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Bacterial lipopolysaccharide-induced systemic inflammation alters perfusion of white matter-rich regions without altering flow in brain-irrigating arteries: Relationship to blood-brain barrier breakdown?

Ibtihel Dhaya[a,b,c], Marion Griton[a,b,d], Gérard Raffard[a,b], Mohamed Amri[c], Bassem Hiba[a,b,1], Jan Pieter Konsman[a,b,*,1]

[a] CNRS, Résonance Magnétique des Systèmes Biologiques, UMR 5536, Bordeaux, France
[b] Univ. Bordeaux, RMSB, UMR 5536, Bordeaux, France
[c] Laboratoire de Neurophysiologie Fonctionnelle et Pathologies, UR/11ES09, Faculté des Sciences Mathématiques, Physiques et Naturelles, Université de Tunis El Manar, Tunis, Tunisia
[d] Service de Réanimation Anesthésie Neurochirurgicale, Centre Hospitalier Universitaire (CHU) de Bordeaux, Bordeaux, France

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ABSTRACT

To better understand brain dysfunction during sepsis, cerebral arterial blood flow was assessed with Phase Contrast Magnetic Resonance Imaging, perfusion with Arterial Spin Labeling and structure with diffusion-weighted Magnetic Resonance Imaging in rats after intraperitoneal administration of bacterial lipopolysaccharides. Although cerebral arterial flow was not altered, perfusion of the corpus callosum region and diffusion parallel to its fibers were higher after lipopolysaccharide administration as compared to saline injection. In parallel, lipopolysaccharide induced perivascular immunoglobulin-immunoreactivity in white matter. These findings indicate that systemic inflammation can result in increased perfusion, blood-brain barrier breakdown and altered water diffusion in white matter.

1. Introduction

Nervous system dysfunction often occurs during sepsis or sepsis-associated encephalopathy (Baron et al., 2006; Levy et al., 2003) with altered mental status, ranging from lethargy to coma, being observed in 25–70% of patients (Ebersoldt et al., 2007; Sprung et al., 1990; Wilson and Young, 2003). Septic patients with encephalopathy display reduced global cerebral blood flow compared to healthy controls (Bowton et al., 1989; Maekawa et al., 1991). Moreover, clinical studies using time-of-flight (TOF) Magnetic Resonance Angiography (MRA) have provided evidence of vasospasms in branches of the anterior and middle cerebral arteries (ACA and MCA) during sepsis-associated encephalopathy (Bartynski et al., 2006; Polito et al., 2013). Furthermore, white matter perivascular hyperintensities on FLuid-Attenuated Inversion Recovery (FLAIR) magnetic resonance (MR) images and altered water diffusion in diffusion-Magnetic Resonance Imaging (dMRI) occur during sepsis-associated encephalopathy indicating edema due to blood-brain barrier (BBB) breakdown (Bartynski et al., 2006; Sharshar et al., 2007). Indeed, autopsies of fatal cases of encephalopathy associated with sepsis have shown white matter perivascular edema and hemorrhages (Jackson et al., 1985; Sharshar et al., 2007).

Reduced global cerebral blood flow and lower flow speed in the territory of the MCA are also observed after intravenous (iv) administration of bacterial lipopolysaccharides (LPS) in human volunteers (Brassard et al., 2012; Moller et al., 2002). Time-resolved 3D TOF MRA allowed us to expand some of these clinical observations in rodents and show that blood flow in the first segments of the mouse ACA and MCA is lower after iv LPS injection as compared to administration of its vehicle (Villega et al., 2017). However, it is unknown how these changes relate to vasodilation of downstream subpial arterioles and venules observed with intravital microscopy and increased resting blood flow in the sensory cortex found with laser Doppler after iv LPS administration in

