Biochemical and Biophysical Research Communications 520 (2019) 47–53

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Prevention of post-ischemic seizure by rapamycin is associated with deactivation of mTOR and ERK1/2 pathways in hyperglycemic rats

Xiao Yang [a, b, 1], Changchun Hei [b, c, 1], Ping Liu [b, d], P. Andy Li [b, *]

[a] Neuroscience Center, General Hospital of Ningxia Medical University, Key Laboratory for Craniocerebral Diseases of Ningxia Hui Autonomous Region, Yinchuan, China
[b] Department of Pharmaceutical Sciences, Biomanufacturing Research Institute Technology Enterprise (BRITE), College of Arts and Sciences, North Carolina Central University, Durham, USA
[c] Department of Human Anatomy, Histology and Embryology, Ningxia Medical University, Yinchuan, China
[d] Department of Endocrinology, General Hospital of Ningxia Medical University, Yinchuan, China

ARTICLE INFO

Article history:
Received 13 September 2019
Accepted 22 September 2019
Available online 26 September 2019

Keywords:
Hyperglycemia
Cerebral ischemia
Post-ischemic seizures
Anti-seizure
mTOR pathway
Rapamycin
ERK1/2

ABSTRACT

Pre-ischemic hyperglycemia increases the occurrence of post-ischemic seizures both in experimental and clinical settings. The underlying mechanisms are not fully delineated; however, activation of mammalian target of rapamycin (mTOR) has been shown to be engaged in the pathogenesis of epilepsy, in which seizures are a regular occurrence. Therefore, we wanted to explore specifically the capacity of an mTOR inhibitor, rapamycin, in preventing post-ischemic seizures in hyperglycemic rats and to explore the underlying molecular mechanisms. The results showed that none of the rats in the sham control, EG ischemic, or within 3 h of I/R in hyperglycemic ischemic groups experienced seizures. Generalized tonic-clonic seizures were observed in all 8/8 of hyperglycemic ischemic rats at 16 h of I/R. Treatment with rapamycin successfully blocked post-ischemic seizures in 7/8 hyperglycemic ischemic animals. Rapamycin also lessened the neuronal death extraordinarily in hyperglycemic ischemic animals as revealed by histopathological studies. Protein analysis revealed that transient ischemia resulted in increases in p-mTOR and p-S6, especially in the hippocampi of the hyperglycemic ischemic rats. Rapamycin treatment completely blocked mTOR activation. Furthermore, hyperglycemic ischemia induced a much prominent rise of p-ERK1/2 both in the cortex and the hippocampi compared with EG counterparts; whereas rapamycin suppressed it. We conclude that the development of post-ischemic seizures in the hyperglycemic animals may be associated with activations of mTOR and ERK1/2 pathways and that rapamycin treatment inhibited the post-ischemic seizures effectively by suppressing the mTOR and ERK1/2 signaling.

© 2019 Elsevier Inc. All rights reserved.

1. Introduction

Post-ischemic seizure is a clinical complication of cerebral stroke. Seizures can occur in acute phase of the cerebral stroke or months after the stroke onset [1]. Patients with early onset seizures have a poor prognosis and experience high in-hospital mortality [2]. Hyperglycemia is not only associated with more severe disability [3], but also increases the susceptibility to post-ischemic seizures. Procaccianti et al. verified that hyperglycemia is an independent predictor of post-stroke seizures [4]. According to numerous experiments, hyperglycemia worsens ischemic brain damage as well as induces post-ischaemic seizures [5]. Butit has been observed that few euglycemia ischemic animals develop post-ischemic seizures, even if they have gone through a prolonged ischemic period with severe brain pathological injuries [6]. This suggests that the extent of brain damage is not the only determinant of post-ischemic seizure development in the hyperglycemic animals [7]. Hyperglycemia may trigger a post-ischemic seizure by activating special cell signaling pathway(s), which have yet to be identified.

