Cytokine 58 (2012) 384–389

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Interleukin-1 receptor antagonist reverses stroke-associated peripheral immune suppression

Craig J. Smith [a,*], Hedley C. Emsley [b], Chinedu T. Udeh [c], Andy Vail [d], Margaret E. Hoadley [a], Nancy J. Rothwell [e], Pippa J. Tyrrell [a], Stephen J. Hopkins [a]

[a] Brain Injury Research Group, School of Biomedicine, University of Manchester, Manchester Academic Health Science Centre, Salford Royal Foundation Trust, Salford M6 8HD, UK
[b] Department of Neurology, Royal Preston Hospital, Preston PR2 9HT, UK
[c] Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Bristol University, Bristol BS1 3NY, UK
[d] Health Methodology Research Group, University of Manchester, Salford Royal Foundation Trust, Salford M6 8HD, UK
[e] Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK

ARTICLE INFO

Article history:
Received 11 October 2011
Received in revised form 28 December 2011
Accepted 22 February 2012
Available online 22 March 2012

Keywords:
Acute stroke
Interleukin-1 receptor antagonist
Inflammation
Immune suppression
Infection

ABSTRACT

Introduction: Infections are common following stroke and adversely affect outcome. Cellular immune suppression associated with acute stroke may increase susceptibility to infection. Cytokines are important contributors to both stroke pathology and the response to infection. Since interleukin (IL)-1 blockade is a candidate treatment for cerebral ischemia, we examined whether administration of interleukin-1 receptor antagonist (IL-1Ra) to patients with acute stroke affected innate cellular immune responses in a phase II placebo-controlled trial.

Methods: Venous blood samples were taken prior to treatment initiation, at 24 h and 5 to 7d. Blood was also drawn from stroke-free controls. Lipopolysaccharide (LPS) stimulation of whole-blood cultures assessed the potential of leukocytes to produce cytokines.

Results: Induction of tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8 and IL-10 by LPS was significantly reduced in patients at admission, compared to controls. At 24 h, cytokine induction remained suppressed in the placebo group. In contrast, for patients treated with IL-1Ra, induction of TNF-α, IL-6 and IL-10 was similar to controls and IL-1β induction was significantly greater than in the placebo group. At 5 to 7d, TNF-α and IL-1β induction remained suppressed only in the placebo group ($p < 0.05$). Plasma cortisol concentrations, elevated at admission in patients compared to controls, were substantially reduced at 24 h in the patients receiving IL-1Ra ($p < 0.05$) and inversely correlated ($p < 0.001$) with either TNF-α ($r = -0.71$) or IL-1β induction ($r = -0.67$) at admission.

Conclusion: Treatment with IL-1Ra reverses peripheral innate immune suppression in the acute phase of stroke, which is associated with attenuated cortisol production. The mechanisms underlying these observations, including the potential impact of IL-1Ra on stroke severity and the clinical significance of immune suppression, require further evaluation in larger studies.

1. Introduction

Cerebral ischemia induces rapid initiation of inflammatory pathways in the brain and its vasculature, as well as systemic responses in the periphery [1]. The pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)-α have pivotal roles in regulating inflammatory and innate immune responses to infectious organisms. IL-1β plays a crucial role in regulating many important inflammatory mediators and pathways, including other cytokines, cellular adhesion molecules, chemokines and the acute-phase response, and is an important therapeutic target in inflammatory disease [2,3].

Infections complicating stroke, particularly pneumonia, are common and have a major impact on outcome [4]. The acute-phase response, characterised by elevated plasma concentrations of IL-6, C-reactive protein (CRP) and activation of the hypothalamic–pituitary–adrenal (HPA) axis, is rapidly activated after stroke onset [5]. By contrast, there is increasing evidence of peripheral immune suppression associated with cerebral ischemia, involving both innate and adaptive pathways [6–12]. We have previously reported that elevation in plasma cortisol concentrations, within hours of ischemic stroke, accompanies impaired production of IL-1, TNF-α and IL-6 by stimulated cultures of whole blood from patients following stroke [6]. The inverse correlation between cytokine

* Corresponding author. Address: University of Manchester, Brain Injury Research Group, School of Biomedicine, Manchester Academic Health Science Centre, Salford Royal Foundation Trust, Room C238, Clinical Sciences Building, Salford M6 8HD, UK. Tel.: +44 161 206 0623; fax: +44 161 707 6534.
E-mail address: Craig.Smith-2@manchester.ac.uk (C.J. Smith).

1043-4666/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2012.02.016

production and elevation of plasma cortisol, and the reversal of impaired TNF-α production by whole blood cultures following glucocorticoid receptor blockade in experimental cerebral ischemia, suggests activation of the HPA axis may be a potential mechanism regulating innate immune suppression [9]. Whilst data from experimental animals suggest that modulating immune suppression prevents bacterial infections following cerebral ischemia [9], the significance of such immune suppression in stroke patients is unclear. Given the potential for reducing incident infections in patients by therapeutically targeting immune suppression, further understanding the regulation of the complex immune-inflammatory interactions in the central nervous system (CNS) and periphery is of considerable clinical relevance.