Abbreviations: ACA, anterior cerebral artery; ADC, apparent diffusion coefficient; ASL, aterial spin labeling; Az, pericallosal azygos artery; BBB, blood-brain barrier; cc, corpus callosum; COX-2, cyclooxygenase-2; CP, caudate putamen; dMCA, dorsal branch of middle cerebral artery; dMRI, diffusion-weighted magnetic resonance imaging; ec, external capsule; FA, fractional anisotropy; fi, fornix; GFAP, glial fibrillary acidic protein; IgG, immunoglobulin G; Iba-1, ionized calcium-binding adaptor molecule; IL-1beta, interleukin-1beta; LPS, lipopolysaccharides; MCA, middle cerebral artery; MO, motor cortex; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; NS, non-significant; PhC, Phase contrast; ROI, Regions Of Interest; Sal, saline; SS, somatosensory cortex; st, stria terminalis; T1, T1-weighted imaging; TOF, time-of-flight; vhc, ventral hippocampal commissure

* Corresponding author at: CNRS, UMR 5536, Résonance Magnétique des Systèmes Biologiques, Université de Bordeaux, 146, rue Léo Saignat, 33076 Bordeaux, Cedex, France.
E-mail address: jan-pieter.konsman@u-bordeaux.fr (J.P. Konsman).
[1] Co-senior authors.

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rodents (Rosengarten et al., 2007; Rosengarten et al., 2008a; Ruiz-Valdepenas et al., 2011). Interestingly, in other neuropathologies involving inflammation increased perfusion has been to be closely associated with breakdown of the BBB in subcortical white matter (Ge et al., 2005; Ingrisch et al., 2012). Although changes of CBF and BBB-breakdown have both been shown to occur in clinical sepsis and after systemic LPS administration, their spatial and temporal relationships remain to be determined.

In the present work, we therefore set out to study blood flow in the dorsal branches of the ACA and MCA with Phase Contrast MRI (PhC-MRI) and perfusion of white and grey matter brain structures with Arterial Spin Labeling (ASL) over time after intraperitoneal (ip) LPS administration. In addition, we analyzed white matter microstructure with dMRI. Then, we performed histological stains on the brains of the same animals to detect the vasoactive prostaglandin-synthesizing enzyme cyclooxygenase-2 (COX-2), the expression of which can be up-regulated in response to LPS (Konsman et al., 2004), to assess the presence of perivascular immunoglobulins (Ig) indicating BBB leakage and to study glia cell morphology.

2. Experimental procedures

2.1. Animals

Experiments were conducted according to European recommendations (European Council Directive of 24 November 1986 (86/609/EEC) and European Parliament and Council Directive of 22 September 2010 (2010/63/UE)). Eighteen male Wistar rats (Charles Rivers, L'arbresle, France), weighing a mean 310 g, were housed two per cage in a temperature-controlled room (22° ± 1 °C; humidity 55–60%) on a 12 h dark-light cycle with free access to water and food during one week before the start of experiments. During this acclimation period, animals were manipulated daily and only by persons who took part in the experiment.

2.2. Anesthesia and animal preparation

Rats were anesthetized with isoflurane (induction 3–5%; maintenance 1.5% in air) and equipped with an intraperitoneal catheter that contained either *E. coli*. LPS or its saline vehicle. Animals were next gently positioned in a body restrainer with a tooth-bar and ear-bar-equipped head holder and provided with ECG electrodes and an anal temperature probe while being positioned on a pressure-sensitive pad. Heart and respiration rate as well as colonic temperature were monitored continuously throughout the experiment with thoracic electrodes and an anally-inserted thermocouple, respectively. A thermostatically regulated water flow system maintained colonic temperature between 35.5 and 39 °C thus allowing for some potential fever and hypothermic responses to occur after LPS administration.

2.3. Magnetic resonance imaging

MRI experiments were performed on a 7T horizontal-bore scanner (Advance III console, Bruker, Ettlingen, Germany) equipped with a magnetic field gradient system providing a maximum gradient strength of 650 mT/m, a quadrature coil for radio-frequency emission and a 4-element phased-array surface coil for MR signal reception. Intracolonic temperature was constantly monitored and the animal was heated when colonic temperature dropped below 35.5 °C by warm water circulating in the bed used to position the rat inside MRI scanner. Respiration was assessed with a ventral pressure sensor and heart rate recorded using an MRI compatible electrode. After careful second-order shimming, the following images were acquired.

