REVIEW

Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review

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Abstract

To review the neuroprotective effects of minocycline in focal cerebral ischemia in animal models. By searching in the databases of PubMed, ScienceDirect, and Scopus, and considering the inclusion and exclusion criteria of the study. Studies were included if focal cerebral ischemia model was performed in mammals and including a control group that has been compared with a minocycline group. Written in languages other than English; duplicate data; *in vitro* studies and combination of minocycline with other neuroprotective agents were excluded. Neurological function of patients was assessed by National Institute of Health Stroke Scale, modified Rankin Scale, and modified Barthel Index. Neuroprotective effects were assessed by detecting the expression of inflammatory cytokines. We examined 35 papers concerning the protective effects of minocycline in focal cerebral ischemia in animal models and 6 clinical trials which had evaluated the neuroprotective effects of minocycline in ischemic stroke. These studies revealed that minocycline increases the viability of neurons and decreases the infarct volume following cerebral ischemia. The mechanisms that were reported in these studies included anti-inflammatory, antioxidant, as well as anti-apoptotic effects. Minocycline also increases the neuronal regeneration following cerebral ischemia. Minocycline has considerable neuroprotective effects against cerebral ischemia-induced neuronal damages. However, larger clinical trials may be required before using minocycline as a neuroprotective drug in ischemic stroke.

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Introduction

Stroke is one of the most common causes of mortality and disability. Ischemic stroke is the second cause of mortality and the third cause of disability worldwide (Jianrong et al., 2019). Cerebral ischemia occurs when blood flow to an area of brain is impaired, which eventually causes neuronal death in the ischemic region. Currently, the only pharmacotherapy in such strokes is the use of thrombolytic agents (Moussaddy et al., 2018). Nevertheless, thrombolytic agents cannot improve cognitive and motor dysfunction in patients with cerebral ischemia (Broome et al., 2016).

Various studies have indicated that inflammation, oxidative stress, and apoptosis are involved in the damage to neurons following cerebral ischemia (Shirley et al., 2014; Kawabori and Yenari, 2015; Radak et al., 2017). After thrombolytic therapy, reperfusion causes increased production of free oxygen and nitrogen radicals, which leads to neuronal apoptosis in the ischemic region (Pan et al., 2007; Sun et al., 2018). Minocycline is a broad-spectrum antibiotic belonging to the group of tetracyclines which has anti-inflammatory, antioxidant, and anti-apoptotic effects (Kelly et al., 2004; Abbaszadeh et al., 2018). Concerning the role of these factors in motor and cognitive disorders caused by cerebral ischemia, numerous studies have been performed about the neuroprotective effects of minocycline against the cerebral ischemia-induced injury in animal models. In addition, several clinical trials have been performed about the protective effects of minocycline against cerebral ischemia damages (Fagan et al., 2010; Switzer et al., 2011, 2012; Amiri-Nikpour et al., 2015).

Microglia account for 5–10 percent of all cells found within the brain. In neurodegenerative disorders including cerebral ischemia, microglia cells are activated in the brain (Hickman et al., 2018). This activation plays a significant role in damage to neurons following cerebral ischemia (Weinstein et al., 2010; Taylor and Sansing, 2013). Furthermore, following cerebral ischemia, the levels of inflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, IL-6, and IL-18 are elevated in the ischemic brain tissue, which can be associated with the activation of microglia (Lambertsen et al., 2012; Taylor and Sansing, 2013). Following cerebral ischemia, expression of cyclooxygenase (COX)-2 enzyme which is involved in inflammatory processes results intheproduction of free radicals and damage to neurons (Vidale et al., 2017). Furthermore, the matrix metalloproteinases (MMPs) produced in microglia is also involved in the damages caused by cerebral ischemia (Dong et al., 2009). Various studies have revealed the anti-inflammatory effects

of minocycline in the brain.

Minocycline is a potent inhibitor of microglial activation and this inhibitory effect causes diminished production of inflammatory cytokines including TNF-α and IL-1β by microglia (Garwood et al., 2010; Abraham et al., 2012). Furthermore, minocycline reduces the production of inflammatory mediators such as COX-2 and inducible nitric oxide synthase *via* its inhibitory effects on the activity of nuclear factor kappa B in microglia (Tikka and Koistinaho, 2001; Nikodemova et al., 2006). On the other hand, with reducing the activity and proliferation of T-cells, minocycline leads to a significant reduction in the production of inflammatory cytokines including TNF-α and interferon-γ (Giuliani et al., 2005; Szeto et al., 2011). The inhibitory effects of minocycline on the activity of inducible nitric oxide synthase, COX-2 and MMPs enzymes have an important role in reducing the neuronal injury caused by inflammatory processes after cerebral ischemia (Yrjänheikki et al., 1999; Koistinaho et al., 2005).

A few minutes following stroke, severe ischemia in the ischemic core zone can result in necrosis of neurons. However, the neurons within the penumbra, a rim of tissue surrounding the ischemic core are metabolically active, in spite of lacking any proper function. These neurons undergo apoptosis during several hours to several days following cerebral ischemia (Radak et al., 2017). Therefore, we can rescue these neurons by anti-apoptotic agents (Kelly et al., 2004; Radak et al., 2017). Following the cerebral ischemia, excitatory amino acids including glutamate are released to the extracellular space. Glutamate then binds to N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors and activates these ligand-dependent calcium channels that results in increased intracellular calcium (Lai et al., 2014). Meanwhile, the acidic conditions caused by cerebral ischemia lead to opening acid sensitive ion channels and entrance of calcium ion through these channels into the cell. Elevation of intracellular calcium concentration leads to release of pro-apoptoticagents including cytochrome C and Smac/DIABLO from the mitochondria to the cytoplasm, which by attaching to apoptotic protease activating factor 1 molecule causes formation of apoptosome containing apoptotic protease activating factor 1, adenosine triphosphate/deoxyadenosine triphosphate, cytochrome C, and inactive form of caspase-9 (Jin et al., 2001; Niizuma et al., 2010). Formation of apoptosome leads to the activation of caspase-9 and eventually caspase-3. By degrading poly(adenosine diphosphate-ribose) polymerase (PARP) protein, caspase-3 causes its deactivation and eventually DNA damage and apoptosis (Broughton et al., 2009; Niizuma et al., 2010). Previous studies have suggested that cerebral ischemia leads to activation of apoptosis by increasing the release of cytochrome C and enhancing the activity of caspases through the mitochondria dependent pathway (Niizuma et al., 2010). On the other hand, cerebral ischemia causes activation of neuronal apoptosis through a caspase-independent pathway. Following cerebral ischemia, adenosine triphosphate depletion from the ischemic cells leads to the release of pro-apoptotic proteins such as apoptosis inducing factor (AIF), endonuclease G, and bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 through the pores in the mitochondrial membrane to the cytoplasm (Cho and Toledo-Pereyra, 2008; Broughton et al., 2009). AIF causes DNA fragmentation and apoptosis after translocation from the mitochondria to the cell nucleus (Cho and Toledo-Pereyra, 2008). Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 is also involved in the apoptosis caused by cerebral ischemia through the impairment of mitochondrial function (Cho and Toledo-Pereyra, 2008; Broughton et al., 2009). Following cerebral ischemia, DNA damage causes the activation of p-53 tumor inhibitor transcription factor in response to oxidative stress that leads to the activation of caspases and pro-apoptotic proteins including p53 upregulated modulator of apoptosis, Bax, Bak and NOX-A (Niizuma et al., 2009). Minocycline causes the inhibition of apoptosis through both caspase-dependent and caspase-independent pathways (Sancho et al., 2011). Previous studies have demonstrated that minocycline reduces the expression of caspase-3 and inhibits its activation mediated by apoptotic protease activating factor 1 (Sancho et al., 2011). Furthermore, minocycline increases the expression of anti-apoptotic proteins (Bcl-2, Bcl-XL), and decreases the expression of pro-apoptotic (Bax, Bak, Bid, and Fas), and P53 proteins (Wang et al., 2004; Sancho et al., 2011; Chen et al., 2012). Minocycline also reduces the release of cytochrome c and SMAC from the mitochondria (Wang et al., 2004; Chen et al., 2012). Moreover, by reducing the activity of extracellular signal-regulated kinases 1/2 activity, minocycline leads to the inhibition of P38-dependent apoptosis (Corsaro et al., 2009). In caspase-independent pathway, minocycline inhibits DNA fragmentation caused by AIF by inhibiting the translocation of AIF to the cell nucleus and the activity of PARP-1 (Heo et al., 2006; Wu et al., 2015). Studies have suggested that the inhibition of PARP-1 may be involved in the protective and anti-inflammatory effects of minocycline (Klöfers et al., 2017).

