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Research Paper

Endotoxemia induces lung-brain coupling and multi-organ injury following cerebral ischemia-reperfusion

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ABSTRACT

Post-ischemic neurodegeneration remains the principal cause of mortality following cardiac resuscitation. Recent studies have implicated gastrointestinal ischemia in the sepsis-like response associated with the post-cardiac arrest syndrome (PCAS). However, the extent to which the resulting low-grade endotoxemia present in up to 86% of resuscitated patients affects cerebral ischemia-reperfusion injury has not been investigated. Here we report that a single injection of low-dose lipopolysaccharide (50 μg/kg, IP) delivered after global cerebral ischemia (GCI) induces blood-brain barrier permeability, microglial activation, cortical injury, and functional decline *in vivo*, compared to ischemia alone. And while GCI was sufficient to induce neutrophil (PMN) activation and recruitment to the post-ischemic CNS, minimal endotoxemia exhibited synergistic effects on markers of systemic inflammation including PMN priming, lung damage, and PMN burden within the lung and other non-ischemic organs including the kidney and liver. Our findings predict that acute interventions geared towards blocking the effects of serologically occult endotoxemia in survivors of cardiac arrest will limit delayed neurodegeneration, multi-organ dysfunction and potentially other features of PCAS. This work also introduces lung-brain coupling as a novel therapeutic target with broad effects on innate immune priming and post-ischemic neurodegeneration following cardiac arrest and related cerebrovascular conditions.

1. Introduction

An estimated 500,000 patients suffer cardiac arrest in the United States annually, and despite the introduction of induced hypothermia, the prognosis for meaningful neurological recovery remains poor in over 80% of cases (Bernard et al., 2002; Hoesch et al., 2008). These dire clinical outcomes reflect the exquisite vulnerability of the central nervous system (CNS) to acute ischemic challenge as well as the delayed effects of reperfusion in the context of systemic injury. Collectively referred to as the post-cardiac arrest syndrome (PCAS), this disorder is characterized by changes in hemostasis and microvascular rheology, adrenal insufficiency with resulting shock physiology, heightened levels of inflammation, and delayed damage to both the CNS and peripheral organs (Adrie et al., 2004; Neumar et al., 2008).

The link between systemic inflammation and poor outcomes in patients presenting with this and other forms of acute neurological injury is well recognized (Becker et al., 2011; Kliper et al., 2013; Rodriguez et al., 2013). In the case of cardiac arrest, tissue ischemia stimulates the activity of transcription factors including hypoxia-inducible factor-1α and nuclear factor-κB that stimulate the production of pro-inflammatory cytokines and enhance other aspects of host immune function (Paradis, 2007; Ridder and Schwaninger, 2009). Ischemic damage also induces a sterile inflammatory response through the release of damage-associated molecular patterns (DAMPs) from the brain and other tissues (Pittman and Kubes, 2013; Tadie et al., 2013; Wang et al., 2015). More recently, studies have revealed detectable levels of endotoxin in the serum of up to 86% of patients within 24–48 h following return of spontaneous circulation (Adrie et al., 2002; Grimaldi et al., 2013; Grimaldi et al., 2015; L'Her et al., 2005). The presence of endotoxin and other pathogen-associated molecular patterns (PAMPs)

Abbreviations: 3VO, three-vessel occlusion; ANOVA, analysis of variance; AU, arbitrary units; BAO, basilar artery occlusion; BBB, blood-brain barrier; BCCAO, bilateral common carotid artery occlusion; CBF, cerebral blood flow; CCA, common carotid artery; CNS, central nervous system; DAMP, damage-associated molecular pattern; EU, endotoxin unit; FSC, forward scatter; GCI, global cerebral ischemia; IHC, immunohistochemistry; IP, intraperitoneal; LPS, lipopolysaccharide; MAP2, microtubule-associated protein 2; OHCA, out-of-hospital cardiac arrest; PAMP, pathogen-associated molecular pattern; PCAS, post-cardiac arrest syndrome; PECAM-1, platelet and endothelial cell adhesion molecule 1; PMN, polymorphonuclear neutrophil; SAL, saline; SSC, side scatter

