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Neuroprotective nature of adipokine resistin in the early stages of focal cerebral ischemia in a stroke mouse model

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ABSTRACT

New evidence suggests that resistin may have a therapeutic potential effect in management of neurodegenerative disease; but its role in the pathophysiology of stroke-induced injuries is not understood. However, further investigations are required to elucidate the effect of resistin and explore its possible molecular mechanisms on the ischemic reperfusion injury. Transient focal cerebral ischemia was induced by the middle cerebral artery occlusion (MCAO) in mice. Animal treated with resistin at doses of 25, 50, 100, 200, and 400 ng/mouse, on the MCAO commencement. Neurological function, infarct size, brain edema and Blood-brain barrier (BBB) disruption were measured. Additionally, content of malondialdehyde (MDA), TUNEL-positive cells and apoptosis-related proteins were assessed by immunohistochemistry and western blot techniques. Resistin mRNA was detected at 3 h, 6 h, 12 h and 24 h after MCAO using real-time QRT-PCR method. Central administration of resistin only at doses of 200 and 400 ng/mouse considerably reduced the infarct size and promoted neurological function ($p < 0.001$). In addition, resistin (400 ng/mouse) significantly decreased brain edema ($p < 0.001$), evans blue (EB) leakage ($p < 0.05$), MDA content ($p < 0.005$), apoptotic cells and apoptosis-related proteins ($p < 0.001$). Resistin mRNA expression markedly increased at 12-h time point and then returned to basal level at 24 h after MCAO. Our findings revealed that treatment with resistin could attenuate ischemic damage in a dose-dependent approach via suppressing apoptosis and oxidative stress. Application of resistin in clinical settings to treat stroke and brain ischemia warrants further research.

1. Introduction

Adipose tissue is an endocrine organ that synthesizes and releases a large number of biological compounds (adipokines) such as leptin, apelin, adiponectin, visfatin, omentin and resistin which are crucial in the regulation of metabolism, inflammation and immunity in a variety of physiological and pathological conditions (Choi and Cohen, 2017; Ruscica et al., 2017).

Resistin is a cysteine-rich polypeptide which is mainly produced by the white adipose tissue in rodents (Zhang et al., 2016) and macrophages in humans (Jung et al., 2006). Several studies reported that rodent and human resistin may be involved in the pathogenesis of insulin resistance and diabetes (Chen et al., 2009; Li et al., 2009; Qatanani et al., 2009; Schwartz and Lazar, 2011). It has been reported that low level of resistin gene expression is identified in the hypothalamus and cortex of mouse brain (Morash et al., 2002). However, its function in the central nervous system is unclear. Recent scientific evidence states that resistin may be associated with ischemic stroke and pathophysiology of cardiovascular and neurodegenerative diseases (Ding et al., 2011; Jamaluddin et al., 2013; Park et al., 2017). Numerous animal and clinical studies evaluated the relationship between resistin and the risk of ischemic stroke as a predictive factor, but these results are still controversial (Bouziana et al., 2016; Dong et al., 2017; Savopoulos et al., 2011; Weikert et al., 2008). Gao et al. reported that pretreatment with mouse recombinant resistin protects the heart against ischemic injury by suppressing apoptosis in a mouse langendorff heart perfusion model (Gao et al., 2007). Other studies have revealed that adipokine leptin also has beneficial effects on the recovery of cerebral ischemic injury in rodents (Zhang et al., 2011, 2012). Two studies exhibited that resistin has the

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neuroprotective effects via reducing apoptosis and oxidative stress signals *in vitro* model of Parkinson's and Alzheimer's diseases (Liu et al., 2013; Lu et al., 2013). Recently, it was reported that pre-ischemic treatment of resistin reduced the infarct size and improved neurological outcome in a dose-dependent manner in MCAO model of mice (Zhu et al., 2017). However, further investigations are required to clarify the effect of resistin and underlying mechanisms on cerebral ischemic damage in a rodent model of stroke.

Therefore, the present study was designed to evaluate resistin expression in brain tissue and dose- and time-dependent effects of recombinant mouse resistin on the recovery of stroke-induced injury, neurological outcome, BBB disruption, MDA content and apoptosis in a mouse model of stroke.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (35–40 g, 2–4 months old) were obtained from the experimental animal center of Semnan University of Medical Sciences (SUMS), Semnan, Iran. The mice were housed in the standard condition (a temperature (22–24 °C), humidity (40–60%) and 12/12 h light/dark) with unlimited access to food and water. All tests were done in agreement with the national guidelines for the care and use of laboratory animals for medical research and the institutional guidelines of SUMS Research Ethics Committee. The SUMS institutional Committee of Research Ethics approved research protocol (ethical code number: IR.SUMUMS.REC. 1395.23).

