Molecular and Cellular Neuroscience 53 (2013) 26–33

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

The effect of stroke on immune function☆,☆☆

Roberta Brambilla [a,1], Yvonne Couch [b,1], Kate Lykke Lambertsen [c,*,1]

[a] The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Miami, FL, United States
[b] The Department of Pharmacology, University of Oxford, Oxford, UK
[c] The Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark

ARTICLE INFO

Article history:
Received 15 March 2012
Accepted 22 August 2012
Available online 30 August 2012

Keywords:
Stroke
Immune responses
Autonomic nervous system
HPA axis

ABSTRACT

Neurological disorders affect over one billion lives each year worldwide. With population aging, this number is on the rise, making neurological disorders a major public health concern. Within this category, stroke represents the second leading cause of death, ranking after heart disease, and is associated with long-term physical disabilities and impaired quality of life.

In this review, we will focus our attention on examining the tight crosstalk between brain and immune system and how disruption of this mutual interaction is at the basis of stroke pathophysiology. We will also explore the emerging literature in support of the use of immuno-modulatory molecules as potential therapeutic interventions in stroke. This article is part of a Special Issue entitled 'Neuroinflammation in neurodegeneration and neurodysfunction'.

Introduction

Ischemic stroke results in a multitude of CNS events characterized ultimately by neuronal and glial cell death, and is also marked by numerous peripheral events including cardiovascular, endocrine and immune dysregulation (Emsley et al., 2008; Stevens and Nyquist, 2007). The contribution of the immune system to the development and progression of cerebral infarcts is well established. However, recent evidence suggests that it may also contribute to recovery and repair in the long term after ischemic damage (Gelderblom et al., 2009; Hug et al., 2009). Stroke patients face severe immunological challenges while still in intensive care units, so much so that the most common, fatal, post-stroke complication is pneumonia (Aslanyan et al., 2004; Johnston et al., 1998; Katzan et al., 2003). Indeed, a recent meta-analysis has revealed that infection after acute ischemia can complicate recovery in up to 30% of cases (Westendorp et al., 2011). While in the past the assumption was that post-stroke infections were dependent on pre-existing co-morbidities and mismanagement of patient care, it is now clear that post-stroke immunodepression represents an independent factor associated with increased susceptibility to infections (Emsley et al., 2008).

Risk factors for developing stroke include co-morbid diseases such as atherosclerosis, obesity, diabetes, hypertension and peripheral infection (Emsley et al., 2008; Hankey, 2006). Common to all is the association with elevated systemic inflammation, which increasing evidence points at having a causative role in the development of these diseases (Hansson and Libby, 2006). Several clinical studies have reported more severe neurological deficits in stroke patients with preceding infection (McColl et al., 2009). Furthermore, elevated systemic concentrations of a number of inflammatory markers have been associated with stroke incidence (Rodriguez-Yanez et al., 2008), emphasizing the role of inflammatory events occurring outside the brain prior to, during and after stroke, on stroke susceptibility and outcome.

Even though these pre-existing conditions are major contributors to stroke incidence and physiopathology, this review will specifically focus on the direct effects of stroke on peripheral immune function, since dysregulation of such immune response has clear negative implications on patient outcome, and a better understanding of these events is critical in devising appropriate and comprehensive therapeutic strategies for the treatment of stroke patients. The effect of inflammation on stroke outcome will be covered separately by Stuart Allan on the review dealing with the afferent pathways in stroke.

Stroke and central inflammation

Since the brain has a very high glucose and oxygen demand, disturbances in the blood supply to the brain rapidly lead to the depletion of these substrates and the development of an ischemic infarct with accompanying necrosis of neurons, glial cells and small vessels within the affected territory. Depletion of cellular energy supplies (such as adenosine triphosphate (ATP)) occurs within minutes, resulting in the

☆ The authors have no conflict of interest.

☆☆ The authors would like to acknowledge financial support from the Lundbeck Foundation and the Danish Medical Research Council to Dr. Kate Lykke Lambertsen and from The Miami Project to Cure Paralysis to Dr. Roberta Brambilla.

