood–brain barrier penetration [3]. As evidence of the lack of serious toxicity of minocycline, it is commonly used chronically for non-life-threatening conditions such as acne vulgaris [4] and rheumatoid arthritis [5].

While minocycline was initially studied for its anti-inflammatory effects in central nervous system disorders [6, 7], research uncovered other beneficial effects in brain injury including anti-apoptotic and neuroprotective effects. Its anti-inflammatory effects are mediated through inhibition of inducible form of nitric oxide synthase and p38 mitogen-activated protein kinase [8, 9], reduction of glutamate toxicity [7, 10], and inhibition of microglial activation [10–12]. Its anti-apoptotic effects are produced through reduction of caspase-1 activation, downregulation of cytochrome c release from mitochondria [13], and inhibition poly(ADP-ribose) polymerase-1 at nanomolar concentrations [14]. Minocycline has also been shown to decrease matrix metalloproteinase (MMP) levels, especially the levels of MMP-9, which are elevated following ischemic stroke and tPA administration [15]. The evidence supporting the use of minocycline as an acute neuroprotectant as well as vasculoprotectant has been reviewed [16]. These actions of minocycline are thought to be responsible for the success of the compound in reducing the ultimate brain injury in models of Parkinson's disease [11, 12, 17], Huntington's disease [18], amyotrophic lateral sclerosis (ALS) [9], and ischemic events such as traumatic brain injury [13], focal cerebral ischemia [7, 19], and global cerebral ischemia [6, 20].

Preclinical Investigation

In the last decade, evidence continued to mount that minocycline demonstrates broad neuroprotective effects in many models of brain injury and in many different laboratories around the world. In a clot model of focal cerebral ischemia in the rat, minocycline was shown to reduce infarct size by more than 40% when administered in multiple, large intraperitoneal doses starting at 1 h after clot injection [21]. Similarly, minocycline started 1 h after traumatic spinal cord injury and continued for 5 days in a mouse model reduced mortality and lesion size and improved neurologic recovery over a 28-day period [22]. Even in a collagenase model of intracerebral hemorrhage, minocycline (starting at 1 h and continued for 2 weeks) improved functional outcome significantly at 7 days and continuing to 28 days [23]. Although no reduction in lesion size was shown in this model, reduction in MMPs in the area surrounding the hematoma was thought to be at least partly responsible for the improved outcome. After rat middle cerebral artery occlusion (MCAO) and reperfusion, Nagel and colleagues found that minocycline preserved the blood–brain barrier and was as effective as hypothermic treatment during ischemic conditions [24]. Further promise for human clinical trials of neuroprotection was revealed in models of ALS [25] and Huntington's disease [21]. The excellent performance of minocycline in these investigations led a multidisciplinary group to choose minocycline as one of the most promising neuroprotective compounds (out of more than 50 candidate agents) for clinical trials in Parkinson's disease [26]. While initially reducing neuroinflammation or neurodegeneration in Parkinson's disease, ALS, and Huntington's disease through mechanisms such as inhibition

of microglial activation, reduction in cytochrome c release from mitochondria, and decreased caspase and iNOS activity, respectively, chronic minocycline therapy in these diseases has proved disappointing [27]. In Parkinson's disease, although oral minocycline was non-futile, it was only mildly so, and not pursued further [28]. In ALS, oral minocycline up to 300 mg daily resulted in even worse outcomes at 1 year [29]. In Huntington's disease, early clinical trials have shown conflicting reports of the benefit of minocycline; however, long-term safety concerns have complicated its use [27]. While minocycline is initially beneficial in these neurodegenerative diseases, the disappointing results of long-term minocycline therapy suggest that other mechanisms of neurodegeneration may predominate in later stages of the diseases. However, this is less relevant to the use of minocycline in ischemic stroke due to its acute and relatively short-term administration.

Time Window

In cerebral ischemia, early research showed the effectiveness of minocycline in decreasing infarct size and inflammation when administered within 5 h of an ischemic event [30, 31]. In 2002, Xu et al. used the MCAO model in rats to evaluate the effectiveness of minocycline in reducing infarct size within certain therapeutic windows. Three-, 10-, and 20-mg/kg doses were evaluated, yielding plasma peak concentrations of 3.6, 13.0, and 28.8 mg/L, respectively. The 3-mg/kg dose, which yields similar plasma concentrations as the standard 200-mg oral dose in humans, reduced infarct size by 42% at 4 h and 34% at 5 h administration. The 10-mg/kg dose was similarly effective in reducing infarct size; however, neither dose yielded a significant reduction when administered at 6 h after the stroke occurrence. Similarly, the 3- and 10-mg/kg doses improved neurological functioning when given within the 4-h time window, but not within the 6-h time window.

