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Research Paper

Differential effects of minocycline on microglial activation and neurodegeneration following closed head injury in the neonate rat

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ABSTRACT

The role of microglia in the pathophysiology of injury to the developing brain has been extensively studied. In children under the age of 4 who have sustained a traumatic brain injury (TBI), markers of microglial/macrophage activation were increased in the cerebrospinal fluid and were associated with worse neurologic outcome. Minocycline is an antibiotic that decreases microglial/macrophage activation following hypoxic-ischemia in neonatal rodents and TBI in adult rodents thereby reducing neurodegeneration and behavioral deficits. In study 1, 11-day-old rats received an impact to the intact skull and were treated for 3 days with minocycline. Immediately following termination of minocycline administration, microglial reactivity was reduced in the cortex and hippocampus ($p < 0.001$) and was accompanied by an increase in the number of fluoro-Jade B profiles ($p < 0.001$) suggestive of a reduced clearance of degenerating cells; however, this effect was not sustained at 7 days post-injury. Although microglial reactivity was reduced in the white matter tracts ($p < 0.001$), minocycline treatment did not reduce axonal injury or degeneration. In the thalamus, minocycline treatment did not affect microglial reactivity, axonal injury and degeneration, and neurodegeneration. Injury-induced spatial learning and memory deficits were also not affected by minocycline. In study 2, to test whether extended dosing of minocycline may be necessary to reduce the ongoing pathologic alterations, a separate group of animals received minocycline for 9 days. Immediately following termination of treatment, microglial reactivity and neurodegeneration in all regions examined were exacerbated in minocycline-treated brain-injured animals compared to brain-injured animals that received vehicle ($p < 0.001$), an effect that was only sustained in the cortex and hippocampus up to 15 days post-injury ($p < 0.001$). Whereas injury-induced spatial learning deficits remained unaffected by minocycline treatment, memory deficits appeared to be significantly worse ($p < 0.05$). Sex had minimal effects on either injury-induced alterations or the efficacy of minocycline treatment. Collectively, these data demonstrate the differential effects of minocycline in the immature brain following impact trauma and suggest that minocycline may not be an effective therapeutic strategy for TBI in the immature brain.

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1. Introduction

With close to half a million children affected annually, traumatic brain injury (TBI) remains one of the most common causes of disability and death in infants and children (Coronado et al., 2011; Faul et al., 2010; Langlois et al., 2005). The youngest age group (≤4 years old) exhibit worse outcome following moderate to severe TBI compared to older children (Anderson et al., 2005; Coronado et al., 2011). Pediatric TBI patients commonly exhibit traumatic axonal injury (TAI) and brain atrophy which are associated with prolonged cognitive deficits such as impairments of learning and memory, attention, and executive function (Anderson et al., 2005, 2009; Catroppa et al., 2007, 2008; Duhaime and Raghupathi, 1999; Ewing-Cobbs et al., 2004, 2006; Tong et al., 2004). Injury to the immature brain may also have adverse effects on the development of cognitive abilities (Babikian et al., 2015). Unfortunately, no specific therapies exist, with supportive care in the acute post-traumatic period being the only current treatment option.

While the mechanisms underlying neuropathologic alterations following TBI in the immature brain are not completely understood,

Abbreviations: TBI, Traumatic brain injury; TAI, traumatic axonal injury; CSF, cerebrospinal fluid; HI, hypoxic-ischemia; eCCI, electronically driven controlled cortical impact; PBS, phosphate-buffered saline; FJB, fluoro-jade B; Iba1, anti-ionized calcium-binding adaptor molecule 1; APP, amyloid precursor protein; HPF, high power field; ANOVA, analysis of variance.

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inflammation may play an important role in the sequelae of secondary injury. Activation of microglia, the resident immuno-competent cells in the brain, is thought to play an important role in the acute and chronic neurodegeneration observed following brain injury (Beynon and Walker, 2012; Graeber and Streit, 2010; Hanisch and Kettenmann, 2007; Kreutzberg, 1996; Nimmerjahn et al., 2005; Ransohoff and Perry, 2009). Markers of microglial/macrophage activation such as sCD163, ferritin, and interleukins-6,-8 and -10 were increased in cerebrospinal fluid (CSF) from children after TBI with more prominent increases observed in the youngest age group (≤4 years of age), suggesting that these patients may be at higher risk for worse neurologic outcome (Bell et al., 1997; Newell et al., 2015; Whalen et al., 2000). In neonatal rodents, hypoxic-ischemia (HI) or ischemia resulted in robust microglial/macrophage activation (Denker et al., 2007; Ferrazzano et al., 2013; Ivacko et al., 1996; McRae et al., 1995; Vexler and Yenari, 2009). Increased microglial reactivity in the injured hemisphere following TBI in the immature mouse brain corresponded to areas containing degenerating neurons and was associated with an expansion of the cortical lesion and spatial learning deficits (Pullela et al., 2006; Tong et al., 2002). In addition, microglial reactivity has also been observed in the white matter tracts that was associated with an increase in tissue loss of the injured hemisphere and working and recognition memory deficits in a rabbit model of pediatric TBI (Zhang et al., 2015). These data suggest that microglial activity may be involved in ongoing pathogenesis following TBI in the immature brain and may potentially serve as a therapeutic target.

