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Research Report

Minocycline mitigates motor impairments and cortical neuronal loss induced by focal ischemia in rats chronically exposed to ethanol during adolescence

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ABSTRACT

Ethanol is an important risk factor for the occurrence of cerebral ischemia contributing to poor prognosis and inefficacy of drug treatments for stroke-related symptoms. Females have a higher lifetime risk for stroke than males. Moreover, female gender has been associated with increased ethanol consumption during adolescence. In the present study, we investigated whether chronic ethanol exposure during adolescence may potentiate the motor impairments and cortical damage induced by focal ischemia in female rats. We also addressed whether these effects can be mitigated by minocycline, which *has been shown to be neuroprotective* against different insults in the CNS. Female rats were treated with distilled water or ethanol (6.5 g/kg/day, 22.5% w/v) for 55 days by gavage. Focal ischemia was induced by microinjections of endothelin-1 (ET-1) into the motor cortex. Animals of both groups were treated daily with minocycline (25–50 mg/kg, i.p.) or sterile saline (i.p.) for 5 days, and motor function was assessed using open field, inclined plane and rotarod tests. Chronic ethanol exposure

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exacerbated locomotor activity and motor coordination impairments induced by focal ischemia in rats. Moreover, histological analysis revealed that microinjections of ET-1 induced pyramidal neuron loss and microglial activation in the motor cortex. Minocycline reversed the observed motor impairments, microglial activation and pyramidal neuron loss in the motor cortex of ischemic rats even in those exposed to ethanol. These results suggest that minocycline induces neuroprotection and functional recovery in ischemic female rats intoxicated with ethanol during adolescence. Furthermore, the mechanism underlying this protective effect may be related to the modulation of neuroinflammation.

1. Introduction

Ethanol is the most widely used drug of abuse among adolescents due to its easy acquisition and wide acceptance by the majority of organized societies (Sanchis and Aragón, 2007; Schuckit, 2009). Early use of this substance has been linked to higher incidence of alcoholism in adulthood, given that over 80% of regular drinkers reported problems related to alcohol before the age of 30 and 40% between 15 and 19 years of age (Chambers et al., 2003).

The high consumption of ethanol during adolescence negatively impacts individuals, such as increased susceptibility to brain damage and cognitive and behavioral disabilities for both rodents and humans (Alfonso-Loeches and Guerri, 2011). This is a critical period of central nervous system (CNS) maturation, in which establishment of neurotransmission pathways and synaptic connections occur, resulting in long-term deleterious effects of ethanol on behavior (Crews et al., 2006; Slawecki et al., 2004; White and Swartzwelder, 2004).

Otero Palleiro and Barbagelata López (2007) showed that prolonged exposure to ethanol was responsible for 16% of cases of cerebral ischemia in patients between 18 and 45 years of age. Other studies have described that consumption of ethanol exacerbates the occurrence of cerebral ischemia in a dose-dependent manner, contributing to poor prognosis and failure of treatment for stroke-related symptoms (Iso et al., 2004; Mandić and Rancić, 2011).

Stroke is among the leading causes of death in Western countries, surpassed only by heart disease and cancer (Donnan et al., 2008). A recent update published in the United States reported that each year, approximately 55,000 more women than men have a stroke. Furthermore, epidemiological studies claim that females have a higher lifetime risk of stroke than males, mainly among those 55–75 years of age with an incidence of 1 in 5 for women (20–21%) compared to 1 in 6 for men (14–17%) (Go et al., 2013; Kleindorfer et al., 2010; Seshadri et al., 2006). In addition to the high risk of stroke among females, recent studies have shown a marked change in the ethanol consumption profile in females, with an early consumption during adolescence often in a heavy binge-drinking manner (INPAD, 2013). Surprisingly, despite the evidence that ethanol exposure is a risk factor for stroke and that females are more susceptible to ethanol's deleterious effects especially during adolescence, there are no studies addressing whether ethanol exposure during adolescence exacerbates stroke-induced damage or alters treatment efficacy for stroke-related symptoms in female subjects.

In addition to the high mortality rate, over 50% of patients with stroke exhibit some motor impairment (Bonita, 1992). Tissue plasminogen activator (tPA) is the only drug approved for the treatment of acute ischemic stroke (Carpenter et al., 2011). However, its clinical application is associated with several limitations such as narrow therapeutic windows (dose should be given no more than 3 h after stroke) and the risk of hemorrhage must be ruled out (Kablau et al., 2011). Furthermore, it has been estimated that only 6 in 1000 patients benefit from this therapeutic resource (Gilligan et al., 2005; O'Donnell et al., 2010).

Considering the paucity of approved therapies for stroke, several studies have investigated new putative therapeutic agents, including minocycline (Moskowitz et al., 2010; Yong et al., 2004). Minocycline is a semi-synthetic tetracycline with pleiotropic actions and neuroprotective effects in acute and chronic neural disorders, including stroke (Cardoso et al., 2013; Franco et al., 2012; Yrjanheikki et al., 1999), amyotrophic lateral sclerosis (Gordon et al., 2008), Parkinson's disease (Thomas and Le, 2004), Huntington's disease (Hodl and Bonelli, 2005), multiple sclerosis (Chen et al., 2011), head trauma (Homsi et al., 2010) and spinal cord trauma (Casha et al., 2012).

The neuroprotective effects of minocycline have been attributed to multiple potential mechanisms (Yong et al., 2004), but its effects on microglial activity is considered of great importance, as neuroinflammation plays a pivotal role in the pathophysiology of several CNS diseases (Block et al., 2007; Gomes-Leal, 2012). For ischemia, minocycline has been shown to decrease the infarct area by about 65% for the cortex and by about 45% for the striatum following middle cerebral artery occlusion (MCAO) (Yrjanheikki et al., 1999). We have reported considerable neuroprotection afforded by minocycline in the endotheline-1 (ET-1) model of focal ischemia in both the cortex (Franco et al., 2012) and striatum (Cardoso et al., 2013). Microglia activation and proliferation have been implicated in the pathophysiology of ethanol intoxication (Blanco and Guerri, 2007; Zhao et al., 2012). It has been shown that a low concentration of ethanol induces an inflammatory response in the CNS with the release of nitric oxide (NO) and proinflammatory cytokines by microglia (Blanco and Guerri, 2007). Activation of toll-like receptors (i.e., toll-like receptors type 4 – TLR-4) and release of transcription factors (i.e., nuclear factor kappa B – **NF-κB**) seem to be involved in the mechanisms of ethanol-induced brain inflammation (Blanco and Guerri, 2007).

Considering that minocycline is a potent anti-inflammatory/antibiotic tetracycline with well-established neuroprotective effects (Yrjanheikki et al., 1999; Franco et al., 2012;