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Beneficial potential of intravenously administered IL-6 in improving outcome after murine experimental stroke

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ABSTRACT

Interleukin-6 (IL-6) is a pleiotropic cytokine with neuroprotective properties. Still, the therapeutic potential of IL-6 after experimental stroke has not yet been investigated in a clinically relevant way. Here, we investigated the therapeutic use of intravenously administered IL-6 and the soluble IL-6 receptor (sIL-6R) alone or in combination, early after permanent middle cerebral artery occlusion (pMCAo) in mice. IL-6 did not affect the infarct volume in C57BL/6 mice, at neither 24 nor 72 h after pMCAo but reduced the infarct volume in IL-6 knockout mice at 24 h after pMCAo. Assessment of post-stroke behavior showed an improved grip strength after a single IL-6 injection and also improved rotarod endurance after two injections, in C57BL/6 mice at 24 h. An improved grip strength and a better preservation of sensory functions was also observed in IL-6 treated IL-6 knockout mice 24 h after pMCAo. Co-administration of IL-6 and sIL-6R increased the infarct volume, the number of infiltrating polymorphonuclear leukocytes and impaired the rotarod endurance of C57BL/6 mice 24 h after pMCAo. IL-6 administration to naïve C57BL/6 mice lead after 45 min to increased plasma-levels of CXCL1 and IL-10, whereas IL-6 administration to C57BL/6 mice lead to a reduction in the ischemia-induced increase in IL-6 and CXCL1 at both mRNA and protein level in brain, and of IL-6 and CXCL1 in serum. We also investigated the expression of IL-6 and IL-6R after pMCAo and found that cortical neurons upregulated IL-6 mRNA and protein, and upregulated IL-6R after pMCAo. In conclusion, the results show a complex but potentially beneficial effect of intravenously administered IL-6 in experimental stroke.

1. Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine, which has been extensively investigated in a variety of diseases (Kishimoto, 1989; Wolf et al., 2014), including ischemic stroke where its role is still disputed (Jung et al., 2011; Loddick et al., 1998; Matsuda et al., 1996; Suzuki et al., 2009). A number of studies have shown an increase in IL-6 expression acutely in the brain after experimental stroke (Suzuki et al., 2009; Erta et al., 2012) while IL-6 receptor (IL-6R) expression has been reported to remain unchanged (Ali et al., 2000; Vollenweider et al., 2003) or increase at later time points (Gertz et al., 2012). Clinical studies show that IL-6 levels in serum and cerebrospinal fluid (CSF) increase significantly within the first 24 h after stroke and that IL-6 levels correlate with final infarct size and mortality (Beridze et al., 2011; Fassbender et al., 1994; Smith et al., 2004). Furthermore, elevated levels of IL-6 in serum correlate with post-stroke infection (Koennecke et al., 2011), which is associated with less favourable outcome (Rohweder et al., 2015). Elevated serum levels of IL-6 are also associated with progression of atherosclerosis (Okazaki et al., 2014), a risk factor for stroke (Bos et al., 2014). However, while elevated serum levels of IL-6 thereby relate to morbidity (Fassbender et al., 1994; Smith et al., 2004), there is also evidence that IL-6 can have neuroprotective functions (Chucair-Elliott et al., 2014; Swartz et al., 2001; Yang et al., 2012), also after experimental stroke (Gertz et al., 2012; Herrmann et al., 2003). This could indicate that systemic and central IL-6, as previously suggested (Suzuki et al., 2009), may either have different effects, or potentially have different effects at different time-points post-stroke.

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The 'Janus face' of IL-6 may be explained by that IL-6 can elicit fundamentally different cellular responses depending on whether the classic or the trans-signalling pathway is activated (Scheller et al., 2011). Classic signalling is responsible for the anti-inflammatory (Rothaug et al., 2016), pro-regenerative (Wolf et al., 2014; Yang et al., 2012) and neuroprotective functions of IL-6 (Chucair-Elliott et al., 2014; Swartz et al., 2001; Yang et al., 2012). This pathway involves binding of IL-6 to the membrane bound IL-6R, which induces dimerization of the glycoprotein (gp) 130. Complex formation of IL-6 + IL-6R with gp130 activates downstream signalling (Schaper and Rose-John, 2015). In contrast, trans-signalling is responsible for the pro-inflammatory (Scheller et al., 2011; Rose-John, 2012) and neurodegenerative (Rothaug et al., 2016; Campbell et al., 2014) effects of IL-6. Trans-signalling involves a cleaved form of the IL-6R, referred to as soluble IL-6 receptor (sIL-6R) (Jones and Rose-John, 2002). The sIL-6R can, upon complex formation with IL-6, stimulate gp130 expressing cells (Jones and Rose-John, 2002), however, the complex can also be neutralized by the antagonistic soluble form of gp130 (sgp130) (Rose-John, 2012). In recent years, therapies aimed at counteracting the trans-signalling pathway have attracted considerable therapeutic interest in general (Rose-John et al., 2007) but also in relation to stroke (Jones and Rose-John, 2002).