Therefore, minocycline is likely to be more successful than other studied neuroprotective compounds in that it has been shown to also be protective at an extended time window in experimental animals [7, 20]. In the murine MCAO model, initiating minocycline treatment at 24 h after the ischemic insult (and continued for 14 days) inhibited atrophy of ischemic brain tissue while improving neurologic functioning and survival [32]. Continuous behavioral and neurologic recoveries were also seen when minocycline was administered 4 days following ischemic insult using the MCAO model and subsequently for 4 weeks [33, 34]. In this case, minocycline decreased activated microglia while preserving stroke-induced hippocampal neurogenesis and neurologic functioning [33]. Because microglia are involved in neurogenesis and neuroplasticity following ischemic insult, inhibition of microglial activation is unlikely to be the sole mechanism of minocycline's long-term neuroprotective effect. The inhibition of apoptosis and inhibition of p38 mitogen-activated protein kinase may also be linked to minocycline's delayed effect [33, 35]. Although the mechanistic pathways have not yet been elucidated, the potential acute and delayed benefit of minocycline in stroke make it an ideal candidate for administration before hospital arrival, during the acute stroke period, and throughout the recovery period.

Pharmacokinetics/Dose

Because minocycline has been used in clinical practice for years, its clinical pharmacokinetics are extensively described in humans. A 200-mg intravenous dose yields an average peak concentration of 4.0 mg/L with steady state concentrations reaching between 1.4–1.8 mg/L after twice daily dosing of 100 mg orally for 3 days [3]. Due to the highly lipophilic nature of minocycline, the CSF concentration after extended dosing is approximately 0.5 mg/L. This level is generally 11–56% of plasma concentrations achieved [16]. Because of minocycline's superior lipophilicity, it would be expected that CSF concentrations would reach levels above those shown to be neuroprotective in rats within

minutes of intravenous administration following ischemic stroke. One may also expect brain concentrations to be higher than CSF concentrations due the tendency of lipophilic compounds to distribute to lipid dense tissue.

It is assumed that the pharmacokinetics of minocycline and other tetracyclines are vastly different in experimental animals compared to humans as a way of explaining the large doses necessary for neuroprotection in rodents [17]. Since 1999, a majority of the published studies of minocycline's neuroprotective effects on cerebral ischemia have utilized intraperitoneal (IP) doses [7, 9, 11–13, 19–23, 25], ranging from a low of 10 mg/kg [9] to a high of 90 mg/kg [13] (Fig. 1). Intraperitoneal administration was even employed in stroke models where expedient medication administration and delivery are known to be important [7, 20, 21, 23]. Since the pharmacokinetics of large IP doses of minocycline in rodents were unknown and necessary in order to translate the experimental research to humans, we performed a study to accomplish this. We found that peak concentrations were achieved approximately 2.5 h after IP injection and yielded fluctuating serum concentrations, suggesting delayed absorption into the systemic circulation. Most likely, frank deposition of the drug in the peritoneal cavity led to an IP bioavailability of 10–80%. Because of the erratic absorption and distribution of IP dosing, a wide range of high doses were reported in the literature. Use of IV dosing was needed to determine a more accurate therapeutic window and dose–response relationship in focal cerebral ischemia. We have determined that peak serum concentrations above 3.5 mg/L are neuroprotective in focal cerebral ischemia in rats, and we may need to maintain trough concentrations above 2 mg/L to maintain the neuroprotective effect throughout the dosing interval [36]. The volume of distribution of minocycline is similar in rats and humans such that administration of 3 mg/kg IV to humans and rats achieves peak concentrations of 3–5 mg/L in both rats and humans. The two species do, however, differ in half-life, which is approximately 17 h in humans [3] and only 3 h in rats [37].