Mammalian/mechanistic target of rapamycin (mTOR) controls several crucial aspects of mammalian cellular processes including be drawn into epileptogenesis in kinds of genetic syndromes and

* Corresponding author. Department of Pharmaceutical Sciences, North Carolina Central University, 1801 Fayetteville Street, Durham, NC, 27707, USA.
E-mail address: pli@nccu.edu (P.A. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2019.09.096
0006-291X/© 2019 Elsevier Inc. All rights reserved.

acquired epilepsy [8]. Rapamycin, a most well-known inhibitor of mTOR, has been shown to suppress seizure, to retard the development of seizure, and to hamper epileptogenesis in mTOR overactive rat models [9]. To date, there are few studies that have addressed on if hyperglycemic ischemia activates mTOR signaling and whether inhibition of mTOR by rapamycin could prevent clinical seizure development in the hyperglycemic ischemic animals. The purpose of the present experiment was to investigate the action of mTOR in mediating post-ischemic seizure in rats who were subjected 10 min forebrain ischemia under hyperglycemic condition.

Extracellular signaling-regulated kinases ½ (ERK1/2) is one of the members of mitogen-activated protein kinases (MAPK). It plays pivotal roles in cell survival and proliferation. Activation of ERK1/2 occurs under both physiological and pathological stimulations. Epilepsy increases the phosphorylation of ERK1/2 in cortical and hippocampal tissues collected from epileptic animal models and intractable epileptic patients [10,11], suggesting that ERK1/2 activation may be involved in epileptogenesis. Much work has been done to demonstrate that hyperglycemic ischemia increases the ERK1/2 protein levels [12,13]. The other objective of this study is to determine whether the anti-seizure effect of rapamycin is related to inhibition of ERK1/2 signaling in the hyperglycemic ischemic animals.

2. Materials and methods

2.1. Animals

Fifty-four adult male Wistar rats (weighing between 230 and 250 g) was supplied by Charles River Laboratories. The animal procedures were approved by the IACUC at North Carolina Central University. According to the study design, the rats were randomly divided into euglycemic (EG) ($n = 18$), hyperglycemic (HG) ($n = 18$) and hyperglycemic + rapamycin treatment (HG + RAPA) groups ($n = 18$). Each subgroup contained a sham control, 3 h of I/R, and 16 h I/R. The rats were kept in light/dark cycle (12:12 h), 50–70% humidity, 24 °C in temperature. The rats had free access to water and food.

2.2. Rapamycin injection

Rapamycin was purchased from LC Laboratories, USA. 100 mg rapamycin was dissolved in 100% ethanol (5 mL, 20 mg/ml) and stored at −20 °C as stock solution. The stock solution was diluted in Tween 80 (5%) and polyethyleneglycol 400 (5%) immediately before use. The final ethanol concentration in the working solution was 4%. Rapamycin (6 mg/kg/d) was intraperitoneally (i.p.) injected daily for 7 days before I/R as we have previously reported [14].

2.3. Operative procedures

The operative procedures and animal ischemic model were completed essentially as previously described [15].

2.4. Seizure observation

After the surgery, the rats were moved to Plexiglas boxes immediately and housed individually. Then the rats were videotaped by aHDR-PJ380/R type 16 GB memory video recorder for seizure recording. Eight rats were decapitated at 3 h of I/R as a pre-seizure group and 8 rats who demonstrated seizure activity were euthanized at 16 h of I/R. The seizure frequency, duration, and grade were recorded and analyzed.

2.5. Histology

After 3 h or 16 h I/R, the rats were perfused with PBS under anesthesia and then the brains were separated. Left brain hemisphere was immersed in liquid nitrogen and kept in a −80 °C freezer until protein analyses could be performed. Another half processed for histological staining as previously described [14,15]. The number of damaged neurons were counted. Dead cells was defined as red acidophilic stained cells with condensed nuclear staining. The numbers of dead neurons were counted in 5 microscopic fields in the parietal cortex and hippocampal CA1 area as we have previously described [15]. The results are presented as percentage of dead neuron.

