Journal of Clinical Neuroscience 41 (2017) 54–59

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

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

Clinical commentary

Monosialotetrahexosy-1 ganglioside attenuates diabetes-associated cerebral ischemia/reperfusion injury through suppression of the endoplasmic reticulum stress-induced apoptosis

Danying Su [a], Jing Ma [b], Jiachen Yang [c], Yingying Kang [d], Manhua Lv [e,*], Yang Li [f,*]

[a] Department of Neurology, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, China
[b] Department of Anatomy, Harbin Medical University, Harbin, China
[c] Harbin Medical University, Harbin, China
[d] Department of Endocrinology, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, China
[e] Department of Neurology, The First Affiliated Hospital of Harbin Medical University, Harbin, China
[f] Department of Cardiology, The Fourth Affiliated Hospital of Harbin Medical University, Harbin, China

ARTICLE INFO

Article history:
Received 13 December 2016
Accepted 13 March 2017

Keywords:
Monosialotetrahexosy-1 ganglioside
Ischemia/reperfusion injury
Endoplasmic reticulum stress
Apoptosis
Brain

ABSTRACT

We aimed to assess the neuroprotective mechanism of monosialotetrahexosy-1 ganglioside (GM1) on focal cerebral ischemia/reperfusion (I/R) injury in rats with diabetes. A total of 54 male Wistar rats were induced with diabetes mellitus by administration of streptozotocin (STZ). The rats were then randomized into three groups, including sham group (n = 18), I/R group (n = 18), and GM1 group (n = 18). Focal cerebral ischemia was modeled using the right middle cerebral artery occlusion method. In the GM1 group, diabetic rats were intraperitoneally administered with GM1 (15 mg/kg) at 20 min prior to reperfusion. GM1 was replaced by an equal volume of saline in the I/R group. Rats from the sham group accepted sham operation and normal saline. The neurological deficit and brain infarct volume and TUNEL-apoptosis were evaluated. The expression of endoplasmic reticulum (ER) stress-related proteins, including caspase-12, GRP78 and CHOP/GADD153, was examined by Western blot. GM1 notably reduced the cerebral infarct size and improved the neurological behavior. In addition, GM1 dramatically reduced TUNEL-positive cell numbers in the cerebral cortex. Furthermore, GM1 treatment modulated protein levels, increasing GRP78 and reducing CHOP/GADD153 expression along with activation of caspase-12 in the ischemic brain hemispheres. These results imply that GM1 attenuates diabetes-associated cerebral I/R injury by suppressing the ER stress-induced apoptosis.

1. Introduction

Diabetes is shown to be a risk factor for cerebral ischemia, and study demonstrates that the relative risk of cerebral ischemia in patients with diabetes is about twice as much as in patients without diabetes [1]. Both acute hyperglycemia and chronic diabetes have previously been demonstrated to exacerbate ischemic brain damage. Diabetes accelerates the development of neuronal damage, increases infarct volume, and induces post-ischemic seizures under diverse mechanisms [2–4]. Indeed, activation of the cell death pathway may play a pivotal role in diabetes-induced brain damage. Evidence shows that the enhanced brain damage following cerebral ischemia/reperfusion (I/R) in diabetic condition was attributed to the activation of cell death pathways by the mitochondria. Furthermore, diabetes-exacerbated ischemic brain damage was recently shown to be mediated by increased endoplasmic reticulum (ER) stress and apoptosis [5].

The ER is a eukaryotic organelle involved in protein synthesis, folding and trafficking, as well as (Ca) homeostasis, and lipid and steroid synthesis [6]. Certain pathophysiological stimuli such as ischemia, hypoxia, ER Ca^{2+} depletion, glucose deprivation, free radicals, and high-fat diet can alter functions of ER and cause accumulation of unfolded proteins, resulting in ER stress [7]. In an attempt to cope with ER stress, cells have self-adaptive mechanisms collectively termed the unfolded protein response (UPR), which includes translational attenuation, induction of molecular

* Corresponding authors at: Department of Neurology, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China. Fax: +86 45153634596.
E-mail addresses: Sudanying2006@sina.com (D. Su), Jingma2009@aliyun.com (J. Ma), 1605711601@qq.com (J. Yang), Zhangyansheng009@sina.com (Y. Kang), chaocuii@163.com (M. Lv), Sulinxi2009@126.com (Y. Li).

http://dx.doi.org/10.1016/j.jocn.2017.03.047
0967-5868/

chaperones (e.g. glucose-regulated protein 78, GRP78), and ER-associated degradation [8]. When ER stress is severe or prolonged, the cell ultimately switches to intrinsic apoptotic pathways, leading to cell death [9]. ER stress-induced cell apoptosis involves the regulation of several pathways, including CCAAT/enhancer binding protein homologous protein (CHOP) (also named growth arrest and DNA damage-inducible gene 153, GADD153), caspase-12 and c-JUN N-terminal kinase (JNK) pathways [10–12]. Attenuation of ER stress, especially the ER stress-induced apoptosis is a treatment strategy against ischemia/reperfusion (I/R) injury [13,14].