Overproduction of free oxygen radicals during cerebral ischemia leads to oxidative stress in the ischemic region of the brain (Li and Yang, 2016). The most important free radicals involved in the damage caused by cerebral ischemia include superoxide anion (O_2^-), hydroxyl radical (OH·), and hydrogen peroxide (H_2O_2) (Nita et al., 2001; Li and Yang, 2016). Under physiological conditions, antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase play important roles in the protection against the brain injury induced by free radicals (Nita et al., 2001). Following ischemia/reperfusion, overproduction of free radicals causes failure of the antioxidant defense system and therefore oxidative stress (Nita et al., 2001; Li and Yang, 2016). Through DNA damage, lipid peroxidation, and changing the structure and function of proteins, oxidative stress causes injury and death of neurons in the ischemic region (Muralikrishna Adibhatla and Hatcher, 2006). Minocycline has radical scavenging properties because of the presence of phenolic ring in its structure (Dai et al., 2017) (**Figure 1**). Previous studies suggested that minocycline causes quenching of free

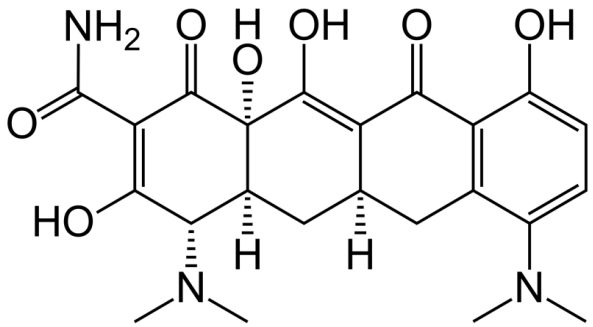

Figure 1 Minocycline structure.

oxygen radicals including superoxide anion, hydroxyl radical, and hydrogen peroxide under oxidative stress conditions (Dai et al., 2017). Studies have also revealed that minocycline decreases the level of lipid peroxidation caused by cerebral ischemia, where this reduction is associated with enhanced activity of antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase (Sonmez et al., 2013; Ortega-Arellano et al., 2017). Therefore, in this study we performed a systematic review of the neuroprotective effects of minocycline against deficits induced by experimental model of focal cerebral ischemia. We also systematically reviewed all available clinical trials that evaluated the neuroprotective effects of minocycline in acute ischemic stroke.

Data and Methods

The studies conducted on neuroprotective effects of minocycline on focal cerebral ischemia injury in animal models were identified from databases of PubMed, Science Direct, and Scopus. The method of searching the papers was based on the following terms and words: "minocycline" AND "cerebral ischemia" OR "focal cerebral ischemia" OR "cerebral ischemia reperfusion" OR "cerebral I/R" OR "transient cerebral ischemia" OR "cerebral ischemic attack" OR "middle cerebral artery occlusion" OR "MCAO" OR "bilateral occlusion of common carotid arteries" OR "stroke" OR "transient ischemic attack".

The inclusion criteria parameters were:
1. The studies were performed in mammals.
2. Focal cerebral ischemia models including middle cerebral artery occlusion, bilateral common carotid arteries occlusion, endothelin-1 induced striatal ischemia, cortical photothrombic vascular occlusion were used.
3. Minocycline should not have been used with another agent with a potential of neuroprotection
4. A control group was included in the experimental design.
5. The experiments were performed *in vivo*.
The exclusion criteria:
1. Non-focal cerebral ischemia models including were used.
2. Minocycline was combined with other drugs or therapeutic methods.
3. Duplicate data.
4. Papers were written in languages other than English.
5. Experiments were conducted *in vitro*.

Results

Data extraction

Paper search was performed based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (**Figure 2**). In total, 755 articles were identified. Relevant studies were selected based on the inclusion criteria of the study. Finally, 35 papers in animal studies and 6 clinical trials were selected and approved for systematic review (**Figure 2**). The oldest and newest papers were related to 1999 and 2019, respectively. Overall, from among all of the papers, 10 (30%) has been published over the past three years (**Table 1**).

Study characteristics

The neuroprotective effects of various doses minocycline in experimental models of focal cerebral ischemia were summarized in **Table 1**. We also listed studies that reported anti-inflammatory, antioxidant, anti-apoptotic, and neurogenesis effects of minocycline in animal models of focal cerebral ischemia in **Table 2**.

The animal models used in cerebral ischemia

In these studies, four animal models including middle cerebral artery occlusion, bilateral common carotid arteries occlusion, endothelin-1 induced striatal ischemia, cortical pho-

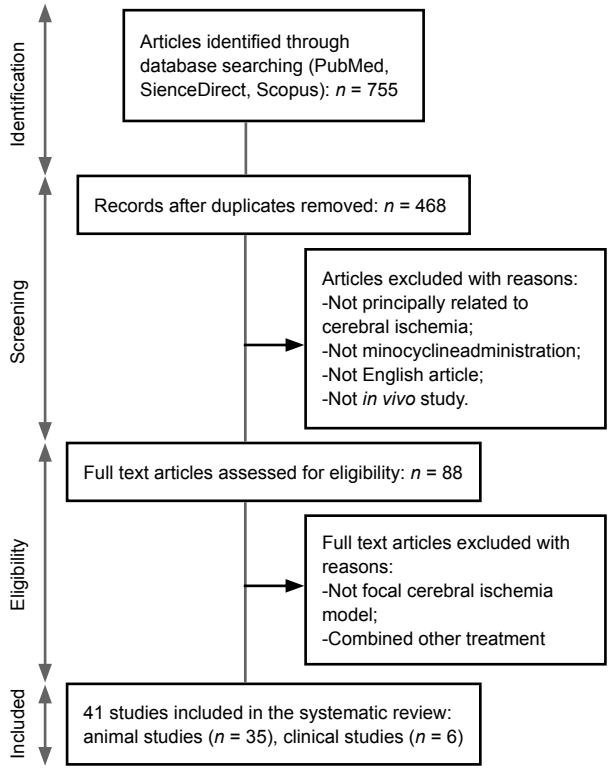

Figure 2 Flowchart of study selection process.

Table 1 Summary of the neuroprotective effects of minocycline in focal cerebral ischemia

