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Full-length Article

Using functional and molecular MRI techniques to detect neuroinflammation and neuroprotection after traumatic brain injury

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ABSTRACT

This study was designed to investigate whether functional and molecular MRI techniques are sensitive biomarkers for assessment of neuroinflammation and drug efficacy after traumatic brain injury (TBI) in rats. We subjected rats to a controlled cortical impact model and used behavioral tests, histology, and immunofluorescence to assess whether flavonoid pinocembrin provides cerebral protection and improves functional recovery. Most importantly, we used multiple noninvasive structural, functional, and molecular MRI techniques to examine whether the pinocembrin-related neuroprotection and attenuation of neuroinflammation can be detected *in vivo*. Significant increases in cerebral blood flow (CBF) and amide proton transfer-weighted (APTw) MRI signals were observed in the perilesional areas in untreated TBI rats at 3 days and could be attributed to increased glial response. In addition, increased apparent diffusion coefficient and decreased magnetization transfer ratio signals in untreated TBI rats over time were likely due to edema. Post-treatment with pinocembrin decreased microglial/macrophage activation at 3 days, consistent with the recovery of CBF and APTw MRI signals in regions of secondary injury. These findings suggest that pinocembrin provides cerebral protection for TBI and that multiple MRI signals, CBF and APTw in particular, are sensitive biomarkers for identification and assessment of neuroinflammation and drug efficacy in the TBI model.

1. Introduction

Traumatic brain injury (TBI) is a serious public health problem that affects an estimated 1.7 million Americans annually (CDC statistics, 2010). It is a complex injury that results in primary injury and secondary injury cascades. Variable outcomes of patients after TBI and the multiple definitions of TBI make interpreting results across research studies challenging (Amyot et al., 2015). Currently, no drug is effective for treatment of TBI, and clinical trials of neuroprotective drugs have not shown clear benefit in reducing or preventing secondary brain damage (McConeghy et al., 2012). Therefore, it is imperative to develop an effective treatment for TBI.

Pinocembrin (5,7-dihydroxyflavanone) is a natural flavonoid compound extracted from honey, propolis, ginger root, wild

Abbreviations: APTw MRI, amide proton transfer-weighted MRI; CBF, cerebral blood flow; CCI, controlled cortical impact; TE, echo time; GFAP, glial fibrillary acidic protein; mNSS, modified neurologic severity score; MRI, magnetic resonance imaging; MTR, magnetization transfer ratio; ROI, region of interest; rpm, revolutions per minute; TBI, traumatic brain injury; TR, repetition time.

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marjoram, and other plants (Lan et al., 2016; Rasul et al., 2013). In the last few years, preclinical studies have shown that it has anti-inflammatory (Lan et al., 2017; Saad et al., 2015) and neuroprotective effects (Kapoor, 2013; Wang et al., 2016b), as well as the ability to reduce reactive oxygen species (Wang et al., 2016b), protect the neurovascular unit (Liu et al., 2014), and modulate mitochondrial function (de Oliveira et al., 2016). It can also regulate apoptosis (Saad et al., 2015; Wu et al., 2013), detoxification, and immunity genes (Mao et al., 2013). The pharmacokinetics and pharmacodynamics of pinocembrin recently have been reported in rats (Sayre et al., 2015) and in humans (Cao et al., 2015; Yan et al., 2014). Furthermore, pinocembrin has been authorized by the State Food and Drug Administration of China for clinical trials in patients with ischemic stroke, and phase II trials have begun (ClinicalTrials.gov Identifier: NCT02059785) (Yan et al., 2014). Nevertheless, its potential efficacy in TBI has not been tested.

The application of neuroimaging techniques, such as magnetic resonance imaging (MRI), has considerably advanced our understanding of various complex neurologic disorders (Yang et al., 2017). Preclinical and clinical data have indicated that MRI is critical for assessing structural and functional changes of TBI that are related to the pathophysiology and clinical manifestations of neurologic disorders (Jaiswal, 2015; Long et al., 2015; Shultz et al., 2015). MRI is commonly used to ascertain measures of structure (T_2 and T_1) and function (apparent diffusion coefficient [ADC, which measures the diffusion rate of water molecules] and cerebral blood flow [CBF]). Further, scientists are investigating whether molecular MRI techniques can be used to assess some neurologic diseases and their response to therapy (Amyot et al., 2015; Zhou et al., 2003, 2011). For example, it has recently been shown that protein-based amide proton transfer (APT) MRI can accurately detect hyperacute intracerebral hemorrhage and distinctly differentiate intracerebral hemorrhage from cerebral ischemia in rats (Wang et al., 2015). APT imaging is a molecular MRI method that can noninvasively detect endogenous mobile protein concentration and tissue pH changes. In this study, we subjected rats to a controlled cortical impact (CCI) TBI model and used behavioral tests, histology, and immunofluorescence to assess whether pinocembrin provides cerebral protection and improves functional recovery. We also used multiple noninvasive structural, functional, and molecular MRI techniques to examine whether the pinocembrin-related neuroprotection and attenuation of neuroinflammation can be detected *in vivo*.

