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Full-length Article

Reparative effects of interleukin-1 receptor antagonist in young and aged/co-morbid rodents after cerebral ischemia

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ABSTRACT

Neuroprotective strategies for ischemic stroke have failed to translate from bench to bedside, possibly due to the lack of consideration of key clinical co-morbidities. Stroke and co-morbidities are associated with raised levels of the pro-inflammatory cytokine interleukin-1 (IL-1). Inhibition of IL-1 by the administration of interleukin-1 receptor antagonist (IL-1Ra) has shown to be neuroprotective after experimental cerebral ischemia. Stroke can also trigger a robust neuroreparative response following injury, yet many of these new born neurons fail to survive or integrate into pre-existing circuits. Thus, we explore here effects of IL-1Ra on post-stroke neurogenesis in young and aged/co-morbid rats. Aged lean, aged Corpulent (a model of atherosclerosis, obesity and insulin resistance) and young Wistar male rats were exposed to transient cerebral ischemia, received subcutaneous IL-1Ra 3 and 6 h during reperfusion, and effects on stroke outcome and neurogenesis were analyzed. Our results show that administration of IL-1Ra improves stroke outcome in both young and aged/co-morbid rats. Furthermore, IL-1Ra not only increases stem cell proliferation, but also significantly enhances neuroblast migration and the number of newly born neurons after cerebral ischemia. Overall, our data demonstrate that systemic administration of IL-1Ra improves outcome and promotes neurogenesis after experimental stroke, further highlighting the therapeutic potential of this clinically approved drug.

1. Introduction

Cerebral ischemia is the second leading cause of death and disability worldwide with treatment limited to fibrinolysis and intravascular therapy, both applicable in a low percentage of patients. Other strategies to improve outcome after stroke have failed to translate from the preclinical setting to the clinic, and with these failures have come calls for more rigorous and transparent approaches to experimental design and conduct (Albers et al., 2011; Howells et al., 2014). Highlighted in many of these calls is the need to consider age and co-morbidities experimentally, as these involve a strong inflammatory response, and are associated with increased risk of stroke and poor post-stroke outcomes.

The pro-inflammatory cytokine interleukin-1 (IL-1) is a major driver of inflammation, with well documented detrimental effects in multiple preclinical models of systemic inflammatory disease as well as in cerebral ischemia (Denes et al., 2010; Fan et al., 2013; McColl et al., 2007). To this end, the selective, naturally occurring competitive inhibitor of IL-1, interleukin-1 receptor antagonist (IL-1Ra) has shown potential as a new treatment for stroke (Emsley et al., 2005; Banwell et al., 2009; Smith et al., 2012). More specifically, in a number of experimental stroke paradigms IL-1Ra reduces infarct volume and improves long term functional outcome, including in co-morbid animals (McColl et al., 2007; Relton and Rothwell, 1992; Pradillo et al., 2012; Girard et al., 2014). However, exact mechanisms by which IL-1Ra is neuroprotective are yet to be fully established.

While much research has focused on limiting ischemic damage in the initial stages of acute reperfusion, it is also important to understand mechanisms that underpin brain repair following injury and develop strategies that enhance reparative endogenous processes, including adult neurogenesis. Ischemic injury elicits a

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robust neurogenic response (Arvidsson et al., 2002) by stimulating production of neuronal progenitor cells (NPCs) in distinct neurogenic regions, which include the subventricular zone (SVZ) and the subgranular zone (SGZ), to generate new functional neurons. Though mechanisms underlying post-stroke neurogenesis and the influence of inflammation on these processes are still poorly understood, it has been observed in young and aged animals that inflammation impairs both basal levels of neurogenesis and attenuates the neurogenic response triggered by CNS injury via induction of the pro-inflammatory cytokines (Vallieres et al., 2002; Ben-Hur et al., 2003; Wong et al., 2004; Bachstetter et al., 2011). IL-1, for example, reduces the proliferation and differentiation of NPCs to neurons in pathologies such as stress and depression, effects reversed by administration of IL-1Ra (Koo and Duman, 2008; Zhang et al., 2013).

Here, we explored how inhibition of IL-1 actions by clinically relevant, delayed administration of subcutaneous IL-1Ra affects stroke outcome and neurogenesis up to 28 days after experimental ischemia, in aged/co-morbid and young rats.

2. Materials and methods

2.1. Animals

All experiments were performed using 13-month-old male, lean (JCR:LA-lean (cp/); 400–500 g) and Corpulent (Cp) (JCR:LA-cp (cp/cp); 900–1000 g) rats (University of Alberta, Edmonton, Canada) and 2-month-old Wistar rats (200–250 g; Charles River, Wilmington, MA, USA). Cp rats are homozygous for the autosomal recessive cp gene (cp/cp), and spontaneously develop obesity, hyperlipidemia, insulin resistance, glomerular sclerosis, and atherosclerosis with enhanced vascular contractility and reduced vascular relaxation (Mangat et al., 2007). Animals were allowed free access to food and water and were maintained under temperature, humidity, and light-controlled conditions. All procedures were performed under appropriate United Kingdom Home Office licenses and adhered to the Animals (Scientific Procedures) Act (1986) Kilkenny et al., 2010.

