Journal of Neuroscience Methods 289 (2017) 75–84

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

A general protocol of ultra-high resolution MR angiography to image the cerebro-vasculature in 6 different rats strains at high field

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HIGHLIGHTS

- MR angiography in six different rat strains at high field.
- Robust time-of-flight protocol to image the cerebro-vasculature of the rat.
- Visualization of the hypothalamic and anterior choroidal artery.
- Detection of the posterior inferior cerebellar artery.
- Simple post-processing to aid visualization.

ARTICLE INFO

Article history:
Received 17 March 2017
Received in revised form 6 July 2017
Accepted 6 July 2017
Available online 8 July 2017

Keywords:
High magnetic field
Ischemia models
Magnetic resonance angiography
Maximum intensity projection
Middle cerebral artery occlusion
Post-processing
Rat
Strain dependence
Ultra-high resolution
Visualization

ABSTRACT

Background: Differences in the cerebro-vasculature among strains as well as individual animals might explain variability in animal models and thus, a non-invasive method tailored to image cerebral vessel of interest with high signal to noise ratio is required.
New method: Experimentally, we describe a new general protocol of three-dimensional time-of-flight magnetic resonance angiography to visualize non-invasively the cerebral vasculature in 6 different rat strains. Flow compensated angiograms of Sprague Dawley, Wistar Kyoto, Lister Hooded, Long Evans, Fisher 344 and Spontaneous Hypertensive Rat strains were obtained without the use of contrast agents. At 11.7 T using a repetition time of 60 ms, an isotropic resolution of up to 62 μm was achieved; total imaging time was 98 min for a 3D data set.
Results: The visualization of the cerebral arteries was improved by removing extra-cranial vessels prior to the calculation of maximum intensity projection to obtain the angiograms. Ultimately, we demonstrate that the newly implemented method is also suitable to obtain angiograms following middle cerebral artery occlusion, despite the presence of intense vasogenic edema 24 h after reperfusion.
Comparison with existing methods: The careful selection of the excitation profile and repetition time at a higher static magnetic field allowed an increase in spatial resolution to reliably detect of the hypothalamic artery, the anterior choroidal artery as well as arterial branches of the peri-amygdoidal complex and the optical nerve in six different rat strains.
Conclusions: MR angiography without contrast agent can be utilized to study cerebro-vascular abnormalities in various animal models.

1. Introduction

Studies of cerebral ischemia are hampered by variability in infarct size 24–48 h following middle cerebral artery occlusion (MCAO) (Fox et al., 1993), importantly affecting all strains to various degrees (Sauter and Rudin, 1995; Howells et al., 2010). Hereby, potential differences in the cerebro-vasculature between strains may explain the difference, especially with respect to collateral

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http://dx.doi.org/10.1016/j.jneumeth.2017.07.003

flow to areas affected by MCAO. One method to visualize anatomical differences of the cerebro-vasculature between individuals and strains is magnetic resonance angiography (MRA) (Reese et al., 1999). Time-of-flight (TOF) MRA methods rely on the flow related enhancement (FRE) of blood entering into an imaging slab from one end (Bernstein et al., 2004). Stationary signal is successfully suppressed by the use of repetitive excitation, whereby freshly inflowing blood gives rise to increased signal intensity in comparison to stationary signal. Alternatively, ultra-high resolution angiograms of the rat cerebral vasculature were recorded in 7 h 16 min using Gd(DTPA) as an intravascular contrast agent (Mellin et al., 1994).

In theory, MRA can be performed before, in naïve animals, as well as during and/or after MCAO. Generally, when animals arrive at the imaging facility, they could be pre-screened by MRI to evaluate their suitability for experimental stroke models. Hereby, animals with abnormal cerebral arteries or enlarged ventricles, hydrocephalus could be identified and excluded from the study prior to surgery to reduce the variability measure of outcome. During MCAO, a successful occlusion could be visualized by MRA (Dittmar et al., 2006; Besselmann et al., 2001) in parallel with measures that detect a reduction in perfusion in the affected area like arterial spin labeling MRI or laser Doppler flowmetry (Kloiber et al., 1993; Lythgoe et al., 2000; Yushmanov et al., 2002; Bardutzky et al., 2005; Weber et al., 2006; Gao et al., 2014; Cuccione et al., 2016). Likewise, immediately following a transient MCAO, a successful reperfusion might be visualized by MRA and confirmed by perfusion readouts to exclude animals and minimize experimental variability within the study cohort (Reese et al., 1999). As putative treatment regimen might influence cerebral perfusion, a characterization of the MCA branching off from the circle of Willis might be the only direct measure to detect abnormalities in the cerebro-vasculature within a study cohort to justify an inclusion or exclusion of a specific animal. An acquisition of MRAs days or weeks following cerebral ischemia would offer the advantage to be able to schedule the imaging session as an extension of routine experiments required to obtain the infarct size and location by T2-weighted MR. Importantly, as longitudinal MR studies often require multiple imaging sessions, one has to keep in mind that they are labor intensive and expensive with respect to data acquisition and analysis, a focus on animals that will be ultimately included in the study report will be beneficial. The benefit to identify unwanted outliers early will increase with the complexity of the study design, e.g. when behavioral and functional MR and positron emission tomography (PET) studies are interleaved. Thus, generic advantages of the use as well as the disadvantages of MRA in experimental stroke models are briefly discussed.