* Corresponding author at: Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, J.B. Winsløwsvej 21, st., DK-5000 Odense C, Denmark.
E-mail address: klambertsen@health.sdu.dk (K.L. Lambertsen).

[1] All three authors contributed equally to this work.

1044-7431/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2012.08.011

accumulation of lactic acid within the tissue. Ischemia also leads to the formation of free radicals that can induce cell damage, and to increased release of excitatory glutamate (for recent review see (Iadecola and Anrather, 2011)). ATP depletion and glutamate release result in uncontrolled calcium-ion influx into the cells leading to activation of intracellular lipases and proteolytic enzymes and, ultimately, destruction of the cell. The ability of glutamate to kill neurons by excessive activation of glutamate receptors is referred to as excitotoxicity (Mergenthaler et al., 2004). The early excitotoxicity induced by the local energy deficit causes fast necrotic cell death in the core area of the infarct (Lipton, 1999). The ischemic penumbra that surrounds the infarct core suffers milder damage, partly due the numerous collaterals and anastomoses, which supply the neurons within the penumbra (Astrup et al., 1981). This area is characterized by compromised blood flow, impaired neuronal functionality, but preserved structural integrity (Astrup et al., 1981). In addition, astrocytes are more resistant to cerebral ischemia than neurons and react to hypoxia by upregulating their glycolytic capacity allowing a continued uptake of glutamate from the synaptic cleft in the penumbral area (Marrif and Juurlink, 1999). It has been observed that the penumbra has suppressed cortical protein synthesis, but preserved ATP content (del Zoppo et al., 2011; Hossmann, 2006). For these reasons the penumbral area is still potentially salvageable and, thus far, has been the target of stroke therapy (del Zoppo et al., 2011).

After ischemia, resident cells, including microglia and astrocytes, are quickly activated and circulating leukocytes are recruited to the ischemic lesion. It is believed that, early on, endogenous signals such as damage-associated molecular patterns (DAMPs; i.e. heat shock protein (HSP)60, HSP70 and high-mobility-group box-1 (HMGB1)) are released from stressed and dying cells and subsequently bind to toll-like receptors (TLRs), especially TLR2 and TLR4, located on resident microglia and astrocytes, resulting in downstream activation of MyD88- and/or TRIF-dependent pathways leading to activation of nuclear factor kappa B- and/or IRF3-dependent gene transcription (for a thorough review on this topic, please refer to Marsh et al. (2009)). This triggers the synthesis of primarily microglia-derived pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF) (reviewed in (Lambertsen et al., 2012)), chemokines (CC and CXC chemokines) (Mirabelli-Badenier et al., 2011), nitric oxide and reactive oxygen species, which, when present at high levels, can exacerbate cell death and cause break down of the blood–brain barrier (BBB) (for recent review see (Iadecola and Anrather, 2011)). Cytokines and chemokines also induce the upregulation of adhesion molecules on the vascular endothelium, favoring diapedesis of circulating leukocytes that may further contribute to brain injury.