Study	Route and dosage	Species (sex)	Model	Treatment time	Neuroprotective effects
Yew et al. (2019)	90 mg/kg (1 h after ischemia), 50 mg/kg (12, 24, 36 and 48 hours after ischemia) i.p.	Male wistar rat	Cortical photothrombic vascular occlusion (permanent focal ischemia)	1, 12, 24, 36 and 48 hours after ischemia	1.Improved the rate of motor function recovery; 2. Decreased number of activated phagocytic cells (macrophage and microglia); 3. Increased number of activated astrocytes.
Tanaka et al. (2018)	100 mg/kg i.p.	Male ICR mice	pMCAO	Single dose, 60 minutes before reperfusion	1.Reduced infarct size; 2. Inhibition of microglial activation
Naderi et al. (2017a)	40 mg/kg i.p.	Male Wistar rat	tBCCAO (20 minutes)	Immediately before I/R	1. Improved learning and memory; 2. Enhanced neuronal viability; 3. Reduction of lipid peroxidation; 4. Decreased IL-1β and TNF- α levels; 5. Inhibition of microglial activation.
Naderi et al. (2017b)	40 mg/kg i.p.	Male Wistar rat	tBCCAO (20 minutes)	Once daily for 7 days after I/R	1. Improved learning and memory; 2. Enhanced neuronal viability; 3. Reduction; of lipid peroxidation 4. Decreased IL-1β and TNF-α levels; 5. Inhibition of microglial activation.
Lu et al. (2016)	25, 50 mg/kg i.p.	Male C57BL/6 mice	tMCAO (60 minutes)	Once daily for 3 days after reperfusion (first dose: 60 minutes after reperfusion)	1. Decreased infarct volume (25, 50 mg/kg); 2. Improvement of neurological deficits (25, 50 mg/kg); 3. decreased brain edema (25, 50 mg/kg); 4. Decreased IL-1β and IL-18 levels (25, 50 mg/kg); 5. Inhibition of microglial activation; 6. Attenuation of NLRP3 inflammasome signaling.
Jin et al. (2015)	90 mg/kg i.p.	Male C57BL/6 mice	tMCAO (2 hours)	12 hours before or after ischemia, then twice daily until sacrificed	1. Decreased infarct size; 2. Improvement of neurological function; 3. Decreased brain edema; 4. Decreased expression of inflammatory cytokines (IL-1β, TNF-α, IL-6, MCP-1); 5. Increased expression of MCPIP-1 in the cerebral cortex; 6. Inhibition of NF-κB-signaling pathway.
Park et al. (2015)	45 mg/kg i.v.	Male Spague-Dawley rat	tMCAO (30 minutes)	Single dose, 30 minutes before ischemia	1. Improved neurobehavioural function; 2. Decreased infarct volume; 3. Enhanced neuronal survival; 4. Reduction of apoptosis; 5. Decreased the number of activated astrocytes.
Soliman et al. (2015)	20 mg/kg i.v.	Male Wistar rat	tMCAO (90 minutes)	Single dose 0 hour after reperfusion	1. Decreased infarct volume; 2. Improved Neurobehavioural and motor functions.
Yang et al. (2015)	3 mg/kg i.v.	Male hypertensive rat	tMCAO (90 minutes)	Single dose, onset of reperfusion	1. Decreased infarct volume; 2. Decreased brain edema; 3. Improvement of cerebral blood flow; 4. Reduction of BBB permeability; 5. Increased tight junction proteins (zonula occluden-1, occludin, clandin-5) in ischemic cortex; 6. Inhibition of microglial activation; 7. Decreased expression of proinflammatory cytokines (IL-1β and TNF-α); 8. Increased expression of antinflammatory cytokines (IL-10 and TGF-β); 9. Decreased activity of MMP-2 and MMP-9.
Zhao et al. (2015)	50 mg/kg p.o.	Female Wistar rat	pBCCAO	Daily for 4 weeks; first dose: 4 days after ischemia	1. Improved learning and memory; 2. Increased expression of BDNF, CREB and pCREB; 3. Enhanced neuronal plasticity.
Hoda et al. (2014)	6 mg/kg i.v.	Male C57BL/6 mice	Thromboembolic MCAO (permanent focal ischemia)	Single bolus at 60 minutes after reperfusion	1. Decreased infarct volume; 2. Improved cerebral blood flow.
Aras et al. (2013)	90 mg/kg p.o.	Male Wistar rat	tBCCAO (20 minutes)	48, 24, 1 hours before ischemia	1. Improved neuronal morphology; 2. Reduced neuronal degeneration; 3. Reduced lipid peroxidation; 4. Inhibition of NO production; 5. Reduced CK levels in blood.
Cardoso et al. (2013)	50 mg/kg i.p.	Male Wistar rat	Endothelin induced striatal ischemia	Twice a day, during the first 2 days after ischemia	1. Inhibition of microglial activation; 2. Improved sensorimotor deficits; 3. Enhanced neuronal survival.
Tao et al. (2013)	3 mg/kg i.v.	Male Sprague-Dawley rat	tMCAO (2 hours)	Twice a day for 14 days; first dose: immediately after reperfusion)	1. Reduction of BBB permeability; 2. Decreased expression of repulsive guidance molecule A; 3. Enhanced axonal regeneration; 4. Improved Sensorimotor deficits.
Zheng et al. (2013)	22.5 mg/kg (first dose: 45 mg/kg) i.p.	Male Sprague-Dawley rats	tBCCAO (2 hours)	Immediately after I/R (first dose), then every 12 hours until sacrificed	1. Improved learning and memory; 2. Decreased infarct volume; 3. Enhanced neuronal viability; 4. Inhibition of apoptosis; 5. Decreased expression of caspase-3 and PARP-1.
Hoda et al. (2011)	6 mg/kg i.v	Male and female C57BL/6 mice	Thromboembolic MCAO (permanent focal ischemia)	Single bolus at 60 minutes after reperfusion	1. Decreased infarct volume in male and female mice; 2. Improved neurological deficits; 3. Reduced mortality at 24 hours post stroke for aged mice; 4. Decreased expression of MMP-9 in male and female mice.
Park et al. (2011)	45 mg/kg s.c.	Male C57BL/6 mice	Cortical photothrombic vascular occlusion (permanent focal ischemia)	30 minutes before ischemia and 2 hours after ischemia	1. Decreased infarct volume; 2. Decreased expression of TNF-α; 3. Decreased expression of MCP-1 and IDO.

Table 1 Continued

Study	Route and dosage	Species (sex)	Model	Treatment time	Neuroprotective effects
Cai et al. (2010)	50 mg/kg p.o.	Female Wistar rat	pBCCAO	Once a day for 4, 8, and 16 weeks	1. Decreased expression of COX-2 and NF-κB; 2. Decreased IL-1β and TNF-α levels.
Chu et al. (2010)	45 mg/kg i.p.	Male Sprague-Dawley rat	tMCAO (90 minutes)	2 and 12 hours after ischemia, then every 12 hours for 5 days	1. Improved motor and neurobehavioural functions; 2. Decreased infarct volume; 3. Enhanced neuronal viability; 4. Decreased expression of 5-LPO in microglia and astrocytes.
Martín et al. (2011)	10 mg/kg i.v.	Male Sprague-Dawley rat	tMCAO (2 hours)	Once a day for 6 days (first dose: 1 hour after ischemia)	Decreased expression of PBR/TSPO
Kim et al. (2009)	45 mg/kg (first dose: 90 mg/kg) i.p.	Male Sprague-Dawley rat	tMCAO (2 hours)	90 mg/kg on reperfusion and 45 mg/kg daily until sacrifice	1. Decreased the number of PMNL cells; 2. Reduction of myeloperoxidase activity; 3. Inhibition of microglial activation; 4. Promotion of neurogenesis.
Matsukawa et al. (2009)	20 mg/kg i.v.	Male Spague-Dawley rat	tMCAO (30 minutes)	Single bolus at 60 minutes after reperfusion	1. Improved neurological deficits; 2. Decreased infarct volume; 3. Increased expression of Bcl-2; 4. Reduction of apoptosis; 5. Enhanced neuronal survival.
Cai et al. (2008a)	50 mg/kg p.o.	Female Wistar rat	tMCAO (60 minutes)	12 or 24 hours after reperfusion	1. Decreased infarct volume; 2. Increased expression of VEGF; 3. Decreased expression of IL-1β and TNF-α.
Cai et al. (2008b)	50 mg/kg p.o.	Female Wistar rat	pBCCAO	Once a day, for 4, 8, or 16 weeks	1. Improved learning and memory; 2. Decreased expression of iNOS; 3. Increased expression of eNOS.
Hayakawa et al. (2008)	10 mg/kg i.p.	Male ddY mice	tMCAO (4 hours)	Once a day for 14 days	1. Improved neurological deficits; 2. Enhanced neuronal viability; 3. Inhibition of microglial activation; 4. Inhibition of apoptosis; 5. Decreased expression of HMGB in striatum and plasma; 6. Improved striatal atrophy.
Chu et al. (2007)	22.5, 45 mg/kg i.p.	Male Sprague-Dawley rat	tMCAO (30 minutes)	30 minutes and 2 hours after reperfusion on the first day an twice daily on the second and third day	1. Improved neurological deficits; 2. Decreased infarct volume; 3. Reduced production of IgG; 4. Decreased expression of 5-LPO; 5. Reduced production of leukoterienes (cysLT and LTB4).
Liu et al. (2007)	50 mg/kg per day for 7 days and 25 mg/kg per day for 21 days i.p.	Male Sprague-Dawley rat	tMCAO (60 minutes)	50 mg/kg once daily (beginning 4 days after reperfusion) for 1 weeks, followed by 25 mg/kg daily for 3 weeks	1. Promotion of neurogenesis in the hippocampus; 2. Inhibition of microglial activation; 3. Improved motor function; 4. Improved learning and memory.
Tang et al. (2007)	45 mg/kg per day i.p.	Male CB6/F1 mice	tMCAO (45 minutes)	45 mg/kg, 30 minutes and 12 hours after reperfusion, then 22.5 mg/kg twice a day for up to 7 days	1. Decreased infarct volume; 2. Improved sensorimotor functions; 3. Inhibition of apoptosis; 4. Inhibition of microglial activation; 5. Decreased uptake of Annexin-V in ischemic neurons.
Cai et al. (2006)	45 mg/kg i.p.	Male Sprague-Dawley rat	tBCCAO (30 minutes)	12 hours before and immediately after the reperfusion and then every 24 hours for 3 days	1. Enhanced neuronal viability; 2. Inhibition of apoptosis; 3. Inhibition of microglial activation; 4. Decreased expression of IL-1β and iNOS; 5. Reduced oxidative and nitrosative stress; 6. Protection from oligodendrocytes against ischemia.
Cho et al. (2006)	First dose: 45 mg/kg, 22.5 mg/kg twice a day for 7 days i.p.	Male Wistar rat	pBCCAO	45 mg/kg immediately after reperfusion, 22.5 mg/kg twice a day from 1–7 days, 22.5 mg/kg once a day from days 8–14	1. Decreased neuronal injury in the white matter (optic tract, internal capsule and corpus callosum); 2. Reduced axonal damage in the ischemic region; 3. Protection from myelin basic protein against damage caused by ischemia; 4. Inhibition of microglial activation; 5. Decreased expression of MMP-2 in the activated microglia.
Machado et al. (2006)	45 mg/kg i.p.	Male Wistar rat	tMCAO (3 hours)	Twice a day (first dose immediately after the onset of reperfusion)	Inhibition of MMPs (MMP-2 and MMP-9) activities
Koistinaho et al. (2005)	First dose: 45 mg/kg; second dose: 60 mg/kg; then 45 mg/kg per day i.p.	Male mice	pMCAO	12 hours before or 2 hours after reperfusion: 45 mg/kg; 24 hours after reperfusion: 60 mg/kg; then 45 mg/kg per day until killed	1. Decreased infarct volume; 2. Decreased expression of MMP-9.
Morimoto et al. (2005)	90 mg/kg i.p.	Male ddY mice	pMCAO	60 minutes before/30 minutes after or 4 hours after reperfusion	1. Decreased infarct volume; 2. Decreased brain edema; 3. Improved neurological deficits.
Xu et al. (2004)	3, 10 mg/kg i.v.	Male Sprague-Dawley rat	tMCAO (90 minutes)	4, 5 or 6 hours after reperfusion	1. Decreased infarct size; 2. Improved neurological deficits.
Yrjänheikki et al. (1999)	45 mg/kg per day i.p.	Male Sprague-Dawley rat	tMCAO (90 minutes)	45 mg/kg twice a day for the first day; 22.5 mg/kg for the subsequent 2 days (first dose: 12 hours before or 2 hours after reperfusion)	1. Decreased infarct volume (post-treatment and pre-treatment); 2. Inhibition of microglial activation (post-treatment and pre-treatment); 3. Decreased expression of IL-1β in microglia (pre-treatment); 4. Decreased PGE2 concentration in the penumbra region (pre-treatment).

