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Neuroprotective effect of agmatine in rats with transient cerebral ischemia using MR imaging and histopathologic evaluation

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ABSTRACT

Purpose: This study aimed to further investigate the effects of agmatine on brain edema in the rats with middle cerebral artery occlusion (MCAO) injury using magnetic resonance imaging (MRI) monitoring and biochemical and histopathologic evaluation.
Materials and methods: Following surgical induction of MCAO for 90 min, agmatine was injected 5 min after beginning of reperfusion and again once daily for the next 3 post-operative days. The events during ischemia and reperfusion were investigated by T2-weighted images (T2WI), serial diffusion-weighted images (DWI), calculated apparent diffusion coefficient (ADC) maps and contrast-enhanced T1-weighted images (CE-T1WI) during 3 h–72 h in a 1.5 T Siemens MAGNETON Avanto Scanner. Lesion volumes were analyzed in a blinded and randomized manner. Triphenyltetrazolium chloride (TTC), Nissl, and Evans Blue stainings were performed at the corresponding sections.
Results: Increased lesion volumes derived from T2WI, DWI, ADC, CE-T1WI, and TTC all were noted at 3 h and peaked at 24 h–48 h after MCAO injury. TTC-derived infarct volumes were not significantly different from the T2WI, DWI-, and CE-T1WI-derived lesion volumes at the last imaging time (72 h) point except for significantly smaller ADC lesions in the MCAO model ($P < 0.05$). Volumetric calculation based on TTC-derived infarct also correlated significantly stronger to volumetric calculation based on last imaging time point derived on T2WI, DWI or CE-T1WI than ADC ($P < 0.05$). At the last imaging time point, a significant increase in Evans Blue extravasation and a significant decrease in Nissl-positive cells numbers were noted in the vehicle-treated MCAO injured animals. The lesion volumes derived from T2WI, DWI, CE-T1WI, and Evans blue extravasation as well as the reduced numbers of Nissl-positive cells were all significantly attenuated in the agmatine-treated rats compared with the control ischemia rats ($P < 0.05$).
Conclusion: Our results suggest that agmatine has neuroprotective effects against brain edema on a reperfusion model after transient cerebral ischemia.

1. Introduction

Magnetic resonance imaging (MRI) technique is by far superior to ultrasound [1–3] and computed tomography [4,5] to describe central nervous system injury and to time the onset of the lesion [6]. MRI techniques have been used in experimental animals for early detection and delineation of the area at risk for ischemic injury in the brain [7]. Experimental studies have utilized a model of unilateral hypoxic–ischemic brain injury in the immature rats [8] and hyper-intensities in both diffusion- and T2-weighted images

Abbreviations: MCAO, middle cerebral artery occlusion; MRI, magnetic resonance imaging; T2WI, T2-weighted images; DWI, diffusion-weighted images; ADC, apparent diffusion coefficient; CE-T1WI, contrast-enhanced T1-weighted images; TTC, triphenyltetrazolium chloride; BBB, blood–brain-barrier; PBS, phosphate-buffered saline; ROI, region of interest; ANOVA, analysis of variance; EB, Evans blue; AQP4, aquaporin 4.

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(DWI and T2WI) reveal a unique alteration in brain water dynamics [9,10]. Early ischemic injury in P7 rat brains was detected as an increase in hyper-intensities in both T2WI and DWI [11]. They promoted that T2WI and DWI allowed to distinguish between different patterns of injury in reversible ischemia [11]. The blood–brain-barrier (BBB) permeability and leakage space can be measured by CE-T1WI (contrast enhanced T1-weighted image) [12,13]. A more recent report demonstrated that ischemic lesion determination by apparent diffusion coefficient (ADC) was more accurate in final infarct prediction [10].

Agmatine is synthesized in the mammalian brain by the decarboxylation of L-arginine [14]. It is known to be neuroprotective against transient focal cerebral ischemia in rodents [15–17]. In a cat model, protective effect of agmatine on a reperfusion after cerebral ischemia was evaluated by using MR perfusion imaging and histopathologic analyses [18]. Additionally, beneficial effect of agmatine on brain apoptosis, astrogliosis, and edema after rat transient cerebral ischemia was observed [19].

Our previous results showed that agmatine treatment was found to accelerate recovery of motor and proprioception deficits and to prevent brain infarction, gliosis, edema (cerebral water contents), apoptosis, and neurotoxicity (inducible nitric oxide synthase expression) during MCAO ischemic injury in rats [15]. The aims of the present study were to further evaluate the neuroprotective effect of agmatine in the same animal model by using MRI (including T2WI, DWI, CE-T1WI, and ADC), measuring transient breakdown of the blood–brain-barrier (BBB) (assessed by Evans blue extravasation), and determining loss of brain cells (assessed by Nissl staining) [20].

2. Materials and methods

2.1. Animals and stroke model

Adult male Sprague–Dawley rats (weight, 253 ± 10 g) were housed under controlled environmental conditions with ambient temperature of 22 ± 1 °C, relative humidity of 65% and 12-h light/dark cycle, with free access to food and water. Brain focal ischemia was induced by MCAO in rats by intraluminal filament, using the relatively non-invasive technique detailed previously [15,16].

