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Research Report

Resveratrol downregulates type-1 glutamate transporter expression and microglia activation in the hippocampus following cerebral ischemia reperfusion in rats

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ABSTRACT

The naturally occurring polyphenol phytoalexin resveratrol (RSV) regulates neuronal inflammation in various disease models and protects the brain against ischemic injury. Cell surface glutamate transporters on perisynaptic astrocytes are important regulators of extracellular glutamate levels and synaptic activation. Following cerebral ischemia, reduced astroglial type-1 glutamate transporter (GLT-1) expression in the CA1 pyramidal layers of the hippocampus contribute to neurotoxic glutamate levels. The current study examined the effects of 21-day RSV pretreatment (1 or 10 mg/kg dose; i.p.) on microglia and astrocyte activation and characterized GLT-1 expression in the dentate gyrus (DG), CA1 and CA3 layers of the hippocampus 7 days following 10 min global ischemia. Male Wistar rats were divided into five groups; sham/saline, ischemia/saline, ischemia/1 mg/kg RSV, ischemia/10 mg/kg RSV and sham/10 mg/kg RSV. Immunohistochemical detection of GLT-1, CD11b/c, glial fibrillary acidic protein (GFAP) assessed type 1 glutamate transporter expression and microglial/glial cell activation following sham surgery or global ischemia. Our findings demonstrate prevention by RSV of ischemia-induced reduction of GLT-1 expression in the vulnerable CA1 layer 7 days following global ischemia, which was accompanied by the polyphenol's inhibition of post ischemic increase in CD11b/c and GFAP expression. RSV also conferred significant CA1 neuronal protection positively correlated with attenuation of glutamate transporter activation. These findings support beneficial effects of RSV in modulation of the excitotoxic cascade postischemia, which are congruent with anti-inflammatory effects observed in various pathological models.

Abbreviations: 4-VO, four-vessel occlusion model; ANOVA, analysis of variance; CA1, cornu ammonis 1; CA3, cornu ammonis 3; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; GLT-1, type-1 glutamate transporter

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1. Introduction

Among nutraceuticals, the naturally occurring polyphenol phytoalexin resveratrol (RSV) demonstrates antioxidant, anti-inflammatory, neuroprotective actions as well as improves learning and memory impairments in a number of disease models (Bhat et al., 2001; Bradamante et al., 2004; Girbovan et al., 2012). In vitro, recent studies have demonstrated its ability to downregulate glial cell activation through inhibition of pro-inflammatory cytokines and key signalling molecules as well as enhance glutamate uptake from astrocytes (Candelario-Jalil, et al., 2007; Lu et al., 2010; Zhang et al., 2010b, 2013). RSV's inhibitory actions on microglia and astrocytes' activation have been proposed as mechanisms fostering neuroprotection against ischemic injury (Zhang et al., 2010a), although this has not been demonstrated in in vivo models of global cerebral ischemia.

The pathogenesis of cerebral ischemia involves a complex sequence of physiological events that includes a well-documented inflammatory cascade, characterized by activation of glial cells, including microglia and astrocytes, and pro-inflammatory cytokines thought to contribute to the selective neuronal injury observed in the hippocampal CA1 region postischemia (Madinier et al., 2009; Zhang et al., 2010b). Often described as sensors of pathological events, microglia make up 5–20% of the glial population in the mature brain (Persson and Ronnback, 2012). Following cerebral ischemia, microglia become positive for markers of activation in the hippocampus as early as 24 h post-insult, and are primarily localized within the CA1 region 7 days post-insult (Anderova et al., 2011; Dos-Anjos et al., 2009; Langdon et al., 2008; Madinier et al., 2009; Moon et al., 2009). Likewise, astrocytes are the most abundant cells of the central nervous system (CNS) and their roles are diverse, including release of neurotrophic factors, control of fluid, ion and pH homeostasis, neurotransmitter scavenging and management of metabolite and waste products (Swanson et al., 2004; Takano et al., 2009). Astrocyte morphology is markedly altered as they become reactive following ischemia, a phenomenon characterized by thickened and retracted processes and an enlarged soma (Panickar and Norenberg, 2005). Increased proliferation of reactive astrocytes to the site of injury along with a sustained and progressive increase in levels of the cytoskeletal protein glial fibrillary acidic protein (GFAP) in astrocytes have been reported to occur in tandem (Panickar and Norenberg, 2005; Petito and Halaby, 1993).