5-LPO: 5-Lipooxygenase; BBB: blood-brain barrier; BDNF: brain-derived neurotrophic factor; CK: creatine kinase; COX-2: cyclooxygenase-2; CPVO: cortical photothrombic vascular occlusion; CREB: cyclic-adenosine monophosphate response element binding; cysLT: cysteinyl leukotrienes; eNOS: endothelial nitric oxide synthase; HMGB: high mobility group box 1; i.p.: intraperitoneal injection; i.v.: intravenous injection; I/R: ischemia/reperfusion; IDO: indoleamine 2,3-dioxygenase; IgG: immunoglobulin G; IL-10: interleukin-10; IL-18: interleukin-18; IL-1β: interleukin-1β; IL-6: interleukin-6; iNOS: inducible nitric oxide synthase; LTB4: leukotriene B4; MCP-1: monocyte chemoattractant protein-1; MCPIP1: monocyte chemotactic protein-induced protein 1; MMP: matrix methalloproteinase; NF-κB: nuclear factor kappa B; NLRP3: NACHT, LRR and PYD domains-containing protein 3; NO: nitric oxide; p.o.: oral administration; PARP-1: poly(adenosine diphosphate-ribose) polymerase 1; pBCCAO: permanent bilateral common carotid arteries occlusion; PBR/TPSO: peripheral benzodiazepine receptor/18-kDa translocator protein; pCREB: phosphorylated CREB; PGE2: prostaglandin E2; pMCAO: permanent middle cerebral artery occlusion; PMNL: polymorphonuclear leukocytes; s.c.: subcutaneous injection; tBCCAO: transient bilateral common carotid arteries occlusion; TGF-β: transforming growth factor-β; tMCAO: transient middle cerebral artery occlusion; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

Table 2 The mechanism of the neuroprotective effects of minocycline

Study	Mechanism of minocycline neuroprotection
Yrjänheikki et al., 1999; Koistinaho et al., 2005; Cai et al., 2006, 2008a, 2010; Cho et al., 2006; Machado et al., 2006; Chu et al., 2007, 2010; Liu et al., 2007; Tang et al., 2007; Hayakawa et al., 2008; Kim et al., 2009; Martín et al., 2011; Park et al., 2011; Cardoso et al., 2013; Jin et al., 2015; Yang et al., 2015; Lu et al., 2016; Naderi et al., 2017a, b; Tanaka et al., 2018; Yew et al., 2019	Anti-inflammation
Cai et al., 2006, 2008b; Aras et al., 2013; Naderi et al., 2017a, b	Anti-oxidant
Tang et al., 2007; Hayakawa et al., 2008; Matsukawa et al., 2009; Zheng et al., 2013; Park et al., 2015	Anti-apoptotic
Liu et al., 2007; Kim et al., 2009; Tao et al., 2013; Zhao et al., 2015	Neurogenesis

tothrombic vascular occlusion have been used for induction of focal cerebral ischemia in 23, 9, 1, 2 and 2 papers, respectively (**Table 1**). In the studies that used the middle cerebral artery occlusion model, middle carotid artery was occluded temporarily (tMCAO) in 18 papers and permanently (pMCAO) in 5 papers. In addition, among the studies that used the bilateral common carotid arteries occlusion model, the occlusion of common carotid arteries were temporary (tBCCAO) in 5 papers and permanent (pBCCAO) in 4 papers. Furthermore, endothelin-1 induced striatal ischemia and cortical photothrombic vascular occlusion led to transient ischemia in the striatum and permanent ischemia in the cortex, respectively (**Table 1**). A total of 25 studies used rats and 10 studies used mice as experimental subjects (**Table 1**). The duration of middle carotid artery occlusion in tMCAO was 30, 45, 60, 90 minutes, 2, 3, and 4 hours in 3, 1, 3, 5, 4, 1, and 1 paper(s), respectively (**Table 1**). In addition, the duration of common carotid arteries occlusion in the tBCCAO model was 30 and 20 minutes as well as 2 hours in 1, 3, and 1 paper(s), respectively (**Table 1**).

Drug administration

In the papers which have been reviewed, minocycline was administered by the intraperitoneal, intravenous, oral, and subcutaneous routes, in 20, 9, 5, and 1 paper(s), respectively. The dose of minocycline for intraperitoneal administration varied between 10 and 100 mg/kg. However, most studies were performed within the dose range of 40–50 mg/kg. In addition, in 7 articles, minocycline was administered intravenously within the dose range of 3–35 mg/kg. For oral administration, the doses of minocycline were 50 and 90 mg/kg in 4 and 1 paper(s), respectively. Furthermore, in one paper, minocycline was administered subcutaneously at 45 gm/kg. The duration and method of minocycline administration in the papers are summarized in **Table 1**.