2.6. Western blotting

The parietal cortices and hippocampi were dissected in a cold room and homogenized using a suspension buffer containing protease inhibitor cocktail (Complete Tablets, Roche). Equal amounts of proteins (25 μg) were loaded for Western blot as we have previously described [14,15]. Primary antibodies include anti-phospho-mTOR (p-mTOR, 1:200, Cell Signaling), anti-phospho-S6 (p-S6, 1:200, Cell Signaling), or anti-phospho-ERK1/2 (p-ERK1/2, 1:1000, Abcam). Protein bands were scanned by Odyssey Infrared Fluorescent scanner (LI-COR Biosciences, Lincoln, NE, USA) and the relative targeting band intensity to loading control band were presented as a ratio.

2.7. Statistical analysis

Data were analyzed by SPSS software (version 17.0). ANOVA followed by Bonferroni *post hoc* test was used to detect differences between the experimental groups.

3. Results

3.1. Blood glucose concentrations

The levels of blood glucose waved between 4.25 and 4.93 mM in the EG, otherwise they were 18.69–23.13 mM ($p < 0.01$ vs. EG) in the HG and were between 17.98 and 21.65 mM ($p < 0.01$ vs. EG) in the HG + RAPA groups. There was no significant difference in glucose concentrations between HG and HG + RAPA.

3.2. Rapamycin suppresses post-ischemic seizures in hyperglycemic rats

As shown in Table 1, none of the sham-operated rats in all three groups and the EG ischemic rats displayed post-ischemic seizures up to 16 h of recovery and they were sacrificed at this terminal point. In the HG, at 3 h of I/R, no seizures were developed in ischemic groups. However, seizures were observed in 8 out 8 rats after 4 h of I/R. The seizures were displayed as generalized tonic-clonic convulsions and scored Racine grade 5 [16]. The average seizure duration was 1 min and average seizure frequency was once every 18 min, which occurred until the pre-determined endpoint of

Table 1
Incidence of post-ischemic seizures among the 3 groups.

Group	Sham (n = 6)	3 h (n = 24)	16 h (n = 24)
Euglycemia	0/2	0/8	0/8
Hyperglycemia	0/2	0/8	8/8
Hyperglycemia + rapamycin	0/2	0/8	1/8

16 h of I/R in this group, at which they were euthanized. In the HG + RAPA, none of the 8 rats developed seizures after 3 h of I/R and only 1 rat displayed post-ischemic seizures at 16 h of I/R. The seizure duration and frequency were much reduced compared to the HG ischemic rats. Indeed, rapamycin reduced seizure duration to an average of 0.3 min and frequency to once an hour. In fact, this seizure rat only displayed 3 convulsions. Clearly, rapamycin was very efficacious in preventing hyperglycemia-induced post-ischemic seizures.

3.3. Rapamycin ameliorates hyperglycemia-enhanced ischemic brain damage

Because cerebral cortices and hippocampi are the most vulnerable regions to ischemic injury, we chose the cortex and the hippocampal CA1 for histopathological assessments. As shown in Fig. 1 in the cortical area, there was virtually no dead neurons observed in the sham-operated EG rats except a few scattered dead neurons. In the EG ischemic rats, after 3 h of I/R, 18.15% ± 4.67% ($p < 0.01$ *vs.* Sham) of neurons appeared as acidophilic and pyknotic neurons (arrows), indicating neuronal death. The percentage of dead neurons increased after 16 h of I/R to 38.98% ± 7.71% ($p < 0.01$ *vs.* 3 h). Pre-ischemic HG aggravated the damage in the cortex both in sham-operated rats and in rats subjected to cerebral ischemia and reperfusion. In sham-operated HG rats, percentage of neuronal death increased to 17.07 ± 4.48%, which is significantly higher than that in sham-operated EG rats ($P < 0.01$). The percentages of dead neurons further increased to 27.70 ± 3.88% at 3 h and 59.89 ± 8.68% at 16 h of I/R ($p < 0.01$). Treatment with rapamycin drastically reduced observed damage in HG ischemic animals. The percentage of dead neurons in HG + RAPA ischemic group decreased to 19.91 ± 2.19% in 3 h and 19.38 ± 2.18% in 16 h of recovery, reducing neuronal death to levels equal or below those observed in the EG ischemic counterparts.