Tolerability

In ambulatory patients taking minocycline chronically, raising the dose beyond 100 mg twice daily has been problematic because of the common (10–78% of patients) side effect of dizziness [4, 38]. This has not been an issue with acute therapy [31]. More recently, all adverse effects of oral minocycline in ALS patients were found to be gastrointestinal in nature [29]. All patients tolerated at least 300 mg daily, with the average tolerated dose being 387 mg daily. No dizziness was experienced, but elevations in blood urea nitrogen and liver enzymes were reported over the 6-month treatment period. In a case series of IV minocycline in the treatment of infectious disease, a dose of 200–400 mg of IV minocycline was used for 2–24 days in 119 patients. Of the 199 patients, 21/119 (18%) experienced side effects, 50% of which were gastrointestinal in nature. Only one patient discontinued therapy prematurely; this was a patient who developed azotemia, and the attribution was complicated by a case of chronic urinary tract infections [39]. We also commissioned a search of all reported adverse effects with intravenous minocycline that had been reported to the World Health Organization (WHO) Collaborating Center for International Drug Monitoring, Uppsala, Sweden. One hundred and twenty-two case reports of adverse drug reactions were reported since 1975. However, no link of causality was provided, and the report does not represent the opinion of the WHO. Hepatic function abnormality was reported most commonly (19 reports), followed by thrombocytopenia (11 times). An injection site reaction was only reported in one case. The WHO data are limited by the fact that the dose and duration of IV minocycline are unknown and no denominator can be ascertained. It is helpful, however, in confirming the importance of monitoring hepatic function tests and complete blood cell counts in patients receiving minocycline.

Combination Studies with tPA

There is great interest in finding agents that can both extend the therapeutic time window of tPA and reduce the hemorrhage risk with tPA. Murata and colleagues reported that minocycline was effective when used in combination with tPA. Using a thromboembolic clot model in male spontaneously hypertensive rats, minocycline 3 mg/kg IV was effective alone at 4 h. When combined with delayed tPA at 6 h, minocycline reduced infarct size, ameliorated tPA-related intracerebral hemorrhage, and reduced plasma MMP-9 levels [40].

We also showed that minocycline was effective at reducing tPA-related hemorrhage and blood–brain barrier injury in a mechanical 3-h temporary occlusion model [41]. Minocycline, at a dose of 3 mg/kg given in combination with tPA at the time of reperfusion, reduced tPA-related hemorrhage, reduced brain MMP-9 levels, improved functional outcome, and reduced mortality [41]. MMP-2 and MMP-9 have been implicated in the degradation of basal lamina and an increased rate of hemorrhagic conversion after stroke [42–44]. Levels of these proteases are elevated after ischemic stroke, and are further increased by tPA administration [15, 45]. Through reduction of MMP levels, minocycline reduces the risk of hemorrhagic conversion increased by tPA administration following ischemic stroke and extends the therapeutic time window of tPA. Moreover, minocycline prevented the degradation of brain collagen IV and laminin levels and preserved vascular integrity [41].

Research has extended to non-tPA-based drug combination therapy. Weng and colleagues studied the effects of a minocycline-based multi-drug therapy following transient and permanent ischemia in mice [46]. The cocktail, which also included nimodipine, a voltage-gated calcium channel blocker, and riluzole, a glutamate antagonist, was more effective than monotherapy of any agent in reducing infarct size and improving functional outcome in transient and permanent ischemia. Minocycline alone showed benefit for both ischemic models, with riluzole being effective in transient ischemia only. While nimodipine showed no beneficial effect in this study as monotherapy, evidence does exist that L-type calcium channel blockers may synergistically increase the neuroprotective effect of minocycline [47], and this synergy may explain the increased efficacy of this combination. Interestingly, tissue analysis 72 h after ischemic insult revealed different biochemical changes and treatment responses in the transient and permanent ischemia groups, suggesting that different pathophysiologic mechanisms may predominate at different time points following stroke [46]. Because of various pathways implicated in ischemia injury, combination studies with minocycline may prove effective.

Clinical Trials

In 2007, Lampl and colleagues conducted an open-label, evaluator-blinded placebo-controlled clinical trial using oral minocycline as monotherapy in 152 acute ischemic stroke patients. An oral dose of 200 mg of minocycline was administered at various times within a 6–24-h therapeutic window and repeated daily for 5 days. Patients were then evaluated on days 7, 30, and 90 using the modified Rankin Scale, Barthel Index, and National Institutes of Health (NIH) stroke scale. The minocycline-treated group showed significant improvement over the placebo at every assessment time and with all monitoring endpoints. In minocycline vs. placebo-treated patients, the NIH stroke scale scores were 6.5 ± 3.8 vs. 8.1 ± 4.4 on day 7, 1.8 ± 2.1 vs. 7.1 ± 4.4 on day 30, and 1.6 ± 1.9 vs. 6.5 ± 3.8 on day 90, respectively (with $p < 0.0001$ at all time points) [35]. When used in patient surveillance following ischemic stroke, a change of two points in the NIH stroke scale may be identified as a clinically significant change in patient outcome [48, 49]. This NIH stroke scale data, along with the modified Rankin scores and Barthel index endpoints, support significant improvement in