The neuroprotective effects of minocycline on focal cerebral ischemia

All of 35 papers that studied the neuroprotective effects of minocycline in animal models of focal cerebral ischemia are presented in **Table 1**. Major neuroprotective effects of minocycline include anti-inflammatory, antioxidant, anti-apoptotic, and neurogenesis effects. As mentioned in **Table 2**, minocycline had anti-inflammatory, antioxidant, anti-apoptotic, and neurogenesis effects in 24, 5, 5, and 4 studies, respectively. In four papers, no mechanism was presented for neuroprotective effects of minocycline as it only decreased infarct volume and improved motor and neurobehavioral function of the animals (Xu et al., 2004; Morimoto et al., 2005; Hoda et al., 2014; Soliman et al., 2015). Severe cerebral ischemia leads to neuronal injury and death in the ischemic region. A large number of studies suggest that minocycline protect neurons against the damage caused by cerebral ischemia. Treatment with minocycline increases the viability of neurons and decreases neurodegeneration caused by cerebral ischemia, and these effects of minocycline lead to significant reduction of the infarct volume and cerebral edema following the cerebral ischemia/reperfusion (**Table 1**). Furthermore, treatment with minocycline improves the neurological deficits and neurobehavioral dysfunction caused by neuronal injury after cerebral ischemia (**Table 1**). Additionally, it significantly improves memory and learning dysfunction caused by cerebral ischemia (**Table 1**).

There were 24 studies that reported anti-inflammatory effects of minocycline in animal models of focal cerebral ischemia (**Table 2**). Fourteen papers reported that minocycline had inhibitory effects on microglial activation induced by cerebral ischemia (**Table 1**). Furthermore, various studies revealed that minocycline decreases production of leukotrienes (one paper), immunoglobulin G (one paper) and inflammatory cytokines including IL-1β (six papers), TNF-α (five papers), IL-18 (one paper) and IL-6 (one paper). However, it increases the production of anti-inflammatory cytokines including IL-10 and transforming growth factor-β (one paper). Furthermore, minocycline decreases the expression of COX-2 (one paper), lipooxygenase (2 papers) and inflammatory mediators including monocyte chemoattractant protein-1 (two papers), indoleamine 2,3-dioxygenase (one paper) and peripheral benzodiazepine receptor/18-kDa translocator protein (one paper). Minocycline also showed neuroprotective effects through a decrease in the activity of MMP-2 and MMP-9 enzymes (six prepares) and the inhibition of NACHT, LRR and PYD domains-containing protein inflammasome signaling (one paper).

Five studies demonstrated that antioxidant properties play an important role in the neuroprotective effects of minocycline against ischemia/reperfusion-induced neuronal damage (**Table 2**). Malondialdehyde, a product of lipid peroxidation, is commonly known as a marker of oxidative damage. In these studies, minocycline decreased lipid peroxidation (three papers), inducible nitric oxide synthase expression (one paper) and nitric oxide production (one paper) caused

by cerebral ischemia (**Table 1**).

In five studies, minocycline had anti-apoptosis effects and decreased the neuronal apoptosis caused by cerebral ischemia (**Table 2**). Zheng et al. (2013) reported that minocycline decreases the number of terminal deoxynucleotidyl transferase dUTP nick end labeling-positive cells and expression of caspase-3 and PARP-1 (**Table 1**). Minocycline also increased expression of Bcl-2 as an anti-apoptotic protein in the ischemic region (Matsukawa et al., 2009). Moreover, Tang et al. (2007) reported that minocycline reduces Annexin-V uptake of ischemic neurons, which represents a reduction in apoptosis induced by cerebral ischemia (**Table 1**).

In 4 studies, minocycline increased neurogenesis following cerebral ischemia (**Table 2**). Zhao et al. (2015) reported that minocycline increases the expression of cyclic-adenosine monophosphate response element binding, phosphorylated cyclic-adenosine monophosphate response element binding, and brain-derived neurotrophic factor, that leads to structural reorganization in ischemic region. Furthermore, minocycline reduces repulsive guidance molecule A expression which causes axonal regeneration in the neurons of the ischemic region (Tao et al., 2013). In addition to these studies, Liu et al. (2017) and Kim et al. (2009) showed that minocycline administration enhances the neurogenesis in the ischemic region of the brain (**Table 1**).

In 15 studies, minocycline improved motor dysfunction and neurological deficits due to focal cerebral ischemia. Furthermore, 9 papers reported that minocycline attenuates cognitive impairments and neurobehavioral dysfunctions caused by cerebral ischemia (**Table 1**).

Clinical studies

In addition to the studies in animal models, clinical trials which had evaluated the neuroprotective effects of minocycline in ischemic stroke were also reviewed individually (**Table 3**). Fagan et al. (2010) performed an open-label, dose-escalation study to investigate the pharmacokinetic properties and the side-effects of minocycline in patients with acute ischemic stroke in Georgia Medical College at Kentucky University, USA. In this study, minocycline was infused for 1 hour at doses of 3, 4.5, 6, and 10 mg/kg, once daily for 3 consecutive days. The patients underwent clinical examination for 90 days. The results of this study showed that minocycline was well tolerated at the studied doses with only one dose limiting toxicity at 10 mg/kg dose. The results also showed that minocycline is well tolerated in combination with tissue plasminogen activators (tPA) and no severe hemorrhage occurred in tPA-treated patients. Furthermore, pharmacokinetic studies on minocycline showed that it has a half-life of 24 hours and can be administered once daily (Fagan et al., 2010). As found in animal studies, minocycline has anti-inflammatory effects and reduces the production of inflammatory cytokines caused by cerebral ischemia in experimental models. Concerning the effects of minocycline on MMP-9 and IL-6 in clinical studies, 2 papers were published in 2011 and 2012. In these studies conducted as non-randomized dose escalation clinical trial in the University of Kentucky by Switzer et al. (2011, 2012), minocycline was infused within six hours after the onset of ischemic stroke at the doses of 3, 4.5, 6, and 10 mg/kg during 1 hrs, and its administration was repeated every 12 hours for 3 days. The results demonstrated that minocycline significantly reduces the levels of MMP-9 and IL-6 at 24 hours after ischemic stroke (Switzer et al., 2011, 2012). In another clinical trial, Padma Sirvastara et al. (2012) performed a single blinded open-label study in India Institute of Medical Sciences in New Delhi, India to investigate the neuroprotective effects of minocycline in 50 patients with ischemic stroke. In this study, the patients were categorized into two groups: one received minocycline at 200 mg/day orally for five days and the other received placebo. Clinical assessments for evaluating the neurological deficits were performed using National Institute of Health Stroke Scale, modified Barthel Index, and modified Rankin Scale. The results demonstrated that oral administration of minocycline for five days improves the neurological function in 30 and 90 days after the onset of stroke (Padma Srivastava et al., 2012). Also, an open-label evaluator blinded clinical study was performed by Ami-

Table 3 Summary of minocycline clinical trials in acute ischemic stroke

Study	Dosage and route	Results
Amiri-Nikpouret al. (2015)	200 mg/d orally for 5 days	Improved the neurological function
Kohler et al. (2013)	100 mg/day intravenously, every 12 hours up to five doses	1. Minocycline is well tolerated in patients with ischemic stroke; 2. Minocycline does not cause any significant improvement in neurological function.
Padma Sirvastara et al. (2012)	200 mg/d orally for 5 days	Improved the neurological function in 30 and 90 days after the onset of stroke
Switzer et al. (2012)	Infusion for 1 hour at doses of 3, 4.5, 6, and 10 mg/kg, repeated every 12 hours for 3 consecutive days	Minocycline significantly reduces the levels of IL-6, 24 hours after ischemic stroke
Switzer et al. (2011)	Infusion for 1 hour at doses of 3, 4.5, 6, and 10 mg/kg, repeated every 12 hours for 3 consecutive days	Minocycline significantly reduces the levels of MMP-9, 24 hours after ischemic stroke
Fagan et al. (2010)	Infusion for 1 h at doses of 3, 4.5, 6, and 10 mg/kg, once daily for 3 consecutive days	1. Minocycline was well tolerated: only one dose limiting toxicity at 10 mg/kg dose; 2. Minocyclineis well tolerated in combination with tPA: no severe hemorrhage occurred; 3. Minocycline has a half-life of 24 hours.

