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Minocycline inhibits 5-lipoxygenase expression and accelerates functional recovery in chronic phase of focal cerebral ischemia in rats

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ABSTRACT

Aims: We previously reported that minocycline attenuates acute brain injury and inflammation after focal cerebral ischemia, and this is partly mediated by inhibition of 5-lipoxygenase (5-LOX) expression. Here, we determined the protective effect of minocycline on chronic ischemic brain injury and its relation with the inhibition of 5-LOX expression after focal cerebral ischemia.
Main methods: Focal cerebral ischemia was induced by 90 min of middle cerebral artery occlusion followed by reperfusion for 36 days. Minocycline (45 mg/kg) was administered intraperitoneally 2 h and 12 h after ischemia and then every 12 h for 5 days. Sensorimotor function was evaluated 1–28 days after ischemia and cognitive function was determined 30–35 days after ischemia. Thereafter, infarct volume, neuron density, astrogliosis, and 5-LOX expression in the brain were determined.
Key findings: Minocycline accelerated the recovery of sensorimotor and cognitive functions, attenuated the loss of neuron density, and inhibited astrogliosis in the boundary zone around the ischemic core, but did not affect infarct volume. Minocycline significantly inhibited the increased 5-LOX expression in the proliferated astrocytes in the boundary zone, and in the macrophages/microglia in the ischemic core.
Significance: Minocycline accelerates functional recovery in the chronic phase of focal cerebral ischemia, which may be partly associated with the reduction of 5-LOX expression.

Introduction

Minocycline, a semi-synthetic tetracycline antibiotic, has neuroprotective effects on cerebral ischemic injury (Hewlett and Corbett 2006; Koistinaho et al. 2005; Morimoto et al. 2005; Yrjanheikki et al. 1998, 1999) and other brain injuries (Wang et al. 2003; Zhu et al. 2002). However, the short-term effects of minocycline have been reported in most studies of cerebral ischemia or other brain injuries (Fox et al. 2005; Morimoto et al. 2005; Yrjanheikki et al. 1998, 1999). To date, only a few studies have investigated the long-term effects of minocycline on cerebral ischemia (Hewlett and Corbett 2006; Liu et al. 2007). Especially, the effect of minocycline on the formation of a glial scar in the chronic changes after cerebral ischemia is unknown. Glial scar results from reactive gliosis (mainly consisting of proliferated astrocytes) in the boundary zone around the ischemic core, and may be a physical and biochemical barrier for the regeneration of axons (Fawcett and Asher 1999; Persson et al. 1989; Silver and Miller 2004).

Minocycline exerts anti-inflammatory, anti-apoptotic, and anti-oxidative activities; however, the cellular and molecular bases for its neuroprotective effects have not been fully elucidated. Among a number of modulation factors, 5-lipoxygenase (5-LOX), a key enzyme metabolizing arachidonic acid to produce leukotrienes, has been reported to be involved in brain injury (Ciceri et al. 2001; Tomimoto et al. 2002; Zhou et al. 2006a). That the gene encoding 5-LOX activating protein confers risk of stroke shows the importance of 5-LOX in stroke (Helgadottir et al. 2004; Lohmussaar et al. 2005). We have reported the increased 5-LOX expression in the ischemic brain, which is located in proliferated astrocytes (Zhou et al. 2006b). The 5-LOX inhibitor caffeic acid inhibits astrocyte proliferation and glial scar formation (Zhou et al. 2006a). 5-LOX metabolites, the cysteinyl leukotrienes (CysLTs), promote astrocyte proliferation through the $CysLT_1$ receptor (Huang et al. 2008). The $CysLT_1$ receptor antagonist pranlukast inhibits glial scar formation in the ischemic brain (Fang et al. 2006; Yu et al. 2005). Therefore, the 5-LOX pathway may be involved in glial scar formation in the chronic phase of focal cerebral ischemia.

Recently, we found that minocycline protects PC12 cells against ischemia-like injury or NMDA-induced excitotoxicity in vitro. This effect is associated with inhibition of 5-LOX translocation to the nuclear membrane (a phenomenon of 5-LOX activation) (Song et al. 2006, 2004). We also found that minocycline attenuates acute brain

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injury and inflammation after focal cerebral ischemia, and this is associated with inhibition of 5-LOX expression and activation (Chu et al. 2007). However, the long-term effects of minocycline on brain injury, behavioral dysfunction, astrogliosis, and 5-LOX expression in the chronic phase of focal cerebral ischemia remains to be clarified. In the present study, therefore, we determined the long-term protective effects of minocycline in rats, and its association with inhibition of 5-LOX expression.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 250–300 g (Experimental Animal Center, Zhejiang Academy of Medicine Sciences) were housed under a controlled temperature (22 ± 1 °C), 12-h light/dark cycle and had free access to water. Food was available ad libitum with the exception that the rats used in the 8-arm radial maze task were subjected to restricted feeding. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Animal Care Committee of Zhejiang University School of Medicine.