In the sham controls of hippocampal CA1 area, almost no dead neurons were observed. The percentage of dead neurons was mildly increased to 13.31 ± 3.65% at 3 h and moderately increased to 20.52 ± 5.84% after 16 h of I/R in EG ischemic animals. The percentage of CA1 neuronal death in HG ischemic animals significantly increased to 48.32 ± 6.33% ($p < 0.01$ *vs.* EG counterpart) at 3 h and further rose to 57.34 ± 4.81% ($p < 0.01$ *vs.* EG counterpart) at 16 h of I/R. Rapamycin effectively halted the HG-exacerbated neuronal impairment in the CA1 area and brought the percentages of neuronal death down to 11.61 ± 3.22% in the sham-operated rats, 13.33 ± 4.60% and 15.38 ± 3.78% in rats subjected to 10 min of forebrain ischemia and followed by 3 and 6 h of I/R, respectively.

3.4. Rapamycin decreases mTOR and p-S6 in HG ischemic rats

Protein contents of p-mTOR and p-S6 were detected in the cortex and hippocampi. In the cortex (Fig. 2 A,C,E), p-mTOR was moderately elevated after 3 h of I/R and then declined. However, p-mTOR remained higher than the control value after 16 h of I/R in the EG. Similarly, p-S6 was elevated at 3 hand recessed at 16 h of I/R in the EG. Compared with EG animals, HG did not further increase p-mTOR or p-S6 except for a surge of p-S6 at 16 h. Treatment with