IL-6: Interleukin-6; MMP-9: matrix metalloproteinase-9; tPA: tissue plasminogen activators.

ri-Nikpour et al. (2015) from January to December 2012 on 60 patients with ischemic stroke at Urmia University of Medical Sciences in Iran. In the study, the neurological function of patients was assessed by National Institute of Health Stroke Scale score and the results showed that patients with acute ischemic stroke who received oral minocycline 200 mg/day for 5 days manifested better neurological function than patients who received placebo (Amiri-Nikpour et al., 2015). Therefore, these studies demonstrated that minocycline improves neurological function following cerebral ischemia. But in a study conducted by Kohler et al. (2013), different results were reported. This study was a randomized multicenter open-label blinded endpoint evaluation pilot study and was performed in 3 hospitals in Perth, eastern Australia. A total of 95 patients with ischemic stroke were studied and minocycline was administered at the dose of 100 mg/day intravenously, every 12 hours up to five doses. The first dose was administered 24 hours following the ischemic stroke. Neurological function of patients was assessed by National Institute of Health Stroke Scale, modified Rankin Scale, and modified Barthel Index. The results suggested that although minocycline is well tolerated in patients with ischemic stroke, it does not cause any significant improvement in clinical signs of these patients. Nevertheless, it was noted that this study was performed on a few patients with ischemic stroke, and more extensive clinical trials should be performed to accurately investigate the neuroprotective effects of minocycline in ischemic stroke (Kohler et al., 2013).

Discussion and Conclusions

In the present study, we conducted a systematic review of all available animal studies and clinical trials to evaluate the neuroprotective effects of minocycline on cerebral ischemia. The results of this study revealed that minocycline can be used to improve neurological outcomes and preventing neuronal damages caused by cerebral ischemia through its anti-inflammatory, anti-oxidant and anti-apoptotic properties.

The results of these studies showed that minocycline reduces the infract volume and enhances neuronal survival through its protective effects against neuronal injury caused by cerebral ischemia (Park et al., 2015). This review shows that the anti-inflammatory effects of minocycline play an important role in its neuroprotective effects. Minocycline is a strong inhibitor of microglial activation (Naderi et al., 2017a, b). After cerebral ischemia, minocycline inhibits the activation of microglia in the ischemic region. It also decreases the production of pro-inflammatory cytokines such as IL-1β and TNF-α and inhibits the activity of enzymes involved in the inflammatory processes such as MMPs and COX (Yang et al., 2015; Naderi et al., 2017a, b). Inhibitory effect of minocycline on microglial activation is accompanied by a promotion of remyelination and an increased survival of oligodendrocytes (Defaux et al., 2011). Cai et al. (2006) reported that minocycline increases survival of oligodendrocytes and promotes neuronal viability and remyelination after cerebral ischemia in rat.

The results of this review suggest that anti-inflammatory mechanisms of minocycline may be involved in its neuroprotective effects against damage caused by ischemia (**Table 1**). The previous studies have also shown that minocycline has anti-oxidant effects and causes scavenging of oxygen free radicals due to the presence of phenolic ring in its structure (Dai et al., 2017). A number of studies suggest that the reduction of lipid peroxidation and the enhancement of the activity of anti-oxidant enzymes such as glutathione peroxidase and superoxide dismutase have an important role in the neuroprotective effect of minocycline (Yrjänheikki et al., 1999; Yang et al., 2015; Zhao et al., 2015; Naderi et al., 2017a, b). Various studies have indicated that neuronal apoptosis plays an important role in brain damage following cerebral ischemia (Radak et al., 2017). On the other hand, minocycline inhibits the apoptosis through Caspase-dependent and independent pathways (Wang et al., 2004; Heo et al., 2006; Sancho et al., 2011; Klöfers et al., 2017). The studied articles showed that minocycline exhibits neuroprotective effects on ischemia/reperfusion by inhibiting apoptosis (Cai et al., 2008a; Park et al., 2015; Yang et al., 2015).

Some studies have also demonstrated that minocycline increases the neurogenesis and accelerates the recovery after cerebral ischemia (Liu et al., 2007; Kim et al., 2009; Tao et al., 2013; Zhao et al., 2015). The results of this review can help us recognize this drug as a neuroprotective agent for the improvement of neurological dysfunction following stroke. Thrombolytic agents such as tPA are currently used in the treatment of acute ischemic stroke (Piccardi et al., 2018). However, reperfusion following thrombolytic therapy may exacerbate the brain injury caused by cerebral ischemia (Piccardi et al., 2018). Therefore, administration of neuroprotectants may lead to the reduction of neuronal injury induced by reperfusion. The clinical trials presented in this review show that minocycline is well tolerated in patients with ischemic stroke, when administered concurrently with tPA (Fagan et al., 2010). Moreover, these studies revealed that treatment with minocycline significantly improves neurological outcome in acute ischemic patients (Padma Srivastava et al., 2012; Kohler et al., 2013; Amiri-Nikpour et al., 2015). Moreover, in 2 clinical trials, treatment with minocycline has reduced the plasma levels of IL-6 and the activity of MMP-9 within 24 hours after ischemic stroke (Switzer et al., 2011, 2012). Therefore, it seems that the anti-inflammatory effects of minocycline have an important role in the improvement of neurological dysfunction caused by cerebral ischemia. According to the results of this systematic review, which indicates the effectiveness of minocycline in reducing neuronal injury and neurological deficits caused by cerebral ischemia, it can be used in the clinical treatment of acute ischemic stroke as a neuroprotectant in the future. However, larger clinical trials are needed to accurately assess the effects of this drug in stroke.

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References

Abbaszadeh A, Darabi S, Hasanvand A, Amini-Khoei H, Abbasnezhad A, Choghakhori R, Aaliehpour A (2018) Minocycline through attenuation of oxidative stress and inflammatory response reduces the neuropathic pain in a rat model of chronic constriction injury. Iran J Basic Med Sci 21:138-144.

Abraham J, Fox PD, Condello C, Bartolini A, Koh S (2012) Minocycline attenuates microglia activation and blocks the long-term epileptogenic effects of early-life seizures. Neurobiol Dis 46:425-430.

Amiri-Nikpour MR, Nazarbaghi S, Hamdi-Holasou M, Rezaei Y (2015) An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect. Acta Neurol Scand 131:45-50.

Aras M, Urfali B, Serarslan Y, Ozgur T, Ulutas KT, Urfali S, Altas M, Yilmaz N (2013) Protective effects of minocycline against short-term ischemia-reperfusion injury in rat brain. Pediatr Neurosurg 49:172-178.

Broome LJ, Battle CE, Lawrence M, Evans PA, Dennis MS (2016) Cognitive outcomes following thrombolysis in acute ischemic stroke: a systematic review. J Stroke Cerebrovasc Dis 25:2868-2875.

Broughton BR, Reutens DC, Sobey CG (2009) Apoptotic mechanisms after cerebral ischemia. Stroke 40:e331-339.

Cai Z, Lin S, Fan LW, Pang Y, Rhodes PG (2006) Minocycline alleviates hypoxic-ischemic injury to developing oligodendrocytes in the neonatal rat brain. Neuroscience 137:425-435.

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

Cai ZY, Yan Y, Yu CY, Zhang J (2008a) Minocycline inhibits neuroinflammation and enhances vascular endothelial growth factor expression in a cerebral ischemia/reperfusion rat model. Neural Regen Res 3:1088-1094.

Cai ZY, Yan Y, Sun SQ, Zhang J, Huang LG, Yan N, Wu F, Li JY (2008b) Minocycline attenuates cognitive impairment and restrains oxidative stress in the hippocampus of rats with chronic cerebral hypoperfusion. Neurosci Bull 24:305-313.

Cardoso MM, Franco EC, de Souza CC, da Silva MC, Gouveia A, Gomes-Leal W (2013) Minocycline treatment and bone marrow mononuclear cell transplantation after endothelin-1 induced striatal ischemia. Inflammation 36:197-205.

Chen SD, Yin JH, Hwang CS, Tang CM, Yang DI (2012) Anti-apoptotic and anti-oxidative mechanisms of minocycline against sphingomyelinase/ceramide neurotoxicity: implication in Alzheimer's disease and cerebral ischemia. Free Radic Res 46:940-950.

Cho BB, Toledo-Pereyra LH (2008) Caspase-independent programmed cell death following ischemic stroke. J Invest Surg 21:141-147.

Cho KO, La HO, Cho YJ, Sung KW, Kim SY (2006) Minocycline attenuates white matter damage in a rat model of chronic cerebral hypoperfusion. J Neurosci Res 83:285-291.

