Experimental Neurology 311 (2019) 44–56

Contents lists available at ScienceDirect

Experimental Neurology

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

Research paper

Metformin attenuates diabetes-induced tau hyperphosphorylation in vitro and in vivo by enhancing autophagic clearance

Jin-Liang Chen[a], Cheng Luo[a], Die Pu[a], Guo-Qiang Zhang[b], Yu-Xing Zhao[a], Yue Sun[a], Ke-Xiang Zhao[a], Zhi-Yin Liao[a], An-Kang Lv[a], Shi-Yu Zhu[a], Jing Zhou[a], Qian Xiao[a,*]

[a] Department of Geriatrics, the First Affiliated Hospital of Chongqing Medical University, Friendship Road 1, Yuan Jiagang, 400016 Chongqing, China
[b] Department of Gastroenterology, Children's Hospital of Chongqing Medical University, No. 136, Zhongshan 2nd Road, 400014 Chongqing, China

ARTICLE INFO

Keywords:
Diabetes mellitus
Alzheimer's disease
Metformin
Autophagy
Tau

ABSTRACT

Diabetes mellitus (DM) can increase the risk of Alzheimer's disease (AD) in patients. However, no effective approaches are available to prevent its progression and development. Recently, autophagy dysfunction was identified to be involved in the pathogenesis of neurodegenerative diseases. This study was designed to investigate the effect of metformin on hyperphosphorylated tau proteins in diabetic encephalopathy (DE) by regulating autophagy clearance. db/db mice were randomly divided into four groups, db/+ mice were used as control group. Twelve-week old male db/db mice received consecutive intraperitoneal injection of 200 mg/kg/d metformin or (and) 10 mg/kg/d chloroquine for eight weeks. Morris water maze (MWM) tests were performed to test cognitive functions before the mice were euthanized. Metformin attenuated cognitive impairment in db/db mice, reduced hyperphosphorylated tau proteins, restored the impaired autophagy in diabetic mice, all of which were reversed by inhibiting of autophagy activity. In high glucose-cultured HT22 cells, metformin increased autophagy in a dose-dependent manner. Besides, metformin enhanced autophagy activity in an AMPK dependent manner. These data show that metformin may reduce tauopathy and improve cognitive impairment in db/db mice by modulating autophagy through the AMPK dependent pathway. These findings highlight metformin as a new therapeutic strategy for the treatment of DE.

1. Introduction

Epidemiological evidence indicates that diabetes mellitus (DM) is associated with a higher risk of cognitive impairment, dementia and Alzheimer's disease (AD) (Leibson et al., 1997), DM-induced cognitive impairment is called diabetic encephalopathy (DE). Two major pathological hallmarks of AD are extracellular amyloid plaques formed by β-amyloid peptides (Aβs) and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein. Interestingly, in diabetic patients, cerebrospinal fluid (CSF) levels of total tau and phosphorylated-tau (pTau) are significantly increased, but is not associated with $A\beta_{42}$ level, which indicates that tau and pTau instead of Aβ plaques are more likely to be pathological hallmarks for diabetes associated dementia (Moran et al., 2015). A recent study reported that leptin resistance-induced diabetes accelerates the development of tauopathy, and may ultimately lead to cognitive impairment (Platt et al., 2016). And other studies have shown that obvious phosphorylation of tau protein in the brain starts as early as 6–8 weeks of age in db/db (BKS.Cg-Dock7m+/+Lepr^{db}/J) mice and in the streptozotocin (STZ)-treated mice (Kim et al., 2009; Planel et al., 2007). Tau is a microtubule-associated protein, abnormally hyperphosphorylated tau forms NFTs which is closely related to cognitive impairment (Ballatore et al., 2007). Hence, targeting hyperphosphorylated tau protein has been considered as one of the promising approaches to diabetes associated dementia.

Metformin is one of the first-line treatments for glycemic control in type 2 diabetes mellitus (T2D) patients. Interestingly, aside from blood glucose control, metformin also has beneficial effects for various central nervous system (CNS) disorders, including ischemic brain disease (Jiang et al., 2014a), Parkinson's disease (Perez-Revuelta et al., 2014), and Huntington's disease (Vazquez-Manrique et al., 2016), etc. Recent clinical trials report that long term use of metformin in T2D patients significantly lower the incidence of dementia (Ng et al., 2014; Hsu et al., 2011), and animal studies have also shown a protective effect of metformin in cognitive impairment in different animal models of diabetes (Pintana et al., 2012; Oliveira et al., 2016; Li et al., 2012). To date, the underlying mechanisms of neuroprotective effect of metformin still remain poorly understood.

* Corresponding author.
E-mail address: xiaoqian1956@126.com (Q. Xiao).

https://doi.org/10.1016/j.expneurol.2018.09.008
Received 21 April 2018; Received in revised form 7 September 2018; Accepted 12 September 2018
Available online 13 September 2018

Macroautophagy (hereafter called autophagy) is a highly conserved catabolic process that degrades the aggregate-prone proteins and damaged cytoplasmic organelles within cells. Autophagy dysfunction in neurons has been reported to contribute to various neurodegenerative diseases, including AD (Kiriyama and Nochi, 2015; Komatsu et al., 2006). Down regulated autophagy in neurons leads to accumulation of misfolded proteins, and then aggravates the deposition of Aβ and formation of NFTs in AD brain (Li et al., 2016). Furthermore, studies have reported that pharmacological induction of autophagy could be a promising therapeutic strategy in neurodegenerative diseases (Jiang et al., 2014b; Jang et al., 2016; Friedman et al., 2015). T2D is also associated with down-regulation of autophagy (Kanamori et al., 2015; Wilson et al., 2014), which could be the pathogenesis of diabetes associated dementia.