2. Materials and methods

2.1. Animals

All animal studies were conducted in accordance with National Institutes of Health guidelines and were approved by the Johns Hopkins University Animal Care and Use Committee. Forty-one adult, male Sprague-Dawley rats (300–350 g) were obtained from Charles River Laboratories (Frederick, MD) and maintained in the Johns Hopkins animal facility. All efforts were made to minimize the numbers of animals used and ensure minimal suffering. Animal experiments were reported in accordance with the ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines (www.nc3rs.org.uk/arrive-guidelines).

2.2. CCI model of TBI

Rats were anesthetized initially with 5% isoflurane and maintained with 1.5%–2% isoflurane in oxygen-enriched air (20% oxygen/80% air) with spontaneous ventilation. CCI injury was induced with a PSI TBI-0310 Impactor (Precision Systems and Instrumentation, Fairfax, VA), which uses electromagnetic force to produce an impact velocity for which speed, depth, and dwell time can each be individually manipulated to produce injuries with different severities (Cheng et al., 2016). The rats were placed on a stereotactic frame with a built-in heating bed that maintains body temperature at 37 °C. The head was mounted in the stereotactic frame. Under aseptic conditions, a midline longitudinal incision was made over the skull, and a 5-mm craniotomy was made using a portable drill and trephine over the left parietal cortex (center of the coordinates of the craniotomy relative to bregma: 1 mm posterior, 1 mm lateral). The bone flap was removed. A pneumatic cylinder with a 3-mm flat-tip impounder produced CCI in the rats at a velocity of 5.5 m/s, depth of 5 mm, and impact duration of 65 ms. The scalp was closed with cyanoacrylate tissue glue. Rats of the sham group received a scalp incision, but the skull was kept intact. After they resumed locomotor activity, the rats were returned to their home cages.

2.3. Experimental groups and administration of drugs

Pinocembrin was dissolved in sterile saline. Rats were randomly divided (http://www.randomization.com) (Han et al., 2016) into four groups: sham (5 rats), TBI + vehicle (sterile saline; 20 rats), TBI + pinocembrin 5 mg/kg (11 rats), and TBI + pinocembrin 10 mg/kg (5 rats). An investigator blinded to treatment administered pinocembrin or an equal volume of saline by tail vein injection at 30 min after CCI and again 1, 2, and 3 days post-CCI. Sham rats were administered an equivalent volume of saline at 30 min after craniotomy. We chose the delivery route, dosing, and treatment regimens for pinocembrin based on previous work (Guang and Du, 2006; Meng et al., 2014; Shi et al., 2011; Wu et al., 2013).

2.4. MRI data acquisition

Imaging experiments were performed on a 4.7 T animal MRI system (Bruker Biospin, Billerica, MA) with an actively decoupled cross-coil setup (a 70-mm body coil for radiofrequency transmission and a 25-mm surface coil for signal reception). MRI data were acquired at six time points (within 1 h and 1, 3, 7, 14, and 28 days after TBI). First, axial/coronal T_2w images were acquired with the following parameters: repetition time (TR) = 3 s; echo time (TE) = 64 ms; 5 slices; thickness = 1.5 mm; field of view = $42/32 \times 32$ mm^2; matrix = $256/192 \times 192$; number of averages (NA) = 2. Then, several quantitative MRI parameters were acquired with previously described methods, including T_2 (TR = 3 s; TE = 30, 40, 50, 60, 70, 80, and 90 ms; NA = 4), T_1 (inversion recovery; predelay = 3 s; TE = 30 ms; inversion recovery times = 0.5, 0.3, 0.6, 1.2, 1.8, 2.5, and 3.5 s; NA = 4), isotropic ADC (TR = 3 s; TE = 80 ms; b-values = 0, 166.7, 333.3, 500, 666.7, 833.3, and 1000 s/mm^2; NA = 8), CBF (arterial spin labeling; 3-s labeling at a distance of 20 mm away from the imaging slice; TR = 6 s; TE = 28.6 ms), APT (frequency-labeling offsets of ±3.5 ppm; TR = 10 s; TE = 30 ms; saturation power = 1.3 μT; saturation time = 4 s; NA = 16), and conventional MTR (with the same experimental parameters as APT, except a saturation frequency offset of 10 ppm, namely, 2000 Hz at 4.7 T).

2.5. Image analysis

All MRI data were processed by using Interactive Data Language V7 (Exelis Visual Information Solutions, Inc., Boulder, CO) and previously described methods (Zhou et al., 2003, 2011). Lesion volumes were measured manually as the sum of all injury voxels in all slices on the high-resolution T_2w images. After being interpolated to 384×384, the T_2 map, T_1 map, and ADC map were fitted by using the following equations: $I = I_0 \exp(-TE/T_2)$, $I = A + B \exp$