Chu LS, Fang SH, Zhou Y, Yu GL, Wang ML, Zhang WP, Wei EQ (2007) Minocycline inhibits 5-lipoxygenase activation and brain inflammation after focal cerebral ischemia in rats. Acta Pharmacol Sin 28:763-772.

Chu LS, Fang SH, Zhou Y, Yin YJ, Chen WY, Li JH, Sun J, Wang ML, Zhang WP, Wei EQ (2010) Minocycline inhibits 5-lipoxygenase expression and accelerates functional recovery in chronic phase of focal cerebral ischemia in rats. Life Sci 86:170-177.

Corsaro A, Thellung S, Chiovitti K, Villa V, Simi A, Raggi F, Paludi D, Russo C, Aceto A, Florio T (2009) Dual modulation of ERK1/2 and p38 MAP kinase activities induced by minocycline reverses the neurotoxic effects of the prion protein fragment 90-231. Neurotox Res 15:138-154.

Dai C, Ciccotosto GD, Cappai R, Wang Y, Tang S, Xiao X, Velkov T (2017) Minocycline attenuates colistin-induced neurotoxicity via suppression of apoptosis, mitochondrial dysfunction and oxidative stress. J Antimicrob Chemother 72:1635-1645.

Defaux A, Zurich MG, Honegger P, Monnet-Tschudi F (2011) Minocycline promotes remyelination in aggregating rat brain cell cultures after interferon-gamma plus lipopolysaccharide-induced demyelination. Neuroscience 187:84-92.

Dong X, Song YN, Liu WG, Guo XL (2009) Mmp-9, a potential target for cerebral ischemic treatment. Curr Neuropharmacol 7:269-275.

Fagan SC, Waller JL, Nichols FT, Edwards DJ, Pettigrew LC, Clark WM, Hall CE, Switzer JA, Ergul A, Hess DC (2010) Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. Stroke 41:2283-2287.

Garwood CJ, Cooper JD, Hanger DP, Noble W (2010) Anti-inflammatory impact of minocycline in a mouse model of tauopathy. Front Psychiatry 1:136.

Giuliani F, Hader W, Yong VW (2005) Minocycline attenuates T cell and microglia activity to impair cytokine production in T cell-microglia interaction. J Leukoc Biol 78:135-143.

Hayakawa K, Mishima K, Nozako M, Hazekawa M, Mishima S, Fujioka M, Orito K, Egashira N, Iwasaki K, Fujiwara M (2008) Delayed treatment with minocycline ameliorates neurologic impairment through activated microglia expressing a high-mobility group box1-inhibiting mechanism. Stroke 39:951-958.

Heo K, Cho YJ, Cho KJ, Kim HW, Kim HJ, Shin HY, Lee BI, Kim GW (2006) Minocycline inhibits caspase-dependent and -independent cell death pathways and is neuroprotective against hippocampal damage after treatment with kainic acid in mice. Neurosci Lett 398:195-200.

Hickman S, Izzy S, Sen P, Morsett L, El Khoury J (2018) Microglia in neurodegeneration. Nat Neurosci 21:1359-1369.

Hoda MN, Fagan SC, Khan MB, Vaibhav K, Chaudhary A, Wang P, Dhandapani KM, Waller JL, Hess DC (2014) A 2 x 2 factorial design for the combination therapy of minocycline and remote ischemic perconditioning: efficacy in a preclinical trial in murine thromboembolic stroke model. Exp Transl Stroke Med 6:10.

Hoda MN, Li W, Ahmad A, Ogbi S, Zemskova MA, Johnson MH, Ergul A, Hill WD, Hess DC, Sazonova IY (2011) Sex-independent neuroprotection with minocycline after experimental thromboembolic stroke. Exp Transl Stroke Med 3:16.

Jianrong S, Yanjun Z, Chen Y, Jianwen X (2019) DUSP14 rescues cerebral ischemia/reperfusion (IR) injury by reducing inflammation and apoptosis via the activation of Nrf-2. Biochem Biophys Res Commun 509:713-721.

Jin K, Graham SH, Mao X, Nagayama T, Simon RP, Greenberg DA (2001) Fas (CD95) may mediate delayed cell death in hippocampal CA1 sector after global cerebral ischemia. J Cereb Blood Flow Metab 21:1411-1421.

Jin Z, Liang J, Wang J, Kolattukudy PE (2015) MCP-induced protein 1 mediates the minocycline-induced neuroprotection against cerebral ischemia/reperfusion injury in vitro and in vivo. J Neuroinflammation 12:39.

Kawabori M, Yenari MA (2015) Inflammatory responses in brain ischemia. Curr Med Chem 22:1258-1277.

Kelly KJ, Sutton TA, Weathered N, Ray N, Caldwell EJ, Plotkin Z, Dagher PC (2004) Minocycline inhibits apoptosis and inflammation in a rat model of ischemic renal injury. Am J Physiol Renal Physiol 287:F760-766.

Kim BJ, Kim MJ, Park JM, Lee SH, Kim YJ, Ryu S, Kim YH, Yoon BW (2009) Reduced neurogenesis after suppressed inflammation by minocycline in transient cerebral ischemia in rat. J Neurol Sci 279:70-75.

Klöfers M, Kohaut J, Bendix I, Herz J, Boos V, Felderhoff-Müser U, Dzietko M (2017) Effects of Poly(ADP-Ribose) Polymerase-1 Inhibition in a Neonatal Rodent Model of Hypoxic-Ischemic Injury. Biomed Res Int 2017:2924848.

Kohler E, Prentice DA, Bates TR, Hankey GJ, Claxton A, van Heerden J, Blacker D (2013) Intravenous minocycline in acute stroke: a randomized, controlled pilot study and meta-analysis. Stroke 44:2493-2499.

Koistinaho M, Malm TM, Kettunen MI, Goldsteins G, Starckx S, Kauppinen RA, Opdenakker G, Koistinaho J (2005) Minocycline protects against permanent cerebral ischemia in wild type but not in matrix metalloprotease-9-deficient mice. J Cereb Blood Flow Metab 25:460-467.

Lai TW, Zhang S, Wang YT (2014) Excitotoxicity and stroke: identifying novel targets for neuroprotection. Prog Neurobiol 115:157-188.

Lambertsen KL, Biber K, Finsen B (2012) Inflammatory cytokines in experimental and human stroke. J Cereb Blood Flow Metab 32:1677-1698.

Li W, Yang S (2016) Targeting oxidative stress for the treatment of ischemic stroke: Upstream and downstream therapeutic strategies. Brain Circ 2:153-163.

Liu Z, Fan Y, Won SJ, Neumann M, Hu D, Zhou L, Weinstein PR, Liu J (2007) Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. Stroke 38:146-152.

Lu Y, Xiao G, Luo W (2016) Minocycline suppresses NLRP3 inflammasome activation in experimental ischemic stroke. Neuroimmunomodulation 23:230-238.

Machado LS, Kozak A, Ergul A, Hess DC, Borlongan CV, Fagan SC (2006) Delayed minocycline inhibits ischemia-activated matrix metalloproteinases 2 and 9 after experimental stroke. BMC Neurosci 7:56.

Martín A, Boisgard R, Kassiou M, Dollé F, Tavitian B (2011) Reduced PBR/TSPO expression after minocycline treatment in a rat model of focal cerebral ischemia: a PET study using [(18)F]DPA-714. Mol Imaging Biol 13:10-15.

Matsukawa N, Yasuhara T, Hara K, Xu L, Maki M, Yu G, Kaneko Y, Ojika K, Hess DC, Borlongan CV (2009) Therapeutic targets and limits of minocycline neuroprotection in experimental ischemic stroke. BMC Neurosci 10:126.

Morimoto N, Shimazawa M, Yamashima T, Nagai H, Hara H (2005) Minocycline inhibits oxidative stress and decreases in vitro and in vivo ischemic neuronal damage. Brain Res 1044:8-15.

Moussaddy A, Demchuk AM, Hill MD (2018) Thrombolytic therapies for ischemic stroke: Triumphs and future challenges. Neuropharmacology 134:272-279.

Muralikrishna Adibhatla R, Hatcher JF (2006) Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. Free Radic Biol Med 40:376-387.