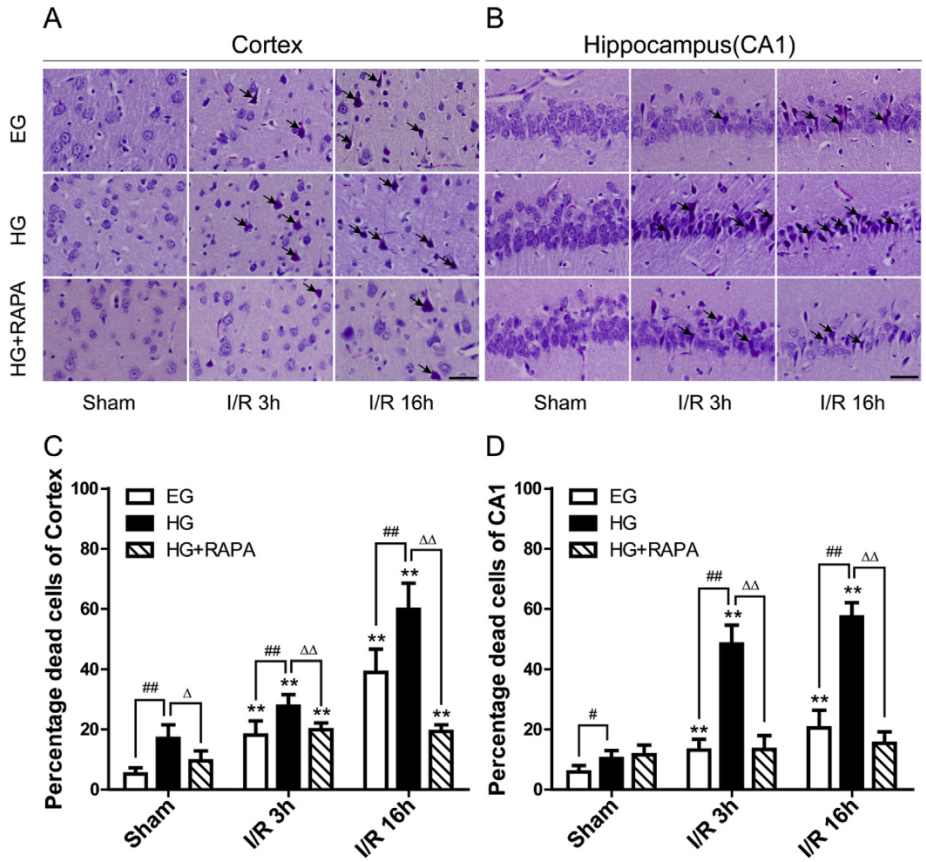

Fig. 1. Pathological damage. A,B. Microphotographs showing pathological results in the cortex (A) and CA1 (B) areas; C,D. Summarized percentage of neuronal death in each group in the cortex (C) and CA1 (D). Lapis lazuli blue and acid fuchsia stain. Arrows show dead neurons. Bar = 50 μm. Data are displayed as means ± s.d. **$p < 0.01$ vs. sham in the same treatment group, #$p < 0.05$ and ##$p < 0.01$ vs. EG at the same reperfusion endpoints. △$p < 0.05$ and △△$p < 0.01$ vs. HG at the same reperfusion endpoints. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