Notwithstanding a close association with the degree of brain injury, the role of glial activation on adjacent neuronal populations postischemia remains a matter of debate. While release of chemokines and reactive oxygen species has conferred a neurotoxic role, enhanced glutamate uptake and upregulation of type-1 glutamate transporter (GLT-1) following glial activation promote neuroprotection (Nakajima et al., 2008). GLT-1 is the predominant subtype of glutamate transporters expressed in astrocytes of the cerebral cortex and hippocampus (Yeh et al., 2005), and plays a key role in terminating synaptic glutamate transmission by mediating 90% of glutamate uptake (Danbolt, 2001). Chronic inhibition of glutamate transporters has been shown to significantly increase excitotoxic neuronal damage following ischemia (Chen et al., 2005). Reduced GLT-1 mRNA expression has been measured for 24 h in the ipsilateral hippocampus and cortex following focal ischemia in mice (Ketheeswaranathan et al., 2011), and GLT-1 protein expression is attenuated in hippocampal CA1 neurons following perinatal hypoxic–ischemic injury in rats (Zhao et al., 2012). An essential role of glial cells and GLT-1 is suggested in attenuating excess synaptic glutamate and excitotoxic cell death.

The current study examined in vivo effects of 21-day pretreatment with RSV on microglia and glial cell activation, and assessed region-dependent changes in the expression of GLT-1 within the hippocampus 7 days following global cerebral ischemia in rats.

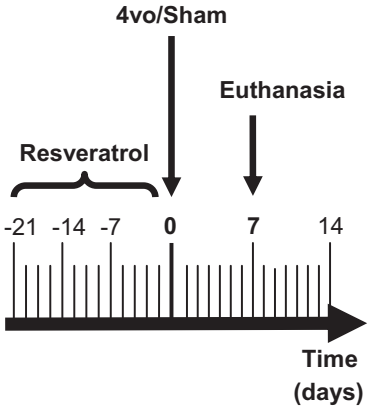

Fig. 1 – Experimental timeline of the study. Daily 21-day pre-surgical period of RSV (1 mg/kg or 10 mg/kg) or saline i.p. injections; 4-vessel occlusion (4vo) or sham occlusion. Day 0 refers to the day of the surgery.

2. Results

2.1. CD11b/c expression in the CA1, CA3 and DG

Simple effects tests indicated that ischemic rats treated with saline had significantly more activated microglia in the CA1 than sham-operated controls ($p<.005$) and ischemic rats treated with 10 mg/kg RSV ($p<.05$). This was evident by the presence of amoeboid-like cells with plump cell bodies and short thick processes which are characteristic features of activated microglia (Fig. 2). Fig. 3 shows the effect of RSV treatment and 10 min global ischemia on microglial activation as measured by CD11b/c expression in the CA1 region of the hippocampus, 7 days postischemia. Statistical analysis revealed a significant surgery × treatment interaction ($F(1,29)=10.93$, $p<.005$). The decrease in CD11b/c (CD11b/c) immunoreactivity in ischemic animals treated with the 10 mg/kg RSV dose is indicative of a dose-related reduction in post-ischemic inflammation. Interestingly, simple effects also indicated that sham rats treated with 10 mg/kg resveratrol displayed an upregulation of activated microglia in the CA1 compared to their saline-treated sham counterparts ($p<.05$). Fig. 4 shows the effect of RSV treatment and 10 min global ischemia on microglial activation in the CA3 and DG regions of the hippocampus, 7 days postischemia. No significant differences were observed in microglial activation in the CA3 region of the hippocampus among the groups. In the DG, analysis of variance revealed a significant main effect of surgery