Monosialotetrahexosy-1 ganglioside (GM1) is the major ganglioside protein family member in the nervous system, particularly around synapses; it acts as a membrane stabilizer, blocking neuronal cell death pathways [15]. GM1 has been used for treating disorders of the central nervous system owing to its ability to traverse the blood–brain barrier [16,17]. A recent study has shown that GM1 reduces neuronal death in diabetic rats after transient forebrain ischemia probably through suppression of the phosphorylation of ERK1/2 [18]. However, potential neuroprotective effects as well as the molecular mechanisms of GM1 therapeutic effect in diabetic rats after cerebral I/R remain unclear. Further studies are necessary to elucidate the protective mechanisms.

In this study, we examined the neuroprotective effects of GM1 on infarction size, neurological deficits, and apoptosis in a combined rat model of diabetes and cerebral ischemia by middle cerebral artery occlusion/reperfusion (MCAO). In addition, we tested whether GM1 was also effective against diabetic stroke by suppressing the augmented ER stress/apoptotic cell death.

2. Materials and methods

2.1. Animals

Fifty-four adult male Wistar rats (weighing 280–300 g) were provided by the Center for Experimental Animals, Harbin Medical College, Heilongjiang, China. Rats were kept at room temperature in a light/dark (12 h/12 h) condition. All animals were fed standard chow and allowed free access to drinking water. All animals care and use protocols were in accordance with the guideline of the local animal ethics for animals experiments.

2.2. Induction of diabetes animal model

The induction of streptozotoxin (STZ)-induced diabetes was performed as previously described [1]. Briefly, diabetes was induced by a single injection of STZ at a dose of 35 mg/kg (Sigma, USA) via the tail vein. Rats typically developed hyperglycemic states 2–3 days after the injection. The rats were fed on their respective diet until the end of study i.e. up to 4 weeks. Blood samples were collected initially and at the end of 4 weeks. The rats with plasma glucose level of $\geq$16.7 mmol/L at the end of 4 weeks were considered diabetic and included in the study.

2.3. Establishment of MCAO model

The diabetic rats were randomized into three groups (18 rats each group), namely sham group, I/R group, and GM1 group. MCAO was utilized to induce focal cerebral ischemia by using the intraluminal occlusion technique [18]. Briefly, rats were anesthetized with 10% chloral hydrate (300 mg/kg). Anylon thread with a distal cylinder (17 mm in length and 0.30 mm in diameter) was inserted into the lumen of the external carotid artery and advanced to the origin of the MCA. The nylon thread was removed 2 h later to allow for reperfusion. For sham-operated animals, the nylon thread was advanced to the origin of the MCA and immediately removed.

Rats in the GM1 group were intraperitoneally administered with GM1 (15 mg/kg, Sigma, MO, USA) at 20 min before the reperfusion. Rats in the I/R group and the sham group were intraperitoneally administered an equal volume of normal saline before the reperfusion.

2.4. Determination of neurological deficit scores

Neurological behavioral test was performed 22 h after reperfusion by using the methods of Longa and Weinstein with a five-point scale [19]. No neurological deficit = 0, limping slightly to the left side = 1, circling if pulled by tail = 2, circling spontaneously = 3, and no spontaneous activity with impaired level of consciousness = 4.

The animals were sacrificed after 2 h of ischemia followed by 22 h of reperfusion for 2,3,5-triphenyl tetrazolium chloride (TTC) analysis (n = 6) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining (n = 6), as well as Western blot analysis (n = 6).

2.5. Evaluation of infarction size

The animals were sacrificed after 2 h of ischemia followed by 22 h of reperfusion. The whole brains were rapidly removed and sectioned into 2-mm-thick coronal blocks in the rostral-caudal direction using a rodent brain matrix. The slices were stained in a solution containing 2% TTC at 37 °C for 30 min in the dark, followed by fixation with 10% formalin at room temperature overnight. The sections were photographed with a digital camera connected to a computer. The infarcted region appeared white, whereas the normal, non-infarcted tissue appeared red. The infarct area from each brain was calculated using image analysis software (image-Pro Plus 6.0) in blinded manner.

2.6. TUNEL staining for apoptosis evaluation

Brain tissues were embedded in paraffin and then sectioned into 5 μm for the TUNEL assay. TUNEL staining was conducted for determination of cellular apoptosis with an *in situ* Apoptosis Detection Kit (Boster Biotechnology Inc., Wuhan, China). Positive staining was identified by brown granules in the cells. Two pathologists independently evaluated and quantified the percentage of apoptosis in a blind manner by using the image-Pro Plus 6.0 software. TUNEL-positive cells were counted using image analysis software (image-Pro Plus 6.0) under the same conditions. Ten randomly selected microscopic fields were chosen under 100 × magnification (n = 6, each). TUNEL-positive cells were counted from randomly selected images over the cortical penumbral region as described below and expressed as percentage of TUNEL-positive cells as compared to total cells.

2.7. Determination of the expression of ER stress proteins by Western blot analysis

Samples from the cortical tissue were obtained and homogenized in ice-cold lysis buffer (Boster, China), followed by centrifugation. Proteins in the supernatant were quantified with a commercial kit (Bio-Rad, Hercules, CA). Equal amounts (15 μg) of protein samples were subjected to 12% sodium dodecyl-sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The separated proteins were transferred onto a PVDF membrane. Membrane was blocked at room temperature with 5% non-fat milk in buffer solution (Tris-buffered saline containing 0.1% Tween 20). Thereafter, the PVDF was incubated overnight at 4 °C with specific antibodies for polyclonal rabbit-anti-rat GRP78 (1:500) (Santa Cruz Biotechnology), polyclonal rabbit-anti-rat caspase-12 (1:1000)