2.2. Treatment

Animals were randomized for all experiments and assessments were performed in a blinded manner. Lean, Cp and young Wistar rats received two doses (subcutaneous) of placebo or IL-1Ra (25 mg/kg, 12.5 mg/kg and 50 mg/kg respectively) at 3 and 6 h post reperfusion and were allocated randomly to the following experimental groups sacrificed on post-stroke day 7: aged lean + tMCAO + placebo ($n = 10$); aged lean + tMCAO + IL-1Ra (n = 10); aged Cp + tMCAO + placebo (n = 9); aged Cp + tMCAO + IL-1Ra (n = 9); young Wistar + tMCAo + placebo (n = 8) and young Wistar + tMCAo + IL-1Ra (n = 8). Another two groups of young Wistar were sacrificed at 14d after stroke: young Wistar + tMCAo + placebo-14d (n = 8) and young Wistar + tMCAo + IL-1Ra-14d (n = 8) to determine neuroblast migration, and two groups at 28d: young Wistar + tMCAo + placebo-28d (n = 8) and young Wistar + tMCAo + IL-1Ra-28d (n = 8) to analyze the number of new integrated neurons. Group sizes were determined by power calculation ($\alpha = 0.05$, $\beta = 0.2$).

The pharmacokinetic profile of subcutaneous human IL-1Ra (r-met-huIL-1Ra: Kineret; Amgen, Thousand Oaks, CA, USA) or placebo (Amgen, Thousand Oaks, CA, USA) was assessed in young and aged-comorbid rats as previously described (Greenhalgh et al., 2010). Owing to the highly lipophobic formulation of the drugs, obese Cp rats received half the dose of IL-1Ra (50%) per body weight compared with aged lean rats (Pradillo et al., 2012). Plasma levels of IL-1Ra (measured by ELISA) in Lean/Cp rats reached a concentration of ~8000 ng/ml 8 h after both injections. Young Wistar rats were given double the dose of the aged lean animals due to the faster metabolism of young animals (Mondon and Starnes, 1992). Following studies by Greenhalgh et al. (2010), a single administration of 100 mg/kg of IL-1Ra to young rats at the time of the MCAO, resulted in a plasma concentration of ~9000 ng/ml 8 h after its administration. Therefore the administration regime of IL-1Ra used here resulted in comparable plasma levels of drug, at what we believe to be clinically therapeutic concentrations (Emsley et al., 2005).

2.3. Focal cerebral ischemia

Focal cerebral ischemia was induced in aged lean and Cp rats by 90 min transient occlusion of the left middle cerebral artery (tMCAO) as described previously (Pradillo et al., 2012). Focal cerebral ischemia was induced in young Wistar rats by 70 min transient occlusion of the left middle cerebral artery and left common carotid artery (CCA). The slight difference in surgical protocol was due to a resistance of young rats to infarction when only the MCA was occluded (data not shown). It was necessary to occlude both the MCA and CCA in these young healthy animals to achieve similar infarcts to aged lean/corpulent rats infarcts to analyze the IL-1Ra effect on neurogenesis, due to the influence of infarct size in the neurogenic response after stroke (Moraga et al., 2014). Isoflurane (2% for induction and 1.5% during surgery) was used in a mixture of 70% N_2O and 30% O_2. Core body temperature was maintained at 37.0 °C ± 0.5 °C throughout the surgery by a heating blanket (Homeothermic Blanket Control Unit; Harvard Apparatus, Kent, UK). Ischemia was induced by a transient ligature of the left MCA trunk and/or CCA with a 10–0 suture (Prolene, Ethicon, Somerville, NJ, USA). Occlusion and reperfusion were confirmed visually under the surgical microscope. After surgery, animals were returned to home cages and allowed free access to water and food. Animals were excluded from statistical analysis based on an a priori exclusion criterion, namely if animals experienced brain hemorrhage, lack of reperfusion or if the surgery took longer than 45 min and there was excessive bleeding (2 aged leans were excluded in total). Only one animal died during the duration of the study due to anesthetic overdose.

2.4. Measurement of infarct volume and BBB damage

Lesion volume and edema were assessed on T_2W brain images obtained at 24 h and 7 d after stroke, using a 7-T, horizontal-bore magnet (Agilent Technologies, UK) interfaced to a Bruker Avance III console (Bruker Biospin, UK) using a surface transmit-receive coil. Images were analyzed using Anatomist software (http://brainvisa.info). In the group of young Wistar rats sacrificed at 28 d, the loss of cortex was determined in brain sections by Nissl staining as described previously (Pradillo et al., 2012).

Blood–brain barrier (BBB) damage was determined by immunohistochemical staining of endogenous rat immunoglobulin G (IgG) as described previously (Greenhalgh et al., 2010) in all the experimental groups at 7d, and in young Wistar rats at 14d of reperfusion.

2.5. Stroke outcome

Neurological status was assessed blinded to drug treatment, before and at different time points up to 28d after stroke, and by the use of motor, behavioral and cylinder tests (Hunter et al., 2000; Madrigal et al., 2003; Schallert et al., 2000). For the motor and behavioral scales, each test was conducted three times per trial and the average taken to determine outcome. For the cylinder test,