2.3.1. Anatomical T1-weighted MRI

T1-weighted images were obtained through a FLASH (Fast Low Angle Shot) pulse sequence with the following parameters: Repetition Time [TR]/Echo Time [TE]: 15/2.68 ms; flip angle: 30°; voxel size: 117 × 117 × 234 μm^3; Field Of View (FOV): 30 × 30 × 30 mm^3; acquisition time: 8 min 12 s.

2.3.2. Flow velocity mapping with phase contrast magnetic resonance imaging

A blood velocity map of a three consecutive 0.5 mm thick axial virtual slices of the rat forebrain centered at the level of the anterior commissure were obtained using PhC-MRI (Dumoulin, 1995; Dumoulin et al., 1991; Underwood et al., 1987). The axial level was chosen because flow in the arteries of interest (ventral anterior and middle cerebral arteries, dorsal branches of the latter and pericallosal artery) was perpendicular to the imaging slice. Mean diastolic velocity map was acquired with ECG triggering and the velocity range (velocity encoding) parameter set to 10 cm/s based on previous studies using Doppler ultrasonography (Kreis et al., 2011). The following acquisition parameters were used for velocity maps: TR/TE: 15/5.5 ms; flip angle: 30°; voxel size: 130 × 154 μm^2; FOV: 30 × 25 mm^2; number of averages: 8; mean acquisition duration: around 1 min.

2.3.3. Cerebral perfusion mapping with arterial spin labeling

ASL data were acquired from a single 1.5 mm-thick axial slice, with a FOV of 3.5 × 3.5 cm^2 centered on phase contrast imaging slices, using a Flow-sensitive Alternating Inversion Recovery – Echo Planar Imaging (FAIR-EPI) pulse sequence (Kim and Tsekos, 1997). This placement allowed for measurement of blood perfusion of those areas that were supplied through smaller arteries and arterioles with the flow measured by phase contrast in the dorsal branches of the MCAs and the pericallosal artery and contained important regions of both white (corpus callosum, external capsule) and grey (cortex) matter. The acquisition was performed twice first with selective inversion of the slice and then with a global inversion recovery technique (Kober et al., 2004). For each of these acquisitions, and based on previous publications (Carr et al., 2007), the following parameters were used: TE/TR 30.5/16000 ms; flip angle: 90°; voxel size: 273 × 547 μm^2; number of averages: 6; inversion times: 0.2, 0.6, 0.8, 0.9, 1.0, 1.1, 1.2, 1.5, 2.0, and 2.5 s; acquisition duration: 3 min 12 s.

2.3.4. Diffusion-weighted imaging

A Stejskal-Tanner Echo Planar Imaging (EPI) pulse sequence was used to collect diffusion-weighted images (Stejskal and Tanner, 1965). A pulse sequence based on 3D sampling of Fourier space was used to increase the sensitivity of diffusion-weighted MRI (dMRI) (Renaud et al., 2013). One image was acquired without diffusion weighting (b-value = 0 s/mm^2) and six with diffusion-weighting with a b-value of 1500 s/mm^2 and six different diffusion encoding directions in space. Diffusion-weighted images were recorded with the following parameters: TE/TR = 58.97/1300 ms; voxel size: 203 × 195 × 750 μm^3; FOV: 30 × 25 × 24 mm^3; acquisition duration 4 min 51 s.

2.3.5. Image processing

All pre-treatments, parametric map computing, definition of Regions Of Interest (ROIs) and measurements of ROIs were performed using ParaVision software (Bruker, Ettlingen, Germany). The phase contrast images calibrated for flow velocity (cm/s) were displayed and mean velocities during the diastole of the heart cycle measured in the center of the ACAs, the pericallosal azygos artery as well as of the MCAs and its dorsal branches (Fig. 1). Two (selective and global) T1 maps were computed using selective and global FAIR data sets. Relative cerebral blood flow (rCBF) map was then computed pixel by pixel according to (Kober et al., 2004). Blood T1 was set to 2070 ms as measured previously on rat and bovine blood (Dobre et al., 2007; Esparza-Coss et al., 2010).

To analyze perfusion-weighted ASL images, ROIs corresponding to the global brain or hemisphere, to the grey matter of the superficial and