IL-1 is strongly implicated in the pathophysiology of ischemic injury, as well as innate immunity [13]. In response to experimental cerebral ischemia, IL-1 is rapidly up-regulated in the CNS and markedly exacerbates injury [14,15]. IL-1 receptor antagonist (IL-1Ra) is induced by the same stimuli as IL-1, but reversibly blocks all of its known actions [16]. IL-1Ra dramatically reduces the extent of cerebral ischemic injury in experimental studies, even when administered peripherally after the onset of ischemia [14,17]. In a phase II study of intravenous (IV) IL-1Ra in acute stroke patients, treatment with IL-1Ra reduced plasma CRP and IL-6 concentrations [18], which are known to correlate with extent of cerebral injury and outcome [19]. The effects of IL-1Ra on peripheral leukocyte immune responses to cerebral ischemia are not known, but there have been some concerns about IL-1Ra treatment increasing risk of infection in other patient groups [20]. The aim of the present study was therefore to investigate the effect of IL-1Ra treatment on measures of peripheral innate immune responsiveness in patients with acute stroke. The objective was to analyse cytokine induction in blood samples collected from acute stroke patients receiving IL-1Ra or placebo in a phase II safety study. This analysis also provided the opportunity to test the proposition that there is a link between immune suppression and cortisol.

2. Materials and methods

2.1. Participants and study procedures

The IL-1Ra in acute stroke study was a randomised, placebo-controlled phase II trial of IV IL-1Ra administered to patients within 6 h of stroke onset [18]. *A priori* secondary analyses included measurement of cytokine production by peripheral whole-blood cultures from these patients and these have subsequently been analysed. The study was approved by the Local Research Ethics Committee. Briefly, patients aged ⩾18 years with a clinical diagnosis of stroke within 6 h of symptom onset and able to provide consent/assent were eligible. Exclusion criteria included National Institutes of Health Stroke Scale (NIHSS) score ⩽ 4/rapidly improving neurological deficit. Patients were randomised 1:1 to recombinant methionylated human IL-1Ra ($n = 17$) or placebo ($n = 17$); stratified by age (<70 and ⩾70 y), baseline stroke severity (NIHSS score 4–9, 10–20, ⩾21) and time since onset (<4 h, ⩾4 h). Test treatment was administered as an IV loading dose of 100 mg over 60 s, followed by consecutive IV infusion at 2 mg/kg/h for a total of 72 h. Infections present at the time of study entry and during the administration of test treatment were recorded prospectively by the study team, diagnosed on the basis of clinical suspicion, laboratory/radiological investigations and antibiotic therapy. Control participants ($n = 13$) of a similar age range, living independently at home without a previous history of stroke or transient ischemic attack, that were free of infection requiring medical treatment and able to provide written informed consent were also recruited. Each control was matched to a patient (6 to patients receiving IL-1Ra; 7 to patients receiving placebo) on basis of age (±5 years), sex and degree of atherosclerosis as previously described (lowest ABPI < 0.92: significant atherosclerosis; lowest ABPI ⩾ 0.92: without significant atherosclerosis) [5].

2.2. Blood sampling and measurement of peripheral cytokine induction and cortisol

Venous blood was collected into tubes containing a final concentration of 10 μ/ml pyrogen-free heparin and immediately wrapped in cool-gel packs, for whole-blood cultures and plasma cortisol measurement. Additional blood samples were collected in EDTA tubes for automated differential leukocyte counts using the Coulter principle method (Coulter GEN-S analyser). Samples were collected prior to initiation of test treatment (admission time point), at 24 h and 5 to 7d (at 09:00). Venous blood was also drawn from resting control participants at 09:00 and at the time of their matched patient admission sample (±2 h) if this was not between 07:00 and 11:00. Whole-blood cultures and lipopolysaccharide (LPS) stimulation to induce cytokine production was as previously described [6]. The cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α were analysed using Luminex technology on a BioPlex 200 analyser. Assays were carried out in Millipore-Multiscreen HTS-96 well filter plates (Millipore, Watford, UK). Standards were captured on carboxy coated polystyrene beads (Bio-Rad, Hemel Hempstead, UK) coupled to specific Pelikine monoclonal antibodies (anti-IL-1β, Cat: M9334; anti-IL-6, Cat: M9316; anti-IL-8, Cat: M191802; anti-IL-10, Cat: M191002; anti-TNF-α, Cat: M192302: MAST Diagnostics, Bootle, UK). The analytes were secondarily bound with Pelikine specific antibodies conjugated with biotin (anti-IL-1β, Cat: M193404; anti-IL-6, Cat: M191604; anti-IL-8, Cat: M191804; anti-IL-10, Cat: M191004; anti-TNFα, Cat: M192304: from MAST Diagnostics), prior to addition of streptavidin, conjugated with phycoerythrin (Stratech Cat: 016-110-084; Stratech Scientific Ltd., Newmarket, UK). Results were read on a Bio-Plex 200 suspension array system analyser. All cytokine measurements are expressed relative to the nominal mass value for the relevant National Institute for Biological Standards Control (NIBSC, Soth Mimms, UK). Plasma cortisol was measured by the routine clinical biochemistry service, using an electrochemiluminescent competitive immunoassay on a Roche Modular E170 analyser.

2.3. Statistical analyses

The primary analysis was LPS-stimulated TNF-α concentration at 24 h in patients treated with IV IL-1Ra versus placebo. Analyses were by intention to treat. Characteristics of the patients receiving IL-Ra or placebo, and the control participants were compared using standard summary statistics. The Mann–Whitney U test was used for between-group comparisons of cytokine induction data and plasma cortisol. Spearman Rank correlation analyses were applied for univariate associations.

3. Results

3.1. Patients and controls

Demographic details and baseline characteristics of the IL-1Ra and placebo-treated patients are presented in detail elsewhere [18]. Briefly, baseline characteristics were similar in the IL-Ra and placebo groups; namely age (median 74 v 71 years respectively), sex (41% v 59% male), NIHSS score (median 13 each group) and symptom onset to treatment (median 3.6 h each group). Five of the patients in the active group (29%) presented with spontaneous intracerebral haemorrhage. There was no evidence of infection