2.2. Drug preparation and delivery

Recombinant mouse resistin was purchased from BioVendor company (BioVendor Research and Diagnostic Products, Brno Czech Republic). The whole drug (0.1 mg) was resolved in 500 μl Phosphate Buffer Saline (PBS). Afterwards, the doses of 25, 50, 100, 200 and 400 ng/2 μl were aliquoted. Under aseptic conditions, 2 μl PBS (as vehicle) or resistin was injected immediately or 1 h and/or 3 h after MCAO into the right lateral ventricle (coordinates: −0.6 mm posterior relative to bregma, 1.1 mm right relative to midline and 2 mm deep from the dura). In order to reduce the post-surgery pain, buprenorphine (4 mg/kg IP, Temad Co. Active Pharmaceutical Ingredients, Iran) was administered about 30 min before the surgery and at 8 h after the MCAO.

2.3. Animal model of stroke

Mice were anesthetized by using chloral hydrate (400 mg/kg, Merck, Germany) intraperitoneally (IP). Transient focal cerebral ischemia was induced by MCA occlusion (Akhoundzadeh et al., 2017; Hadadha et al., 2015). A midline neck incision was made under a surgical microscope, and then the right common carotid artery and its branches were isolated. By using Laser Doppler Flowmetry (LDF, Moor instruments, Devon, UK), a silicone-coated 8–0 nylon monofilament was inserted into the internal carotid artery and advanced to the origin of MCA to produce focal cerebral ischemia. The middle cerebral artery was occluded for 60 min and re-circulation was conducted for 23 h. During surgery, the body temperature was controlled with an electrical pad.

2.4. Experimental protocol and treatment plan

The current study was performed in the following eight experiments. In the first experiment, to determine the effects of different doses of resistin on the brain injury and neurological outcome, 56 mice were randomly divided into 7 equal groups (n = 8, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. Group 2, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Groups 3 to 7, the treatment groups, received resistin at doses 25, 50, 100, 200, and 400 ng/mouse, ICV at the beginning of MCAO.

In the next experiment, to assess the effect of resistin on the brain edema, 24 mice were randomly divided into 3 equal groups (n = 8, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. Group 2, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Group 3, the treatment group, received resistin at the dose of 400 ng/mouse, ICV at the commencement of MCAO.

For evaluating the effect of resistin on BBB disruption, 21 mice were randomly divided into 3 equal groups (n = 7, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. Group 2, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Group 3, the treatment group, received resistin at the dose of 400 ng/mouse, ICV at the commencement of MCAO.

For investigating the effect of resistin on apoptosis, 18 mice were randomly divided into 3 equal groups (n = 6, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. Group 2, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Group 3, the treatment group, received resistin at the dose of 400 ng/mouse, ICV at the commencement of MCAO.

For the assay level of proteins associated with apoptosis signaling (caspase-3 and caspase-8), 18 mice were randomly divided into 3 equal groups (n = 6, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. Group 2, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Group 3, the treatment group, received resistin at the dose of 400 ng/mouse, ICV at the commencement of MCAO.

For the next experiment, the effect of resistin on MDA content was investigated. Eghiteen mice were randomly divided into 3 equal groups (n = 6, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. Group 2, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Groups 3, the treatment group, received resistin 400 ng/mouse, ICV at the commencement of MCAO.

For investigating resistin gene expression in the brain cortex at different time points, 25 mice were randomly divided into 5 equal groups (n = 5, each) as followed: Group 1, the sham-operated group which surgery was done without MCAO. Groups 2, 3, 4 and 5, the ischemic groups that animals were decapitated at 3 h, 6 h, 12 h and 24 h after the MCAO, respectively.

For evaluating the effect of resistin on brain damage at various time intervals, 32 mice were randomly divided into 4 equal groups (n = 8, each) as followed: Group 1, the control group, received PBS (2 μl, ICV) at the commencement of MCAO. Groups 2 to 4, the treatment groups, received resistin 400 ng/mouse, ICV at 0 h, 1 h and 3 h after MCAO, respectively.

2.5. Infarct size measurement

The animals were sacrificed under deep anesthesia at 24 h after ischemia. The brains were isolated and then sectioned coronally into five 2-mm thick slices by a mouse Brain Matrix. The focal ischemic lesion was distinguished by immersing the slices into 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich, Germany) for 15 min, and transferring to 10% buffered formaldehyde solution (Merck, Germany) for 12 h. The sections were photographed using a digital camera (Cannon, Japan)