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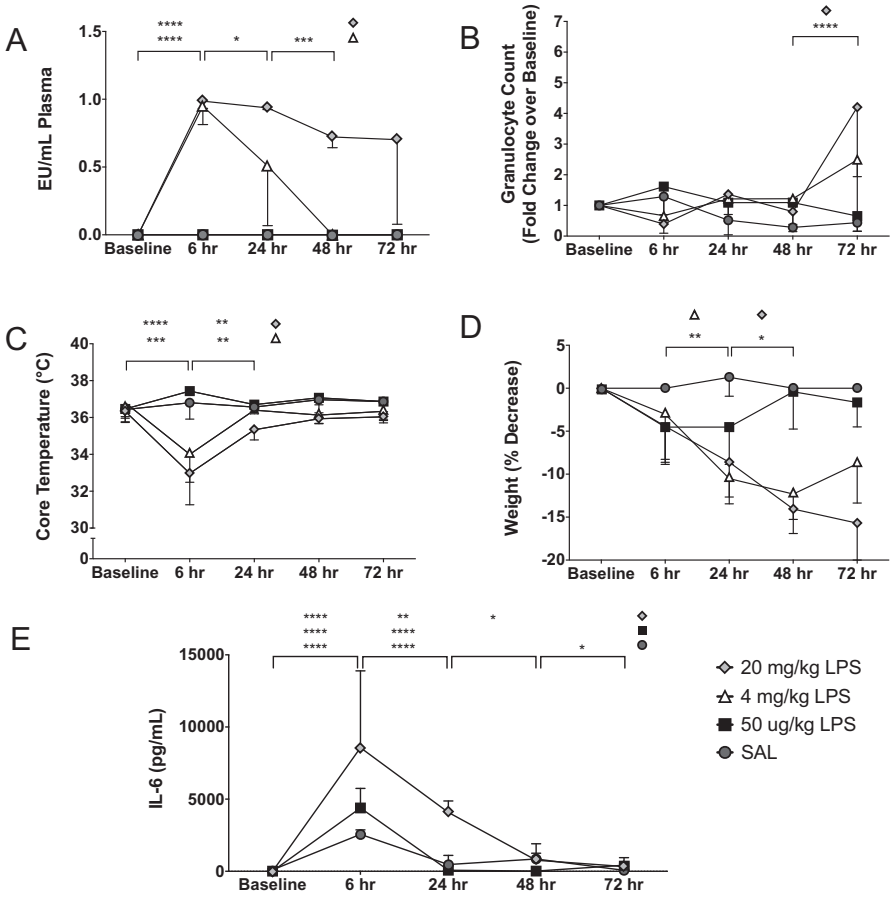

Fig. 1. LPS dose titration studies. IP administration of 50 μg/kg LPS yielded undetectable plasma endotoxin (A) and no changes in granulocyte count (B), core temperature (C), or weight (D), while effects were seen with 4 mg/kg and 20 mg/kg. (D) Elevated IL-6 was seen in all mice after 6 h and remained elevated at 24 h in mice receiving 20 mg/kg LPS. Values represent means ± SD ($n = 3$). 1-way ANOVA: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

has been linked with ischemia-induced growth of gram-negative intestinal flora and loss of mucosal barrier function with secondary bacteremia (Adrie et al., 2002; Karhausen et al., 2013; Wang et al., 2012). While endotoxemia has been associated with durable neurocognitive deficits in patients surviving *Escherichia coli* sepsis (Annane and Sharshar, 2015), the levels of lipopolysaccharide (LPS) detected in cases of sepsis are typically higher than those reported following cardiac arrest (2.6 *vs.* < 0.6 EU/ml) (Casey et al., 1993; Grimaldi et al., 2015). And while linked to increased risk of shock and organ failure (Grimaldi et al., 2013), the effects and consequences of serologically undetectable endotoxemia on cerebral ischemia-reperfusion injury remain unexplored.

The pathological changes in the brain caused by global cerebral ischemia (GCI) have been widely studied and involve both the cell-autonomous and non-cell-autonomous effects of cardiac arrest (Blomqvist and Wieloch, 1985; Hossmann and Hossmann, 1973). Models of GCI are often used to model delayed neurodegeneration within the CA1 field of the hippocampus; however, additional regions of the brain including the thalamus, basal ganglia, and cortex may also be affected (Deierborg et al., 2008; Thal et al., 2010). And while selective neuron vulnerability is the *sine qua non* of GCI, associated post-ischemic changes include microgliosis, and damage to the cerebrovasculature characterized by endothelial dysfunction, and loss of blood-brain barrier (BBB) integrity (Yao et al., 2015). Preclinical models of GCI typically involve the isolation of the cerebral and systemic circulation *via* occlusion of 2 or more extracranial vessels (Ito et al., 2013; Pulsinelli and Brierley, 1979; Thal et al., 2010; Yonekura et al., 2004). While intrinsic CNS mechanisms leading to neuronal loss remain an important therapeutic target (Coultrap et al., 2011), GCI models do not recapitulate multi-organ injury and other features of PCAS. Thus, making headway in identifying therapies to disrupt post-arrest neurological decline will require a better understanding of the complex interplay between the brain's intrinsic response to ischemia and the extrinsic changes in the periphery involving cellular immunity as well as the production of other factors with long-range signaling properties.

To better understand the mechanism(s) underlying catastrophic brain injury in PCAS, we studied the effect of low-dose endotoxin challenge on brain injury in a mouse model of GCI. Our results indicate that serologically undetectable endotoxemia increased levels of cortical injury, microglial activation, microvascular injury, and behavioral compromise beyond what is observed after ischemic injury alone. We also observed marked neutrophil (PMN) activation, lung injury, and the accumulation of PMNs in the brain, lung, and other non-ischemic tissues with combined challenge. Our data suggest that therapies geared towards blocking the effects of systemic endotoxin in patients surviving cardiac arrest may be an effective strategy to limit multi-organ dysfunction commonly observed in the post-resuscitation period. Furthermore, these results implicate lung-brain coupling as an important determinant of post-ischemic neurodegeneration mediated through effects on innate immunity.

2. Materials and methods

2.1. Endotoxin titration

All mouse work was performed according to federal regulations with approval by the University Committee on Animal Resources. C57BL/6 mice (5–8-week-old males, 25–30 g, $n = 3$) received intraperitoneal (IP) injections of either saline (SAL) or LPS (50 μg/kg, 4 mg/kg, or 20 mg/kg; derived from *E. coli* O111:B4; Sigma-Aldrich, St. Louis, MO) to mimic endotoxin leak during gut injury. LPS doses were tested to determine which yielded serum endotoxin levels comparable to those observed in patients admitted after cardiac arrest (Adrie et al.,