Biochemical and Biophysical Research Communications 474 (2016) 377–383

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Seinpin knockout exacerbates cerebral ischemia/reperfusion damage in mice

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ARTICLE INFO

Article history:
Received 6 April 2016
Accepted 20 April 2016
Available online 22 April 2016

Keywords:
Seipin
Knockout mice
Lipodystrophy
Ischemic stroke
Transient middle cerebral artery occlusion

ABSTRACT

Background and purpose: Seipin, which regulates adipocyte differentiation and lipolysis, inducing severe lipodystrophy and metabolic syndromes, is also highly expressed in the nervous system and affects some neurological diseases. However, the impacts of seipin in stroke remain unclear.
Methods and results: In this study, we subjected seipin knockout mice to cerebral ischemia/reperfusion injury and found that seipin knockout mice exhibited exacerbated neurological disorder and enlarged infarct size, companied by blood–brain barrier (BBB) damages. Furthermore, we showed that seipin knockout aggravated endoplasmic reticulum (ER) stress and significantly increased glucose levels, decreased leptin and adiponectin levels in mouse plasma.
Conclusions: Our findings reveal that seipin knockout exacerbates cerebral I/R-induced damages by increasing BBB permeability, amplifying ER stress and increasing glucose levels, as well as decreasing leptin and adiponectin levels, indicating that seipin may be a potential therapeutic target for stroke.

1. Introduction

Stroke is the leading cause of death and disability worldwide. However, stroke therapies have made little progress. While 1000 compounds were found effective in animal studies, nevertheless, apart from thrombolytics, none of these compounds were effective under clinical conditions [1]. Brain injury following stroke originates from the complex interplay of multiple mechanisms [2]. Development of new treatments requires more comprehensive understanding of the complex mechanisms of ischemic brain damage.

Seipin/BSCL2 was originally identified as a candidate gene of congenital generalized lipodystrophy type 2(CGL2) [3]. Patients with homozygous null mutations in *seipin* show lipodystrophy, hypertriglyceridemia, insulin resistance and mental retardation. Experimental studies have indicated that seipin locates in endoplasmic reticulum(ER) and regulates adipocyte differentiation and lipolysis, determining the size and distribution of lipid droplets [4]. Like the patients of congenital generalized lipodystrophy type 2, seipin deficiency in mice results in severe lipodystrophy and metabolic syndromes. Furthermore, the seipin gene is highly expressed in the nervous system [5,6]. Several studies have indicated its role in neurological diseases. Lack of seipin in neurons results in anxiety- and depression-like behaviors [7] and gain of function mutation in seipin causes some kinds of motor neuron diseases [8]. Nevertheless, it is unclear whether seipin is also involved in stroke.

In this study, we subjected both seipin knockout mice and wild-type mice to cerebral ischemia/reperfusion injury with middle cerebral artery occlusion (MCAO) model and to investigate the impacts of seipin on ischemic stroke.

2. Materials and methods

2.1. Experimental animals

Seipin knockout (*seipin* −/−, SKO) mice with C57BL/6 background were generated as described in our previous study [4]. Mating between *seipin* ± mice produced the SKO mice and their wild type (*seipin*+/+, WT) littermates. Male SKO and WT mice, all aged 12–14 weeks were used. Four to five mice were housed per cage in a specific pathogen free (SPF) room with controlled

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http://dx.doi.org/10.1016/j.bbrc.2016.04.116
0006-291X/

environment: 23–25 °C; relative humidity 55 ± 5%; 12:12 h light:dark cycle. Pelleted diet and sterilized water were provided ad libitum. The Principles of Laboratory Animal Care (NIH publication no.85Y23, revised 1996) were followed, and the experimental protocol was approved by the Animal Care Committee, Peking University Health Science Center (LA2010-059). All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

2.2. Transient middle cerebral artery occlusion (tMCAO)

Mice were anesthetized with isoflurane (3% for induction and 1.5% for maintenance) in N_2O:O_2 (3:1). Rectal temperature was maintained at 37.0 ± 0.5 °C with a heating pad (Harvard Apparatus, Holliston, MA). Transient focal cerebral ischemia was induced by middle cerebral artery occlusion [9]. Briefly, the right external, internal and common carotid arteries were exposed and ligated through a midline incision in the neck. The right external carotid artery was coagulated and cut distally. A small hole was made in the external carotid artery stump and a silicone-coated 6-0 monofilament (Doccol Corp, Redlands, CA) was inserted into the lumen and gently advanced *via* the right internal carotid artery approximately 8–9 mm distal to the carotid bifurcation to occlude the origin of the middle cerebral artery with laser Doppler flow (LDF; Perimed PF 5000, Stockholm, Sweden) monitored to confirm the successful occlusion. The monofilament was carefully withdrawn 60 min after MCAO and the circulation of right internal carotid artery was recovered. The reperfusion of the MCA was confirmed by LDF. Sham-operated mice underwent an identical procedure without the monofilament insertion. After completely regaining consciousness, mice were returned to the cages. No death occurred during the operation. After the operation, mice were monitored hourly for the first 4 h following the MCAO surgery, and observed every 8 h thereafter. Human endpoints were defined by a lack of spontaneous movement and a depressed level of consciousness. Post-operative care consisted of keeping the animals warm in a quiet environment with access to sufficient water and a soft diet as well as the close monitoring of the incision site for hemorrhage.

