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Voxel-based morphometry and histological analysis for evaluating hippocampal damage in a rat model of cardiopulmonary resuscitation

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ABSTRACT

Cardiac arrest and subsequent cardiopulmonary resuscitation (CPR) induce hippocampal damage, which has been identified using histological analysis of post-mortem brains. Voxel-based morphometry (VBM), an in-vivo assessment of regional differences in the concentration or volume of a particular tissue such as gray matter, has revealed CPR-induced decreases in gray matter in the hippocampus, where histopathological findings were observed. However, the potential link between the changes in gray matter detected by VBM and hippocampal damage has not been investigated directly. In this study, we compared results obtained using VBM directly to results from histological analyses in the same CPR rat brains, which exhibited neuronal loss and microglial invasion in the CA1 region of the hippocampus (CA1). T2-weighted images were obtained and preprocessed for VBM to produce gray matter concentration (GMC) maps in rats with asphyxia-induced cardiac arrest and CPR and sham-operated controls ($n = 12$ each). Brains were fixed, and the number of neurons and microglia in CA1 were counted. VBM revealed a significant decrease in GMC in CPR rats compared to sham-operated controls. The CPR-induced decrease in GMC was localized to CA1, which is the same brain region where neuronal loss and microglial invasion were noted in response to CPR. GMC values were positively correlated with the number of neurons and tended to be negatively correlated with the number of microglia in CA1 of CPR rats. In conclusion, these results indicate that VBM-detected alterations in gray matter can be used as a surrogate marker for hippocampal damage following CPR.

Introduction

Sudden cardiac arrest is a disastrous situation that results in death without immediate efforts of cardiopulmonary resuscitation (CPR). One of the most significant problems reported by survivors after CPR is the complication of long-term neurological and cognitive deficits (Lim et al., 2004; Mateen et al., 2011; Roine et al., 1993) that can result from damage to gray matter regions of the brain that are vulnerable to hypoxia, such as the hippocampus (Cummings et al., 1984; Horstmann et al., 2010; Press et al., 1989; Volpe and Petito, 1985; Zola-Morgan et al., 1986). Hippocampal damage following CPR has been originally identified using histological analysis of post-mortem brains (Cummings et al., 1984; Press et al., 1989; Volpe and Petito, 1985; Zola-Morgan et al., 1986). More recently, voxel-based morphometry (VBM), an unbiased objective technique based on magnetic resonance imaging (MRI), has been used to assess regional differences in the concentration or volume of a particular tissue such as gray matter in animals and humans (Ashburner and Friston, 2000, 2001; Biedermann et al., 2012; Quallo et al., 2009; Sawiak et al., 2009; Yang et al., 2011). In survivors after CPR, VBM has revealed decreases in gray matter in several brain regions, including the hippocampus (Horstmann et al., 2010), where histopathological findings were observed. However, the potential link between the changes in gray matter detected by VBM and CPR-induced hippocampal damage has not been investigated directly.

A rat model of cardiac arrest and subsequent CPR has been shown to be useful in investigating the mechanisms underlying CPR-induced brain damage (Empey et al., 2012; Katz et al., 1995; Keilhoff et al., 2010, 2011; Liu et al., 2012; Shoykhet et al., 2012). Studies using this model have revealed cellular events in the hippocampus that are similar to those observed in humans, such as neuronal cell death and microglial invasion in the CA1 region of the hippocampus (CA1) (Katz et al., 1995; Keilhoff et al., 2010, 2011; Shoykhet et al., 2012). However, VBM has not revealed CPR-induced alterations in gray

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matter in rats. We previously reported an in-vivo rat T2 MRI template that includes three classes of probability segmentations, which enables us to perform VBM on rat brains (Valdés-Hernández et al., 2011). In animal VBM studies, it is possible to use histological analyses of post-mortem brains to validate VBM-detected gray matter changes. In the present study, we compared results obtained using VBM directly to results from histological analyses of CA1 in the same CPR rat brains. The purpose is to investigate whether VBM-detected alterations in gray matter can be used as a surrogate marker for hippocampal damage in CPR rats. The hypotheses are as follows: (1) CPR induces gray matter changes that can be detected by VBM in brain regions including CA1, and (2) the gray matter changes detected by VBM are associated with the number of neurons and/or microglia in CA1.

Materials and methods

Animals

A total of 24 male Sprague–Dawley rats (11 week-old; SLC, Shizuoka, Japan) were assigned to either the CPR group or the sham-operated group, which received anesthesia and vessel cannulations but not cardiac arrest (n = 12 each). No significant differences in the body weight were observed in CPR (365 ± 7 g) and sham-operated rats (362 ± 7 g) (P = 0.731). All procedures and protocols were performed in accordance with the policies established by the Animal Care Committee at Tohoku University, Sendai, Japan (approval number: 2012-241).