2.2. Experimental design

Animals were randomly assigned to sham-operated group (n = 32), MCAO rats treated with saline (1 mg/kg, i.p.) (n = 32), or MCAO rats treated with agmatine (Sigma Chemical Co., St. Louis, Mo, USA) (100 mg/kg, i.p.) 5 min after beginning of reperfusion and again once daily for the next 3 post-operative days [15,21].

2.3. Cerebral infarction assessment

Rats were with deep anesthesia and were transcardially perfused with heparinized 0.05-mol/L phosphate-buffered saline (PBS) followed by ice-cold 15% sucrose in PBS. The brains were rapidly removed and frozen in liquid nitrogen and then sectioned for immunohistochemistry as detailed previously [15]. In brief, brain slices were stained in a solution containing 2% 2,3,5-triphenyltetrazolium chloride (TTC) in saline, at 37 °C. Infarct volume (mm^3), as revealed by negative TTC stains indicating dehydrogenase-deficient tissue, was measured in each slice and summed using computerized planimetry (PC-based image tools software). The volume of infarction was calculated as 2 mm (thickness of the slice) × (sum of the infarction area in all brain slices [mm^2]) [22].

2.4. MR imaging

MRI was performed immediately at 3, 24, 48 and 72 h after reperfusion. MRI was conducted with a 1.5 T Siemens MAGNETOM Avanto scanner (Erlangen, Germany) using a commercially available CP extremity coil. Rats were lightly anesthetized using urethane (500 mg/ml) and were pronely positioned in a plastic holder which was placed into the coil. The right femoral vein of rats was cannulated for contrast agent injection.

For each imaging sequence, 10 coronal images were acquired with a slice thickness of 2 mm gapped at 0.2 mm. T2-weighted turbo-spine echo images (T2WI) were obtained with a repetition time ms/echo time ms of 3920/116, field of view (FOV) of 80 × 80 mm and image acquisition matrix of 192 × 192, which led to an in-plane resolution of 0.4 × 0.4 mm. Diffusion-weighted images (DWI) were obtained by using a two-dimensional spin-echo echo-planar imaging sequence (SE-EPI) with 3000/96, field of view of 104 × 104 mm and image acquisition matrix of 100 × 50, which led to an in-plane resolution of 1 × 2 mm. Quantitative maps of the apparent diffusion coefficient (ADC) were calculated based on three different *b*-values (0,400 and 800 s/mm^2). Contrast enhanced T1-weighted images (CE-T1WI) were obtained with 300/10, field of view of 80 × 80 mm and image acquisition matrix of 128 × 128. Delayed (10–20 min) postcontrast T1-weighted images, after a triple-dose (0.3 mmol/kg) of manual bolus injection of Omniscan (Ameasham S.A., Norway) in less than 2 s with 1 cc saline flush, were conducted to detect contrast enhancement of the cerebral ischemic lesions due to blood–brain-barrier (BBB) breakdown and angiogenesis.

The image analysis was performed using dedicated Onis 2.3 software (ONIS™, Digital Core Co., Ltd., Tokyo, Japan). For the quantification of lesion size on T2WI, DWI, CE-T1WI, and ADC maps, the lesion and brain areas in the same slice were delineated by two authors with consensus using an operator-defined region of interest (ROI) on each of the lesion-containing slices, and the volume of the lesion and brain regions were then summed for each animal. The entire contra lateral brain areas were also measured by using the same methods. The area of the different structures involved in the ischemic process was delineated on the images according to rescaled drawings from the Paxinos and Watson atlas [23]. The hyper intense pixels in the ipsilateral cortex were then selected as those with a significantly higher signal ($P < 0.05$) than in the contra lateral cortex. The volumes of hyper intensities were determined as the sum of the hyper intense area in each slice multiplied by the slice thickness. For comparison between the different animals, these volumes were then expressed as a percentage of the volume of the contra lateral hemisphere (mean ± S.E.M.).

2.5. Evans blue extravasation assay

The integrity of the blood–brain-barrier was investigated using Evans-Blue extravasation as previously described with some modifications [20]. Evans Blue dye (Sigma-Aldrich, St, Louis, MO, USA; 10%, 5 mL/kg) in 0.9% saline was injected into the right femoral vein at either 3, 24, 48 or 72 h after the onset of MCAO. After the dye had circulated for 120 min, rats were anesthetized with pentobarbital sodium (0.1 g/kg, i.p.) and transcardially perfused with physiological saline. Brains were removed and each hemisphere was weighted, homogenized in PBS, and centrifuged at 2000 × g for 15 min at 4 °C. Following homogenization and centrifugation, the extracted dye was diluted with formaldehyde (1:5). After overnight incubation and centrifugation at 3500 × g for 30 min at 4 °C, the supernatant was taken for spectrophotometric quantification