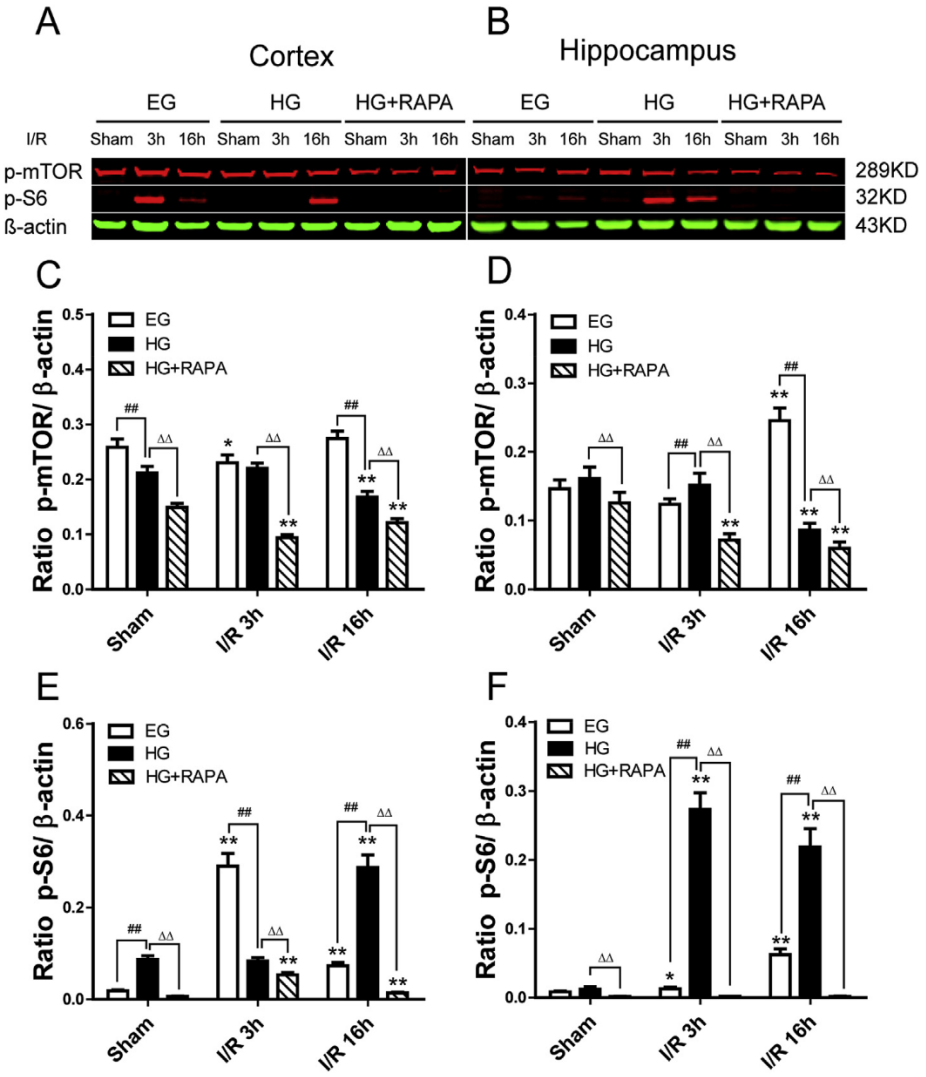

Fig. 2. Protein blotting of p-mTOR and p-S6 in the cytosolic fractions. A&B, Representative protein blots of p-mTOR and p-S6 in the cortex and hippocampi. C–F, Corresponding band densities of the proteins. $^{**}p < 0.01$ *vs.* sham in the same treatment group. $\#\#p < 0.01$ *vs.* EG at the same reperfusion endpoints. $\triangle\triangle p < 0.01$ *vs.* HG at the same reperfusion endpoints.

rapamycin completely blocked the increases of p-mTOR and p-S6 in the cortex of the HG animals.

In the hippocampi (Fig. 2 B,D,F), both p-mTOR and p-S6 increased moderately at 3 h and 16 h of I/R in the EG animals. HG further increased the p-mTOR at 3 h and p-S6 was increased at 3 h and 16 h compared to EG counterparts. Treatment with rapamycin completely blocked the ischemia-caused elevations of p-mTOR and p-S6 in the hippocampi of the hyperglycemic animals.

3.5. *Rapamycin suppressesERK1/2 in HG ischemic rats*

Phospho-ERK1/2 was measured in the nuclear fractions of the brain samples collected from the cerebral cortex and hippocampi using a specific antibody. In the cortex, cerebral ischemia caused a moderate transient raise in p-ERK1/2 at 3 h I/R and then slightly recessed at 16 h of I/R. HG led to a mild increase in p-ERK1/2 in sham controls and a drastic elevation at 16 h after ischemia and reperfusion. Treatment with rapamycin dampened the elevation of p-ERK1/2 substantially in shams and at 16 h after I/R in the HG animals (Fig. 3 A,D).

In the hippocampi, EG ischemia resulted in a moderate increase in p-ERK1/2 after 16 h of I/R. HG caused a much higher increase of p-ERK1/2 than the EG counterparts after 3 h and 16 h of I/R. Rapamycin significantly suppressed the p-ERK1/2 at 3 h and completely abolished its elevation at 16 h in the HG animals (Fig. 3 B,D).

4. Discussion

Our results demonstrate that pre-ischemic hyperglycemia, but not the euglycemia, caused 100% (8 out of 8) of rats to develop post-ischemic seizures after a 10-min transient forebrain ischemia. Euglycemic ischemia caused mild damages to neurons located in the cerebral cortex and hippocampi and increased moderately the protein contents of p-mTOR, p-S6 and p-ERK1/2. By contrast, hyperglycemia resulted in more profound damages to the cortex and hippocampi and led to significant surges of p-S6 and p-ERK1/2 in the EG animals, especially in the hippocampal tissue. Rapamycin treatment in HG animals successfully prevented post-ischemic seizures in 7 out of 8 rats, substantially reduced the neuronal death, and partially or completely blocked the hyperglycemia-induced activations of mTOR and ERK1/2 signaling pathways.