The purpose of this work is to establish a robust protocol of 3D-time-of-flight (TOF) MRA suitable to image the arterial vasculature of the rat without the use of contrast agents in different strains. Several imaging protocols differing in the required spatial and temporal resolution were utilized to improve upon previously reported protocols (Reese et al., 1999). The methods were then applied to an experimental stroke model of 60 min transient MCAO using an intra-luminal thread (Koizumi et al., 1986; Belayev et al., 1996). The 3D-TOF MR angiograms were obtained in the rat 24 h after re-perfusion, in the same anesthetic session that acquired the T2-weighted images to derive the infarct size.

2. Material and methods

2.1. Animals

All animal procedures had been reported according ARRIVE guidelines and were approved by the ethical committee of CIC biomaGUNE, an AAALAC accredited institution, and local authorities, according to the specific Spanish (RD 53/2013) and European Union (Directive 2010/63/EU) legislation.

2.1.1. Different rat strains

Healthy male rats of six different strains (n = 2 of each strain, 240–345 g) were used in this study. Sprague Dawley (SD), Wistar Kyoto (WK), Spontaneous Hypertensive Rats (SHR) and Long Evans (LE) provided by Janvier Labs (Saint-Berthevin Cedex, France) and Fischer 344 (FI) and Lister Hooded (LH) rats by Charles River Laboratories (Calco, Italy) were allowed to rest in individually ventilated cages, with an enriched environment for animal welfare, for at least five days following transport and had free access to food and water.

2.1.2. Transient ischemia in Sprague Dawley rats

The experimental procedure was performed following criteria derived from the Stroke Therapy Academic Industry Roundtable (STAIR) group guidelines for preclinical evaluation of stroke therapeutics (Liu et al., 2009; Fisher et al., 2009): (1) the vascular recirculation was confirmed by MRA, as an index of the reliability of the ischemic model; (2) silicone rubber coated filaments were used to produce consistent ischemic injuries; (3) standard sized occluder were used depending on the animal strain and size; (4) the temperature was controlled during the ischemic period; (5) this study serves as a pilot study to evaluate putative neuroprotective treatment regimen in the future

Transient MCAO was carried out according to published procedures with minor modifications (Koizumi et al., 1986). Briefly, rats were anesthetized with isoflurane (4–5% induction, 2–2.5% maintenance) in O_2/N_2 (30/70) administered via a face mask. Through a ventral midline neck incision the right common carotid artery (CCA) was exposed up to the bifurcation of internal (ICA) and external carotid artery (ECA) and clamped. The ECA was ligated with a 6–0 silk suture. A silicone rubber coated thread (Doccol Corp. Sharon MA, USA) was introduced into the ECA and advanced into the lumen of the ICA up to a length of 17–18 mm to occlude the MCA. Recirculation was initiated after 1 h by removing the thread and the CCA clamp. Using this procedure, the CCA is reopened and the blood flow to the cerebral hemisphere is re-established. Only vascular branches fed by the ipsilateral ECA remained permanently occluded. The incision wound was finally closed with a 4.0 silk suture and the rat brought back to its home cage.

2.2. Magnetic resonance imaging of different rat strains

Experiments were carried out using Biospec spectrometer (Bruker, Karlsruhe, Germany). The 11.7 T 16 cm bore magnet is equipped with a 9 cm gradient capable of switching 750 mT/m in 90 μs. A 40 mm 1H-resonator was used for RF-transmission and reception.

Animals were anesthetized prior to imaging using 3–4% v/v isoflurane and maintained at 2–2,5% in a gas mixture of O_2/N_2 1:1. The anesthetized rats were placed in sphinx position in a MRI compatible cradle. Rectal temperature was kept at $36 \pm 1\,^{\circ}C$ using heated water blanket and the respiration rate was continuously monitored (MRI compatible animal monitoring system Model 1030, SA Instruments Inc., Stony Brook, NY, USA) to assure the anesthesia depth. Following the automatic calibration (pulse power, shim, and resonance frequency) a high resolution RARE (rapid acquisition with relaxation enhancement) sequence was used with the following parameter: field of view (FOV) 40 mm, image matrix 256×256, 24 slices of 1 mm thickness, pixel resolution 0.156 mm/pixel, either to determine the brain volume in naïve animals or to determine the stroke volume 24 h of 60 min tran-