2.3. Evaluation of neurological status

Neurological deficit score was evaluated 23 h after reperfusion according to a neurological grading scale ranging from 0 to 4 [10]. A score of 0 was defined as no observable neurological deficit, whereas a score of 4 corresponded to an inability to walk spontaneously and a depressed level of consciousness. The mice were scored in a double-blinded manner.

2.4. Measurement of cerebral infarction volume

After neurological scoring, mice were deep anesthetized with an intraperitoneal injection of sodium pentobarbital and sacrificed. Mice brains were harvested and sectioned coronally with a mice brain slicer matrix (Zivic Instruments, Pittsburgh, PA). Sections were incubated in 1% of 2, 3, 5-triphenyletrazolium chloride (TTC) for 15 min at 37 °C. Infarction volumes were then determined by an observer blinded to the experimental groups and edema were corrected by using an image analysis software (ImagePro Plus, OLYMPUS). Infarction volumes were expressed as percentage of the ipsilateral hemispheric volume. To detect brain edema, the increased percentage of ischemic hemisphere volume was calculated according to the formulation: (ipsilateral volume − contralateral volume) × 100%/contralateral volume.

2.5. Blood–brain-barrier (BBB) permeability

The BBB permeability was measured according to a previously reported procedure [11]. Briefly, 20 h after operation, 2% Evan's blue dye (Sigma–Aldrich, Saint Louis, MI, USA) was injected intravenously into the tail vein at a dose of 4 ml/kg and allowed to circulate for 4 h, followed by perfusion with PBS *via* the left cardiac ventricle. The mice were then sacrificed, and their brains were harvested and weighed. Subsequently, the brains were homogenized in 1 mL 50% trichloroacetic acid (Sigma–Aldrich, Saint Louis, MI, USA), followed by centrifugation at 15,000 rcf and 4 °C for 30 min. The supernatant was collected and the concentrations of Evan's blue dye was measured by a spectrophotometer and calculated according to a standard curve. The results were expressed as Evans blue (μg)/tissue (g).

2.6. Western blotting

The protein was extracted by using protein extraction kit and the supernatants were separated by Tris–glycine SDS-PAGE, transferred to PVDF membranes and blocked with 10% nonfat dry milk in Tris-HCL Buffer Saline for 1 h at room temperature. Then, the PVDF membranes were incubated with primary antibody against matrix metalloproteinases (MMP-9; 1:100, Santa Cruz), GRP78 (1:1000, CST, USA), CHOP (1:1000, CST, USA), p-eIF2α (1:1000, CST, USA), eIF2α (1:1000, CST, USA) and β-actin (1:500, Santa Cruz) overnight at 4 °C. After being washed in TBS-T, the membranes were incubated with corresponding secondary antibody for 1 h at room temperature. The protein bands were analyzed using densitometry and Image J image analysis software.

2.7. Measurement of plasma adiponectin, leptin and glucose levels

Mouse plasma adiponectin and leptin levels were determined with adiponectin and leptin enzyme-linked immunosorbent assay (ELISA) kit (Linco Research, St Charles, MO). Plasma glucose was determined by an enzymatic method (Sigma kits, USA). For these measurement, blood (100 ul) was collected from ophthalmic venous plexus before MCAO surgery and at 24 h after operation.

2.8. Data analysis

The software SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to conduct all statistical analyses. Parametrical data were presented as means ± S.D. Student's t-test was used to compare difference between two groups. For groups over two, between-group differences were detected by one-way analysis of variance with the post hoc Student–Newman–Keuls test. The neurological deficit scores were analyzed with the Mann–Whitney rank-sum test. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Seipin deficiency results in increased cerebral injury after ischemia-reperfusion

Direct effects of genotype on development of vasculature could severely bias our ischemia model and needed to be ruled out. We neither found differences in vascular anatomy nor in blood flow reduction/reperfusion between SKO and WT mice (Supplemental figure 1).

To elucidate the impact of *seipin* deletion on cerebral ischemic damage, we examined the response to ischemic injury in SKO mice. WT and SKO mice were subjected to 1 h of MCA occlusion *via* intraluminal filament followed by 23 h of reperfusion.