Naderi Y, Sabetkasaei M, Parvardeh S, Moini Zanjani T (2017a) Neuroprotective effects of pretreatment with minocycline on memory impairment following cerebral ischemia in rats. Behav Pharmacol 28:214-222.

Naderi Y, Sabetkasaei M, Parvardeh S, Zanjani TM (2017b) Neuroprotective effect of minocycline on cognitive impairments induced by transient cerebral ischemia/reperfusion through its anti-inflammatory and anti-oxidant properties in male rat. Brain Res Bull 131:207-213.

Niizuma K, Endo H, Nito C, Myer DJ, Chan PH (2009) Potential role of PUMA in delayed death of hippocampal CA1 neurons after transient global cerebral ischemia. Stroke 40:618-625.

Niizuma K, Yoshioka H, Chen H, Kim GS, Jung JE, Katsu M, Okami N, Chan PH (2010) Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. Biochim Biophys Acta 1802:92-99.

Nikodemova M, Duncan ID, Watters JJ (2006) Minocycline exerts inhibitory effects on multiple mitogen-activated protein kinases and Ikappa-Balpha degradation in a stimulus-specific manner in microglia. J Neurochem 96:314-323.

Nita DA, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L (2001) Oxidative damage following cerebral ischemia depends on reperfusion - a biochemical study in rat. J Cell Mol Med 5:163-170.

Ortega-Arellano HF, Jimenez-Del-Rio M, Velez-Pardo C (2017) Minocycline protects, rescues and prevents knockdown transgenic parkin Drosophila against paraquat/iron toxicity: Implications for autosomic recessive juvenile parkinsonism. Neurotoxicology 60:42-53.

Padma Srivastava MV, Bhasin A, Bhatia R, Garg A, Gaikwad S, Prasad K, Singh MB, Tripathi M (2012) Efficacy of minocycline in acute ischemic stroke: a single-blinded, placebo-controlled trial. Neurol India 60:23-28.

Pan J, Konstas AA, Bateman B, Ortolano GA, Pile-Spellman J (2007) Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. Neuroradiology 49:93-102.

Park CH, Shin TK, Lee HY, Kim SJ, Lee WS (2011) Matrix metalloproteinase inhibitors attenuate neuroinflammation following focal cerebral ischemia in mice. Korean J Physiol Pharmacol 15:115-122.

Park SI, Park SK, Jang KS, Han YM, Kim CH, Oh SJ (2015) Preischemic neuroprotective effect of minocycline and sodium ozagrel on transient cerebral ischemic rat model. Brain Res 1599:85-92.

Piccardi B, Arba F, Nesi M, Palumbo V, Nencini P, Giusti B, Sereni A, Gadda D, Moretti M, Fainardi E, Mangiafico S, Pracucci G, Nannoni S, Galmozzi F, Fanelli A, Pezzati P, Vanni S, Grifoni S, Sarti C, Lamassa M, et al. (2018) Reperfusion Injury after ischemic Stroke Study (RISKS): single-centre (Florence, Italy), prospective observational protocol study. BMJ Open 8:e021183.

Radak D, Katsiki N, Resanovic I, Jovanovic A, Sudar-Milovanovic E, Zafirovic S, Mousad SA, Isenovic ER (2017) Apoptosis and acute brain ischemia in ischemic stroke. Curr Vasc Pharmacol 15:115-122.

Sancho M, Herrera AE, Gortat A, Carbajo RJ, Pineda-Lucena A, Orzáez M, Pérez-Payá E (2011) Minocycline inhibits cell death and decreases mutant Huntingtin aggregation by targeting Apaf-1. Hum Mol Genet 20:3545-3553.

Shirley R, Ord EN, Work LM (2014) Oxidative stress and the use of antioxidants in stroke. Antioxidants (Basel) 3:472-501.

Soliman S, Ishrat T, Fouda AY, Patel A, Pillai B, Fagan SC (2015) Sequential therapy with minocycline and candesartan improves long-term recovery after experimental stroke. Transl Stroke Res 6:309-322.

Sonmez E, Kabatas S, Ozen O, Karabay G, Turkoglu S, Ogus E, Yilmaz C, Caner H, Altinors N (2013) Minocycline treatment inhibits lipid peroxidation, preserves spinal cord ultrastructure, and improves functional outcome after traumatic spinal cord injury in the rat. Spine (Phila Pa 1976) 38:1253-1259.

Sun MS, Jin H, Sun X, Huang S, Zhang FL, Guo ZN, Yang Y (2018) Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization therapy. Oxid Med Cell Longev 2018:3804979.

Switzer JA, Sikora A, Ergul A, Waller JL, Hess DC, Fagan SC (2012) Minocycline prevents IL-6 increase after acute ischemic stroke. Transl Stroke Res 3:363-368.

Switzer JA, Hess DC, Ergul A, Waller JL, Machado LS, Portik-Dobos V, Pettigrew LC, Clark WM, Fagan SC (2011) Matrix metalloproteinase-9 in an exploratory trial of intravenous minocycline for acute ischemic stroke. Stroke 42:2633-2635.

Szeto GL, Pomerantz JL, Graham DR, Clements JE (2011) Minocycline suppresses activation of nuclear factor of activated T cells 1 (NFAT1) in human CD4+ T cells. J Biol Chem 286:11275-11282.

Tanaka M, Ishihara Y, Mizuno S, Ishida A, Vogel CF, Tsuji M, Yamazaki T, Itoh K (2018) Progression of vasogenic edema induced by activated microglia under permanent middle cerebral artery occlusion. Biochem Biophys Res Commun 496:582-587.

Tang XN, Wang Q, Koike MA, Cheng D, Goris ML, Blankenberg FG, Yenari MA (2007) Monitoring the protective effects of minocycline treatment with radiolabeled annexin V in an experimental model of focal cerebral ischemia. J Nucl Med 48:1822-1828.

Tao T, Xu G, Si Chen C, Feng J, Kong Y, Qin X (2013) Minocycline promotes axonal regeneration through suppression of RGMa in rat MCAO/reperfusion model. Synapse 67:189-198.

Taylor RA, Sansing LH (2013) Microglial responses after ischemic stroke and intracerebral hemorrhage. Clin Dev Immunol 2013:746068.

Tikka TM, Koistinaho JE (2001) Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. J Immunol 166:7527-7533.

Vidale S, Consoli A, Arnaboldi M, Consoli D (2017) Postischemic Inflammation in Acute Stroke. J Clin Neurol 13:1-9.

Wang J, Wei Q, Wang CY, Hill WD, Hess DC, Dong Z (2004) Minocycline up-regulates Bcl-2 and protects against cell death in mitochondria. J Biol Chem 279:19948-19954.

Weinstein JR, Koerner IP, Möller T (2010) Microglia in ischemic brain injury. Future Neurol 5:227-246.

Wu Y, Chen Y, Wu Q, Jia L, Du X (2015) Minocycline inhibits PARP1 expression and decreases apoptosis in diabetic retinopathy. Mol Med Rep 12:4887-4894.

Xu L, Fagan SC, Waller JL, Edwards D, Borlongan CV, Zheng J, Hill WD, Feuerstein G, Hess DC (2004) Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. BMC Neurol 4:7.

Yang Y, Salayandia VM, Thompson JF, Yang LY, Estrada EY, Yang Y (2015) Attenuation of acute stroke injury in rat brain by minocycline promotes blood-brain barrier remodeling and alternative microglia/macrophage activation during recovery. J Neuroinflammation 12:26.

Yew WP, Djukic ND, Jayaseelan JSP, Walker FR, Roos KAA, Chataway TK, Muyderman H, Sims NR (2019) Early treatment with minocycline following stroke in rats improves functional recovery and differentially modifies responses of peri-infarct microglia and astrocytes. J Neuroinflammation 16:6.

Yrjänheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho J (1999) A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci U S A 96:13496-13500.

Zhao Y, Xiao M, He W, Cai Z (2015) Minocycline upregulates cyclic AMP response element binding protein and brain-derived neurotrophic factor in the hippocampus of cerebral ischemia rats and improves behavioral deficits. Neuropsychiatr Dis Treat 11:507-516.

Zheng Y, Xu L, Yin J, Zhong Z, Fan H, Li X, Chang Q (2013) Effect of minocycline on cerebral ischemia-reperfusion injury. Neural Regen Res 8:900-908.

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