patient status following minocycline therapy compared to placebo. The earliest benefit was detected on day 1 using the NIH stroke scale, with significant benefit detected on days 2 and 7 using the modified Rankin Scale and Barthel Index, respectively. The study also found no significant difference in minocycline response based on magnitude of focal deficits, location of ischemic onset, or medical cause, and attributed this effect to minocycline's multiple mechanisms of action. Although the results of this study were promising, other studies focusing on the optimal minocycline dose and the most effective administration window were needed [35].

IND

Dose-finding studies in a rat model of focal cerebral ischemia suggested that minocycline's neuroprotective effect increased at doses higher than the usual antimicrobial dose (3 mg/kg) [30]. In order to administer high intravenous doses to acute stroke patients in a clinical trial, it was necessary to submit an investigator-sponsored investigational new drug application (IND) to the United States Food and Drug Administration (FDA). This was complicated by the fact that the manufacturer of the sterile vials of minocycline hydrochloride for intravenous administration did not market the product in the United States at the time of the initiation of the exploratory trial. With the assistance of Wyeth, the manufacturers of the product for distribution in Japan, the investigators imported an adequate number of 100-mg vials to conduct the 60 patient exploratory trial (Minocycline to Improve Neurologic Outcome in Stroke (MINOS)). The investigators were given permission to reference the manufacturer's original IND for the purity and safety of the product. Unlike the Lampl trial described above, however, the investigators intended to enroll patients whether or not they had received intravenous tPA. The investigators were required to conduct additional ex vivo studies to ensure that minocycline, a known protease inhibitor, would not negate the ability of intravenous tPA to lyse clots. The need to design, execute, and interpret these additional studies [15] resulted in a 12-month FDA clinical hold for the MINOS trial. Minocycline, in concentrations up to 30 mcg/mL, did not reduce the extent or rate of fibrinolysis of tPA [15].

Exploratory Trial

In 2010, the MINOS trial found minocycline to be safe and well tolerated in intravenous doses up to 10 mg/kg alone and when administered with tPA. The patients received an intravenous loading dose of minocycline within a 6-h time window followed by maintenance dosing of 3, 4.5, 6, or 10 mg/kg divided in two equal doses for 3 days. Minocycline was well tolerated, with "mild and self-limiting" adverse effects and only one dose-limiting toxicity at the 10-mg/kg dose. No thrombophlebitis was observed, and infusion-related events were rare. While possibly significant in other studies, dizziness was not observed at any of the doses studied. Over 60% of the enrolled patients received tPA simultaneously, with no cases of severe intracerebral hemorrhage. Further analysis revealed linear kinetics with a half-life of 24 h. These results suggested that minocycline is a safe and feasible agent for stroke therapy, and a large efficacy trial is needed for further development [31].

Summary and Conclusion

The anti-inflammatory, anti-apoptotic, and neuroprotective effects of minocycline have improved the neurologic outcome of stroke in both animal and human models. This effect is seen in both acute and delayed minocycline and is hypothesized to stem from the multiple mechanisms of action of minocycline. While many of these mechanisms are identified, elucidation of the time course of their effects in relation to ischemic onset would help pinpoint minocycline's potential place in stroke therapy. Early clinical trials produced

significant improvements in neurologic tests such as the NIH stroke scale, the modified Rankin scale, and the Barthel index correlating with improvement in functional status and overall patient outcome. Due to the limited treatment options for ischemic stroke, an agent producing even modest improvements in outcome may substantially increase patient functional recovery and quality of life while decreasing public financial burden. While the results of both early phase human trials are promising, a large double-blinded, placebo-controlled trial is needed. A multinational trial with 1,500–2,000 patients receiving fixed doses of minocycline in combination with tPA is planned [31]. The minocycline dose will be administered within a 4.5-h time window, where preclinical neuroprotection was previously demonstrated [30]. Powering the study for subgroup analysis, specifically in relation to gender [50], is essential in identifying certain patient populations that would receive maximal benefit from minocycline.

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Fig. 1.
Key milestones in the development of minocycline as an acute treatment for ischemic stroke