Focal cerebral ischemia

Transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) according to a previously reported method (Longa et al. 1989) with modification. Briefly, rats were anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg). A midline incision was made in the neck, the right external carotid artery (ECA) and the right internal carotid artery (ICA) were carefully exposed and dissected, and a 3-0 monofilament nylon suture was inserted from the ECA into the ICA to occlude the origin of the right MCA. The suture was withdrawn to allow reperfusion, the ECA was ligated, and the incision was closed after 90 min of occlusion. Sham-operated rats underwent identical surgery, except that the intraluminal filament was not inserted. After the surgery, rats were kept for about 2 h in a warm box heated by lamps to maintain the body temperature.

Drug administration

Minocycline (Syowa Hakko, Tokyo, Japan) dissolved in sterile saline was injected intraperitoneally (45 mg/kg) 2 and 12 h after ischemia on the operation day, and once every 12 h for 5 consecutive days from the next day (total, 6 days). An equal volume (5 ml/kg) of saline was injected intraperitoneally as the control.

Functional assessment

Limb-placing test

This test was a modified version of a described test (De Ryck et al. 1989). The rats were habituated to handling before the induction of ischemia. In the test, seven limb-placing tasks were done to assess the integration of forelimb and hindlimb responses to tactile and proprioceptive stimulation. The tasks were scored as follows: 2, normal performance; 1, delayed (>2 s) and/or incomplete performance; and 0, no normal performance. Both sides of the body were tested. In the first task, the rat was suspended 10 cm over a table. Rats normally stretch both forelimbs toward the table. In the second task, the rat was positioned toward the table and its forelimbs were placed on the table. Each forelimb was gently pulled down, and retrieval and placement were checked. Rats normally replace the limb on the table. The third task was the same as the second except that, by keeping the rat's head upward at a 45° angle, the rat was prohibited from seeing the table or contacting it with its vibrissae. Next, the rats were placed along the table edge to check for lateral placement of each forelimb (fourth task) and hindlimb (fifth task). In the sixth task, the rat was again positioned toward the table with the hindlimbs just over the table edge. Each hindlimb was pulled down and gently stimulated by pushing it towards the side of the table. In the seventh task, the forelimbs were placed on the edge of the table and the rat was gently pushed from behind toward the edge. Rats normally resist the pushing, but injured rats cannot keep their grip and the injured limb slips off the edge.

Foot-fault test

A modified forelimb foot-fault placing test was used to examine forelimb function (Ding et al. 2001). The foot placing apparatus consisted of an elevated (100 cm) grid surface ($10 \times 110\ cm^2$, with a square opening of $9\ cm^2$ and grid wire diameter of 1.0 mm) connected to platforms at each end ($15 \times 20\ cm^2$). In each trial, the animal was encouraged by noise or prodding to traverse the grid surface for 1 min. Occasionally, animals inaccurately placed a forelimb, which fell through one of the openings in the grid. These mistakes were considered foot faults. The number of contralateral forelimb foot faults made per meter in 1 min was calculated: 0, no fault; 1, <1 fault in 1 min per meter; 2, ≥1 fault; 3, >2 faults; 4, >3 faults; and 5, unable to proceed.

Adhesive tape test

An adhesive tape test was used to measure somatosensory deficits (Ding et al. 2001) both pre- and post-operatively. All rats were familiarized with the testing environment. In the initial test, two small pieces of adhesive-backed paper dots ($113.1\ mm^2$) were used as bilateral tactile stimuli occupying the distal–radial region on the wrist of each forelimb. The rats were then returned to their cages. The time it took to remove each stimulus from the forelimbs was recorded on five trials per day. Individual trials were separated by at least 5 min. Before surgery, the animals were trained for 3 days. Once the rats were able to remove the dots within 10 s, they were subjected to MCAO. The time to remove the left dot was recorded.

Eight-arm radial maze

The apparatus used was described in a previous report (Chen et al. 1999) and consisted of a radial eight-arm maze with four baited arms (1, 2, 4 and 7). The rats were allowed to familiarize with the radial maze for 2 days before training. Food pellets (45 mg each, Bio-Serv, Frenchtown, NJ, USA) were scattered over the entire maze surface, and three or four rats were simultaneously placed in the maze and allowed to explore and take food freely for 10 min. After adaptation, all rats were trained with four trials per day. A rat was placed on the center platform that was closed off by a door. After 15 s, the door was opened and the rat was allowed to choose which arm to obtain food pellets until all four pellets had been eaten or 5 min had elapsed. The number of entries into the unbaited arms was regarded as total error (TE).

Histological examination

Rats were anesthetized 36 days after ischemia and perfused transcardially with saline followed by 4% paraformaldehyde. Brains were removed and photographed with a digital camera (FinePix S602 Zoom, Fuji Film, Japan). Then, 6 serial coronal sections (20 μm) at 2 mm intervals from the frontal to the occipital poles were cut by cryomicrotomy (CM1900, Leica, Germany) for gross photographic examination after being stained with 1% toluidine blue. The infarct area was defined as an area with reduced Nissl staining and confirmed by light microscopy to have dark pyknotic–necrotic cell bodies. The infarct areas were determined using an image analysis program (AnalyPower1.0, Zhejiang University, Hangzhou, China). The infarct volume in each section was calculated as: infarct area × slice thickness (2 mm), and total infarct volume was the summation of the infarct