The CPR rats

CPR rats were subjected to cardiac arrest and a subsequent CPR protocol that has been described previously (Empey et al., 2012; Katz et al., 1995; Keilhoff et al., 2010, 2011; Liu et al., 2012; Shoykhet et al., 2012). Rats were anesthetized with isoflurane, and were placed in the prone position on a hotplate (AS ONE, Osaka, Japan) to maintain their rectal temperature at 37.0 ± 1.0 °C throughout the entire procedure as monitored using a thermometer (Unique Medical Co., Ltd., Tokyo, Japan). Surgical preparations were performed under 3% isoflurane as follows. Polyethylene catheters were inserted into the femoral artery and vein for examining arterial blood pressure and systemic drug delivery, respectively. Blood pressure waves transmitted from the arterial polyethylene catheter were digitized by a pressure transducer (DTXPlus™, BD, Franklin Lakes, NJ, USA), amplified by an MEG-6108 amplifier (Nihon Kohden Corporation, Tokyo, Japan), and analyzed by a PowerLab/16SP and LabChart 6 device (ADInstruments, Inc., Colorado Springs, CO, USA). Subsequently, rats were orally intubated, mechanical ventilation was initiated at a respiration rate of 50 breaths/min by a ventilator (Harvard Apparatus, Holliston, MA, USA), and isoflurane was washed out with room-air for 5 min after the intravenous administration of vecronium (2 mg/kg) for immobilization. After the 5-min washout, asphyxia was induced by disconnecting the ventilator and plugging the tracheal tube, which resulted in cardiac arrest within approximately 4 min (Fig. 1). After 8 min of this apnea and airway obstruction, CPR was initiated by starting mechanical ventilation with 100% O_2 at a frequency of 50 breaths/min, administering epinephrine (0.02 mg/kg) followed by sodium bicarbonate (1 mmol/kg) intravenously, and applying sternal compression at a rate of 200/min until restoration of spontaneous circulation (ROSC) was achieved. CPR was stopped if ROSC was not obtained within 2 min. At 60 min after the start of CPR, ventilation was withdrawn, extubation was performed with no reversal medication for vecronium, the catheters were removed, the vessels were ligated, and the skin was closed. The CPR rats were returned to their cages where they were housed for 4 weeks in a room on a 12-h light–dark cycle until the time of MRI recordings.

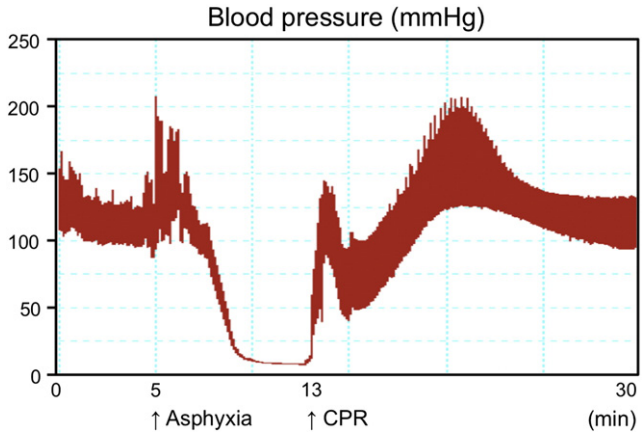

Fig. 1. A representative image of blood pressure monitoring during the cardiac arrest and subsequent CPR protocol. CPR, cardiopulmonary resuscitation.

MRI recordings

Four weeks after the CPR or sham protocol, T2 anatomical MRI images for VBM were obtained from CPR and sham-operated rats (n = 12 each, 429 ± 11 g and 456 ± 11 g, respectively, P = 0.083). Subsequent animal preparations for MRI recordings were performed as described in our previous studies (Sumiyoshi et al., 2012). Briefly, rats were initially anesthetized with isoflurane, and polyethylene catheters were inserted into the femoral artery and vein to examine blood pressure and systemic drug delivery, respectively. The rats were orally intubated for artificial ventilation, were placed in the prone position on a custom-built MRI bed with a bite bar, and mechanically ventilated at a respiration rate of 60 ± 1 breaths/min using a ventilator (SAR-830/AP, CWE Inc., Ardmore, PA, USA). After the rats received a bolus injection of pancronium (2 mg/kg), anesthesia was maintained with 1.5% isoflurane and the continuous administration of pancronium (2 mg/kg/h).

All MRI data were acquired using a 7.0-T Bruker PharmaScan system (Bruker BioSpin, Ettlingen, Germany) with a 38-mm-diameter bird-cage coil. Prior to all MRI acquisitions, we first performed global magnetic field shimming inside the core and later completed it at the region of interest (ROI) using a point resolved spectroscopy protocol (Sumiyoshi et al., 2012). The line width (full width at half maximum) at the end of the shimming procedure ranged from 10 to 16 Hz in the ROI (approximately 300 μl). T2-weighted images (T2WI) were obtained using a 2D-RARE sequence with the following parameters: TR = 4600 ms, TEeff = 30 ms, RARE factor = 4, SBW = 100 kHz, flip angle = 90°, FOV = 32 × 32 mm^2, matrix size = 256 × 256, voxel size = 125 × 125 μm^2, number of slices = 54, slice thickness = 0.5 mm, slice gap = 0 mm, and number of repetitions = 10.

VBM analysis

All T2WIs were analyzed using the statistical parametric mapping software (SPM8, Wellcome Department of Cognitive Neurology, London, UK) and custom-written software in MATLAB (MathWorks Inc., Natick, MA, USA). In the present study, VBM was performed using a method that was modified as previously applied in humans (Taki et al., 2012). First, the T2WIs were resized by a factor of 10 (Biedermann et al., 2012) and were realigned and resliced to adjust for head motion. The realigned anatomical images were then averaged to produce mean images. Second, after the mean images were aligned with the Wistar rat template brain (Valdés-Hernández et al., 2011), they were segmented into images of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) by applying a unified segmentation approach (Ashburner