Minocycline is a second generation tetracycline derivative antibiotic with anti-inflammatory properties, effectively crosses the blood-brain barrier after systemic administration and has demonstrated neuroprotection in many models of brain injury and neurodegenerative diseases (Elewa et al., 2006; Garrido-Mesa et al., 2013; Kim and Suh, 2009; Plane et al., 2010). Following neonatal rodent models of HI, minocycline decreased microglial activation which was associated with a reduction in injury-induced neuronal damage, oligodendroglial cell death, hypomyelination, white matter atrophy and locomotor deficits (Cai et al., 2006; Carty et al., 2008; Cikla et al., 2016; Fan et al., 2006). In a model of pediatric cardiac arrest, acute treatment with minocycline reduced microglial activation along with neurodegeneration and apoptosis (Tang et al., 2010). Treatment with minocycline following TBI in the adult mouse resulted in a reduction of microglial activation and proliferation which was associated with a decrease in pro-inflammatory cytokine response, cerebral edema, lesion volume and attenuation of locomotor and spatial memory deficits (Homsi et al., 2009, 2010; Siopi et al., 2011, 2012). Similarly, minocycline administration following moderate-severe contusive trauma to the adult rat brain reduced microglial activation and improved behavioral function (Abdel Baki et al., 2010; Lam et al., 2013). In contrast, minocycline did not attenuate cell death, axonal injury or tissue loss despite reducing active microglia following either diffuse brain trauma in the adult mouse (Bye et al., 2007) or repetitive brain trauma in the neonate rat (Hanlon et al., 2016). Depleting the brain of its resident microglia exacerbates cell death following neonatal stroke (Faustino et al., 2011) but appears to not affect the extent of white matter injury following TBI (Bennett and Brody, 2014). It must be noted that not all effects of minocycline can be attributed to its effects on reducing microglial activation following brain injury. Fox et al. (2005) observed that minocycline administration following focal ischemia in the neonate rat did not reduce the extent of microglial activation but did reduce the volume of injury. Although microglial activation was not evaluated, minocycline treatment reduced both apoptotic and excitotoxic cell death following HI in the neonatal rat (Arvin et al., 2002), reduced caspase activation following contusive brain trauma in the adult mouse (Sanchez Mejia et al., 2001) and attenuated inflammatory protein expression in a rat model of mild blast TBI (Kovesdi et al., 2012). By contrast, minocycline worsened injury-induced infarction and tissue atrophy in a mouse model of HI (Tsuji et al., 2004). Collectively, these data underscore the complicated relationship between injury-induced microglial activation and ensuing brain damage.

With the goal of understanding the role of microglial activation in neonatal brain trauma, the present study sought to test the hypothesis that minocycline treatment following TBI will attenuate microglia reactivity along with neuronal and axonal degeneration leading to decreases in brain atrophy and a reversal of spatial learning and memory deficits. The effects of both short-term (3 days) and extended (9 days) administration of minocycline (45 mg/kg/dose) were tested in a well characterized model of single TBI in the 11-day-old rat. This injury results in neuronal and axonal degeneration, TAI, microglial reactivity, tissue atrophy and cognitive deficits that last up to 4 weeks post-injury (Raghupathi and Huh, 2007).

2. Methods

2.1. Brain injury

All surgical and behavioral procedures were done in accordance with the rules and regulations of the Institutional Animal Care and Use Committee of Drexel University College of Medicine and were in compliance with the Guide for the Care and Use of Animals. Animals were placed on a heating pad set to 37 °C to maintain body temperature throughout all procedures and recovery. Brain injuries were induced in isoflurane-anesthetized 11-day-old male and female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) using the electronically driven controlled cortical impact (eCCI) device (Custom Design International, Richmond, VA), as previously described (Raghupathi and Huh, 2007); the convex indenter (5 mm diameter) was driven 3 mm from the point of contact with the skull with a velocity of 5 m/s and a dwell time of 100 ms. Animals were injured at 45 s following the removal of anesthesia, and the total time from anesthesia to impact was 5 min; sham-injured animals were exposed to anesthesia for 4 min during surgical preparation and the zeroing of the impactor tip to the skull (which was not fired). On the day of injury, animals were randomly assigned to injury and treatment conditions. Animals had similar weights on the day of injury irrespective of sex and injury/treatment status ($F_{2,129} = 0.76$, $p = 0.38$; Table 1). Apnea latency times were recorded for brain-injured animals from the time of impact to when they took their first breath. The loss of a righting reflex was measured

Table 1
Acute neurologic status of groups used in studies 1 and 2.
For purposes of comparison, animals used in study 1 and 2 were combined. Apnea times and latency to regain righting reflex were calculated as described in the Methods. Values represent group means and standard errors of the mean.

Group	N	Sex	Body weight at injury (g)	Apnea (s)	Latency to righting reflex (s)	Hematoma		Herniation (N)
						Mild (N)	Moderate (N)	
Sham-injured	19	Male	23 ± 1	n/a	137 ± 21	n/a	n/a	n/a
Sham-injured	19	Female	22 ± 0	n/a	119 ± 14	n/a	n/a	n/a
Brain-injured + vehicle	21	Male	23 ± 1	13 ± 2	168 ± 23	11	8	2
Brain-injured + vehicle	20	Female	22 ± 0	13 ± 2	183 ± 18	9	7	4
Brain-injured + minocycline	29	Male	23 ± 1	14 ± 2	185 ± 17	11	16	2
Brain-injured + minocycline	25	Female	22 ± 1	15 ± 2	187 ± 22	9	13	3

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