To better understand the potential mechanisms of metformin on diabetes associated dementia, high glucose-treated mouse HT22 cells and db/db mice were used as in vitro and in vivo models of diabetes. We demonstrated that metformin treatment enhances autophagic clearance of hyperphosphorylated tau in high glucose-incubated HT22 cells and in brain of db/db mice through the activation of the AMP-activated protein kinase (AMPK), ultimately attenuates cognitive impairment in db/db mice.

2. Materials and methods

2.1. Reagents

Metformin and chloroquine (CQ) were purchased from Sigma (Sigma, St. Louis, MO, USA). 3-methyladenine (3-MA) and compound C (CC) were purchased from Selleckchem (Selleck, Westlake Village, CA, USA). In vitro studies, 3-MA and CC were dissolved in DMSO and adjusted to the final concentration with culture medium.

2.2. Cell culture and treatments

Mouse hippocampal neuron cells HT22 were a generous gift from prof. Wei-Lin Jin (Institute of Bio-Nano-Science and Engineering, Department of Instrument Science and Engineering, School of Electronic Information and Electrical Engineering, and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China). The cells were cultured in standard Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and incubated at 37 °C with 5% CO_2. The glucose concentration of 25 mM in DMEM was considered the normal glucose (NG), additional 25 mM glucose was added into DMEM (50 mM) and considered the high glucose (HG). Cells were incubated with either NG or HG medium for 24 h, and then were treated with vehicle (DMSO) or metformin (3.2 mM) in the presence or absence of 3-MA (10 mM) or compound C (10 μM) for another 24 h.

2.3. Transfection of cells with Beclin 1 siRNA

Specific small interfering RNA (siRNA) targeting Beclin 1 (Sense: 5′-CAGUUUGGCACAAUCAAUAUUTT-3′; Anti-sense: 5′-AAUAUUGAU UGUGCCAAACUGTT-3′) and control siRNA (Sense:5′-UUCUCCGAACG UGUCACGUTT-3′; Anti-sense: 5′-ACGUGACACGUUCGGAGAATT-3′), which should not knock down any known proteins, were purchased from Gene Pharma, Inc. (Gene Pharma, Shanghai). The above-mentioned siRNA molecules were transfected into the cells using Lipofectamine 2000 (Invitrogen, USA). 48 h after transfection, cells were exposed to various treatments as specifically indicated.

2.4. Animals and drug treatments

Six-week old male db/db (BKS.Cg-Dock7m+/+Leprdb/Nju) mice and their age-, sex-matched wild-type db/+ control mice were purchased from Model Animal Research Center of Nanjing University. All animals were housed five per cage in a temperature-controlled room (22 ± 2 °C) with a 12 h light/dark cycle (lights on 8 a.m.-8 p.m.), and given free access to food and water. All experimental procedures were carried out during the light phase. The animal protocol was approved by the Animal Care and Use Committee of The First Affiliated Hospital of Chongqing Medical University. All animal experiments followed Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal studies complied with the ARRIVE guidelines.

The mice (n = 60) were randomly selected and allocated to five groups: (Leibson et al., 1997) db/+ group (control group, n = 12); (Moran et al., 2015) db/db group (DM group, n = 12); (Platt et al., 2016) DM + metformin treatment group (Met group, n = 12); (Kim et al., 2009) DM + metformin + CQ treatment group (Met + CQ group, n = 12); DM + CQ treatment group (CQ group, n = 12). CQ is one of the most widely used autophagy inhibitors in vivo which can inhibit the fusion of autophagosomes with lysosomes. Drug treatment was started when mice were 12 weeks old. Metformin (200 mg/kg/d) and CQ (10 mg/kg/d) were dissolved in saline. Mice were injected intraperitoneally with vehicle (control group, DM group), metformin (Met group), metformin + CQ (Met + CQ group) and CQ (CQ group) every day for 8 consecutive weeks, respectively. Their body weights were measured every one week and fasting blood glucose levels were measured every one month.

2.5. Morris water maze

Cognitive performance was evaluated by the Morris water maze (MWM) during the last 6 days of experimental period. The mice (n = 10 per group) were given 4 training trials per day for 5 consecutive days. For each trial, the mouse was placed in the pool (facing pool wall) at one of the selected quadrants. The duration of each trial lasted until the mouse found the platform or until a maximum of 60 s. If the mouse failed to find the platform within 60 s, it was guided to the platform by technician for 15 s. Once a mouse mounted the platform, it was allowed to remain there for 5 s. Acquisition was measured as escape latency to reach the platform. Twenty-four hours after the last trial, mice were subjected to a probe trial in which the platform was removed and they were allowed to swim freely for 60 s. The frequency of an individual mouse passing the platform area and the time the animal spent in the target quadrant were recorded as a measure of spatial memory.

After probe trials, all mice performed a visible platform test to detect possible deficits in visual acuity and motor ability. The platform was fixed in a new quadrant 1 cm above the water level, and the latency to reach the platform was recorded.

2.6. Brain tissue preparation

Following behavioral testing, all mice were deeply anesthetized with 1% sodium pentobarbital (40 mg/kg) at 21 weeks of age. For western blotting, hippocampi were dissected out quickly and stored in liquid nitrogen. For immunohistochemistry (IHC) and hematoxylin & eosin (HE) staining, rats were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde (PFA). Brains were removed and fixed in 4% PFA at 4 °C.

2.7. Western blotting

Cells and hippocampal tissues were homogenized in RIPA buffer (Beyotime Inc.) supplemented with a protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche). Protein concentrations were determined by using BCA Protein Assay Kit (Beyotime Inc.). Equal amount of protein from different samples were loaded onto 8–10% SDS polyacrylamide gels and separated by electrophoresis at 100 V for 120 min. The proteins were transferred onto PVDF