Stroke-associated infection

Post-stroke infections represent one of the principal complications adversely affecting the clinical outcome in stroke patients. Although some infections may occur as a direct consequence of dysphagia causing aspiration, or may be linked to the advanced age of the patient, it is increasingly apparent that stroke itself represents a risk factor for infections due to the induction of a so-called post-stroke immunodepression syndrome, which occurs immediately after stroke (Chamorro et al., 2007; Vermeij et al., 2009). Indeed, the fact that the majority of post-stroke infections manifest within three days of hospitalization is further indication that immunodepression is involved, and infections are not simply a secondary outcome related to patient care (Westendorp et al., 2011). A recent meta-analysis of the 87 clinical studies conducted thus far, where rates of post-stroke infections were examined, has indicated that infection complicated acute stroke in 30% of patients, with rates varying considerably between 5 and 67% (Westendorp et al., 2011). Pneumonia and urinary tract infections each occurred in 10% of patients, with pneumonia significantly associated with death. It is also clear that the severity of post-stroke infections is directly correlated with the magnitude of the stroke itself, and specifically with how extensive the infarct area is (Hug et al., 2009). The size of the infarct area also parallels the severity of leukocytopenia, which directly correlates with stroke-associated immunodepression. The occurrence of leukocytopenia following stroke has been well documented in patients, with reports dating back over 40 years (Czlonkowska et al., 1979). Rapid reduction of lymphocyte counts and functional deactivation of monocytes and T helper type 1 cells have been observed in acute stroke patients, with more pronounced immunodepression in patients with severe clinical deficit or large infarction (Haeusler et al., 2008). Lymphocytopenia does correlate with the occurrence of post-stroke infections (Haeusler et al., 2008; Hug et al., 2009). In some instances, rather than a generalized reduction in total lymphocyte counts, only selective lymphocytopenia in the NK cell subset was reported immediately after stroke (Hug et al., 2009).

The tight relationship between lymphocytopenia, size of infarct and the occurrence of post-stroke infections has been demonstrated also in animal models of stroke. In mice, severely reduced lymphocyte counts (B cells and $CD4^+$ T helpers, especially) were found in lymphoid organs (spleen and thymus) within 12 h after stroke (Prass et al., 2003). This was paralleled by spontaneous bacteremia and pneumonia (often leading to death), which were completely prevented by administration of a sympathetic blocker, but not by inhibition of the hypothalamic-pituitary-adrenal (HPA) axis (the paravetricular nucleus in the hypothalamus, the anterior pituitary gland and the cortex of the adrenal glands), suggesting that a catecholamine-mediated defect in early lymphocyte activation is the key factor in the impaired antibacterial immune response after stroke (Prass et al., 2003). A recent study by Wong and colleagues has highlighted the role of invariant natural killer T (iNKT) cells in the defense against post-stroke infections (Wong et al., 2011). Modulation of hepatic iNKT through blockade of noradrenergic neurotransmitters or directly with administration of β-galactosylceramide results in reduced infection and associated lung injury after stroke, demonstrating that these cells act as conductor of immunity, meaning that their acute responses modulate and facilitate the adaptive immune response (Wong et al., 2011).

Because of the correlation between post-stroke infections and clinical outcome, the prophylactic use of antibiotics to prevent such infections and improve the outcome has been proposed. The data emerging from clinical studies, however, are contradictory. For example, no benefit was found with prophylactic administration of the fluoroquinolone levofloxacin, a broad-spectrum antibiotic, in acute stroke patients admitted within 24 h after symptom onset. The rate of stroke-related infections at 7 days was identical to the placebo group, and levofloxacin administration was directly correlated with poor clinical outcome (Chamorro et al., 2005). On the other hand, prophylactic treatment with another fluoroquinolone, moxifloxacin, resulted in the significant reduction of post-stroke infections, although not in the improvement of the clinical outcome (Harms et al., 2008). This is in contrast with data obtained in a mouse model of middle cerebral artery occlusion (MCAO), where moxifloxacin, a similar broad-spectrum antibiotic, administration significantly reduced infarct size (Bao et al., 2010). A possible explanation for the failure of these molecules in human therapy is the neurotoxicity of fluoroquinolones, which could be offsetting their beneficial antimicrobial effect. In contrast, other classes of broad-spectrum antibiotics (e.g. penicillins and tetracyclines) have shown protective effects. Indeed, prophylactic administration of the broad spectrum semisynthetic penicillin mezlocillin in combination with the beta-lactamase inhibitor sulbactam decreased incidence and severity of fever within the first 3–4 days after stroke and was associated with a lower rate of post-stroke infection and improved long term outcome (Schwarz et al., 2008). Finally, minocycline, a semisynthetic second generation tetracycline, is the antibiotic that perhaps holds the highest promise. After being proven effective in numerous experimental models of