Ischemia-induced upregulation of IL-6 has been suggested to represent an endogenous neuroprotective mechanism against N-methyl-D-aspartate (NMDA) receptor-mediated injury (Ali et al., 2000). Furthermore, it appears that IL-6 produced in brain cells promotes post-stroke angiogenesis thereby improving long-term outcome after stroke (Gertz et al., 2012). A few intervention studies exist, and they all report a neuroprotective effect of exogenous IL-6 (Jung et al., 2011; Loddick et al., 1998; Matsuda et al., 1996; Feng et al., 2015). However, these studies have major translational limitations, due to initiation of treatment before induction of ischemia (Jung et al., 2011; Loddick et al., 1998; Matsuda et al., 1996) or the use of administration routes, such as intracerebroventricular (Jung et al., 2011; Loddick et al., 1998; Matsuda et al., 1996) and intraperitoneal (Feng et al., 2015) administration, which are not routinely used clinically. To our knowledge, no studies have investigated the effect of sIL-6R administered in a clinically relevant way post-stroke and none of the previous intervention studies have investigated how IL-6 treatment influences the inflammatory response which is an integral part of stroke pathophysiology (Clausen et al., 2016; Nguyen et al., 2016; Veltkamp and Gill, 2016). In addition the behavioral outcome has largely been ignored (Jung et al., 2011; Loddick et al., 1998; Matsuda et al., 1996; Herrmann et al., 2003). We therefore investigated how IL-6, sIL-6R and a combination thereof, administered intravenously (iv.) as a single injection or repeated injections within the first hour post-stroke, affect infarct size, and behavioral outcome in mice. We also investigated neutrophil recruitment into the brain and central and systemic inflammatory responses. Overall, our results are indicative of a beneficial effect of repeated i.v. treatment with IL-6 in experimental stroke.

2. Materials and methods

2.1. Mice

C57BL/6 (C57BL/6JBomTac) mice (8 weeks old males) were obtained from Taconic A/S (Ry, Denmark), and B6.129S2-*Il6*$^{tm/Kopf}$ (IL-6 (KO)) mice (8 weeks old males) from Jackson Laboratories (Bar Harbor, ME, USA). Mice were individually caged at a 12 h light/dark cycle under controlled temperature and humidity, with free access to food and water. Mice were 10 weeks old when experiments were carried out. Procedures were approved by the The Danish Animal Inspectorate under the Ministry of Food and Agriculture (J. no. 2011/561-1950) and reported in accordance with the ARRIVE guidelines.

2.2. Focal cerebral ischemia model

General anaesthesia was with the exception of Intervention study 1 (see below) induced by subcutaneous injection of Dormicum (diazepam 5 mg/ml, Actavis, Gentofte, Denmark), Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml, Jansen-Cilag, Birkerød, Denmark) and distilled H_20 mixed 1:1:2 in a volume of 0.16 ml/10 g mouse. In Intervention study 1 the mice were anaesthetized by use of isoflurane. Temperature was measured prior to (baseline) and 10 min after induction of anaesthesia. Mice were placed under a surgical microscope (Zeiss, Göttingen, Germany) on a 37 °C ± 0.5 °C warm heating pad and the distal part of the left middle cerebral artery (MCA) was permanently occluded by electrocoagulation (pMCAo) through a craniectomy (Lambertsen et al., 2005). Following surgery, 1 ml physiologic saline (0.9% NaCl) and 0.1 ml of Temgesic® (Buprenorphinum 0.3 mg/ml, RB Pharmaceuticals, North Chesterfield, VA, USA) were subcutaneously administered. Post-surgical analgesia was administered three times during the first 24 h. Sham mice underwent the same procedure, except that the electrocoagulation was made in the brain parenchyma next to the MCA.

2.3. Experimental design

2.3.1. Temporal profiles of mRNA and protein after pMCAo

Eighty-one C57BL/6 mice were subjected to pMCAo (n = 8–13/group) and 55 mice to sham-surgery (n = 6–9/group) before euthanized after 1, 2, 4, 6, 12, 24 and 72 h. Nine mice served as naïve controls. For immunohistochemistry (IHC), 28 mice were euthanized 1, 2, 4, 6, 12, 24 and 72 h (n = 4/group) after pMCAo. Three mice served as naïve controls. For *in situ* hybridization (ISH), 9 mice were euthanized 2, 24, and 72 h (n = 3/group) after pMCAo. Three mice served as naïve controls.

2.3.2. Treatment studies

2.3.2.1. Drugs. For the treatment was used carrier-free recombinant mouse IL-6 (406-ML-025/CF, R&D Systems) and recombinant mouse soluble IL-6R (1830-SR-025/CF, sIL-6R, R&D Systems) diluted in saline (NaCl) (500 ng drug/100 μL). The choice of source and dose of IL-6 and sIL-6R was guided by other studies, reporting beneficial effects of IL-6 dosages between 40 and 400 ng/mouse (Ellingsgaard et al., 2011) and maximum behavioral response for a dosage of 500 ng of sIL-6R (Patel et al., 2012). Saline was used as vehicle treatment. Investigators were blinded to the treatment of the mice.

2.3.2.2. Adverse effects and drug detection in blood. Forty mice were randomized into four groups (n = 10/group). *Group 1* was injected i.v. with 100 μL of saline, *group 2* i.v. with 500 ng IL-6, *group 3* i.v. with 500 ng sIL-6R and *group 4* i.v. with 500 ng sIL-6R followed by 500 ng IL-6. After 30 min, mice were tested in the open field test (see below). Blood was sampled by percutaneous perforation from the left jaw vein after 45 min. The temperature was monitored closely for three hours after drug injection.

2.3.2.3. Blood brain barrier (BBB) permeability of drugs. Naïve mice were injected with IL-6 or sIL-6R labelled with 5-carboxyfluorescein (5-FAM) using the AnaTag™ 5-FAM Microscale Protein Labelling Kit (ANA-72054, BioNordika Denmark A/S) (Bach et al., 2012). Mice were randomized into: *Group 1* injected i.v. with 500 ng 5-FAM + IL-6, *group 2* injected i.v. with 500 ng 5-FAM + sIL-6R, and *group 3* i.v. injected with saline added