The Effects of Mouse Recombinant Resistin on mRNA Expression of Proinflammatory and Anti-Inflammatory Cytokines and Heat Shock Protein-70 in Experimental Stroke Model

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Background: Our recent research showed that resistin has a neuroprotective effect against stroke-induced injury through suppressing apoptosis and oxidative stress. However, the molecular mechanism of neuroprotection of resistin is unclear. This work was designed to examine the effect of mouse recombinant resistin on mRNA expression of Tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), Interleukin-10 (IL-10), Transforming growth factor-β1 (TGF- β1), and Heat shock protein-70 (HSP-70) in mouse model of stroke. *Materials and Methods:* Transient focal cerebral ischemia was induced by the middle cerebral artery occlusion (MCAO) in mice. TNF-α, IL-1β, IL-10, TGF-β1, and HSP-70 mRNA were detected at sham (0 hour), 3 hours, 6 hours, 12 hours, and 24 hours after MCAO using real-time QRT-PCR method. Moreover, animals were treated with resistin at the dose of 400 ng/mouse at the commencement of MCAO, and mRNA expression of the cytokines and HSP-70 was measured 24 hours after MCAO. *Results:* Tumor necrosis factor-α and IL-1β mRNA expression markedly increased at 12-hour time point and then returned to the basal level at 24 hours after MCAO; but HSP-70 mRNA expression increased at 24-hour time point. Furthermore, resistin (400 ng/mouse) significantly increased TGF-β1 and IL-10 and decreased HSP-70 gene expression at 24 hours after MCAO. *Conclusions:* Our findings revealed that a molecular mechanism of attenuating ischemic damage by resistin administration probably is increased mRNA expression of anti-inflammatory cytokines. However, applying resistin in the clinical settings for the treatment of stroke deserves further researches in the future.

Key Words: Resistin—cerebral ischemia—gene expression—cytokines—heat shock protein-70—mice

Introduction

Adipokine resistin, a cysteine-rich polypeptide, is mainly released from adipose tissue and macrophage cells, and has an important link with insulin resistance, diabetes, obesity and atherosclerosis.[1,2] A number of human studies reported that there might be an association between plasma level of resistin and risk of cardiovascular and cerebrovascular diseases.[3,4] Based on some scientific evidence, resistin has protective effects against the heart injury[5] neurodegenerative insults[2,6] and brain ischemia.[7,8]

Adipokine resistin is a fat-brain axis regulator.[9] Although resistin expression was primarily identified in adipocytes, low level of resistin gene expression is also found in the normal mouse brain.[10] Our recent finding indicated that resistin mRNA expression considerably increased at 12 hours after cerebral ischemia in cortex of

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mouse brain.[8] Moreover, our results demonstrated that central administration of mouse recombinant resistin could reduce ischemic injury and improve neurological function through attenuating apoptosis and oxidative stress.[8] Taken together, resistin probably plays an effective role in the pathophysiology of stroke.

In the brain tissue, heat shock protein-70 (HSP-70) expression increases in ischemia. HSP-70 is an important component of the interior response to cellular stress. According to some studies, pretreatment with resistin induces up-regulation of HSP-70 in a dose- and a time-dependent approach.[2] On the other hand, the previous researches reported that human resistin stimulates the synthesis and release of proinflammatory cytokines, such as IL-6, TNF-α, and IL-12 from macrophages.[11,12] It has been approved that in the cerebral stroke proinflammatory cytokines possess the destructive effects; but anti-inflammatory cytokines exert the constructive effects.[13-15] Therefore, the present study was designed to explore the effect of central administration of mouse recombinant resistin on mRNA expression of TNF-α, IL-1β, IL-10, TGF-β1, and HSP-70 in a mouse model of stroke.

Materials and Methods

Animals

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

Medication and Delivery

Recombinant mouse resistin was purchased from Bio Vendor Company (Bio Vendor Research and Diagnostic Products, Brno Czech Republic). The whole drug (.1 mg) was resolved in 500 μl Phosphate Buffer Saline (PBS). Afterward, the drug was aliquoted into 400 ng/2μl concentrations. Under aseptic conditions, 2 μl PBS (as the vehicle) or resistin was injected immediately after MCAO into the right lateral ventricle (coordinates: .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 injected about 30 minutes before the surgery and at 8 hours after the MCAO.

The Experimental Model of Ischemic Stroke

Mice were anesthetized by using intraperitoneal (IP) injection of chloral hydrate (400 mg/kg, Merck, Germany). Transient focal cerebral ischemia was induced through middle cerebral artery occlusion.[16] 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 Flowmeter (LDF, Moor Instruments, Devon, UK), a silicone-coated 8-0 nylon monofilament was inserted into the internal carotid artery and sent toward the origin of MCA to produce focal cerebral ischemia. The middle cerebral artery was blocked for 60 minutes and recirculation was conducted for 23 hours. During surgery, the body temperature was preserved with an electrical pad.

Regional Cerebral Blood Flow Measurement

Before starting the microsurgery and under deep anesthesia, the right temporal bone was exposed, and LDF probe was placed on the temporal bone to record the basal regional cerebral blood flow. Cerebral blood flow (CBF)in the ischemic hemisphere was recorded at 15 minutes before MCAO, during 60 minutes of MCAO, up to 15 minutes after reperfusion. During occlusion, CBF decreased to lower than 20% of baseline. After extracting monofilament, CBF increased and reached approximately baseline.[8]

Experimental Protocol and Treatment Plan

For investigating time course of mRNA expression of cytokines (TNF-α, IL-1β, IL-10, and TGF-β1) and HSP-70 in the brain cortex, mice were randomly divided into 5 equal groups (n = 5, each) as followed: In group 1, the sham-operated group, surgery was done without MCAO. In groups 2, 3, 4, and 5, the ischemic groups, PBS) 2 μl/ICV) was administrated at the commencement of MCAO and animals were decapitated at 3 hours, 6 hours, 12 hours, and 24 hours after MCAO initiation, respectively.

For evaluating the effect of resistin on mRNA expression of cytokines and HSP-70, mice were randomly divided into 3 equal groups (n = 5, each) as followed: Group 1, the sham-operated group, which surgery was done without MCAO. In group 2, the control group, PBS (2 μl, ICV) was administrated at the commencement of MCAO and mice were decapitated at 24 hours after MCAO initiation. The animals in group 3, the treatment group, received resistin 400 ng/mouse, ICV at the commencement of MCAO and were decapitated at 24 hours after MCAO initiation. Then the levels of proinflammatory (TNF-α, IL-1β) and anti-inflammatory cytokines (IL-10, TGF-β1) and HSP-70 were measured using real-time QRT-PCR technique.

Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (QRT-PCR) Analysis

At the end of every time point (sham, and 3 hours, 6 hours, 12 hours, and 24 hours after ischemia), mice were killed and cortex tissue of ischemic hemisphere