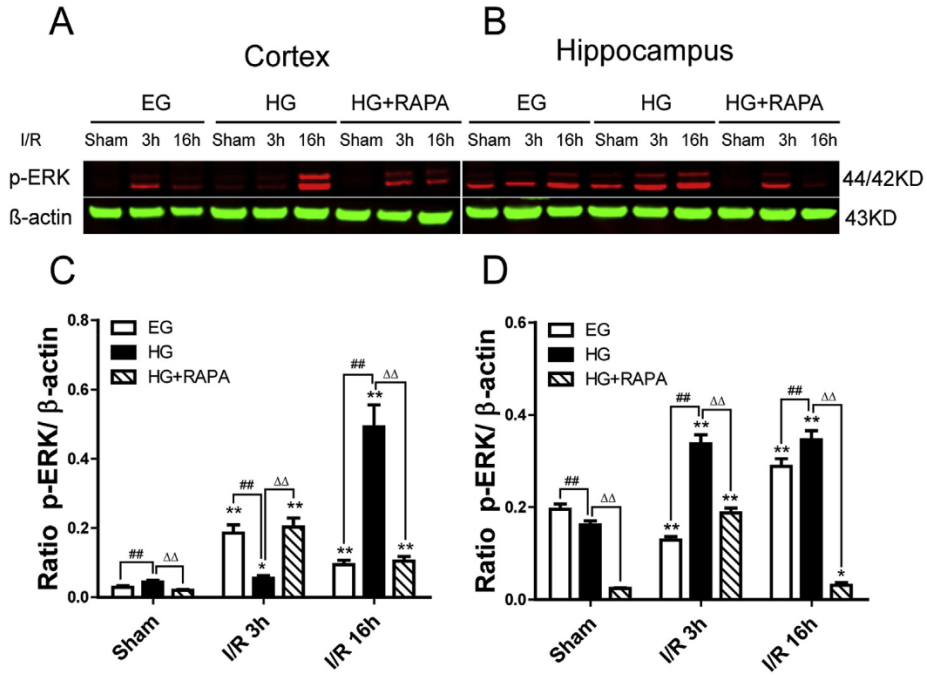

Fig. 3. Western blotting for p-ERK1/2 in the nuclear fractions of the cortex and hippocampi. A&B, Representative protein blots of p-ERK1/2. C&D, Summarized bar graph showing p-ERK1/2 band intensities. $^{**}p < 0.01$ *vs.* sham in the rapamycin treatment group, $\#\#p < 0.01$ *vs.* EG at the same reperfusion endpoint. $\triangle\triangle p < 0.01$ *vs.* HG at the same reperfusion endpoint.

Clinical studies have revealed that patients with hyperglycemia have a higher incidence of seizure than those without hyperglycemia and the post-stroke seizure is inclined to occur more in diabetes mellitus patients than in those without diabetes [17,18]. We have previously observed that euglycemic animals rarely developed post-ischemic seizure after 10 min of forebrain ischemia, while about 50% of hyperglycemic ischemic animals developed seizures when plasma glucose increased to 10–16 mM and the post-ischemic seizure incidence reached to 100% when plasma glucose content surpassed 16 mM [18]. In the present study, the glucose levels ranged from 18 to 23 mM and 8 out of 8 rats developed post-ischemic seizures, which is consistent with our previous findings. Importantly, we have demonstrated for the first time in this study that rapamycin is capable of preventing post-ischemic seizures in hyperglycemic ischemic rats, providing a new therapeutic approach to prevent clinical post-stroke seizures in diabetic patients.

Hyperglycemia worsens the pathological outcomes in brain regions after stroke by greatly shortening the time of maturation of ischemic destruction, impairing resistant structures and triggering post-ischemic seizures [19,20]. In this study, EG ischemia induced a mild damage in the cortex and hippocampi at 16 h of I/R. As expected, hyperglycemia significantly aggravated the neuronal damage in these two areas and more extensive damage appeared after only 3 h of I/R in the CA1 sub-region. Rapamycin treatment significantly reduced neuronal death in the cortex and hippocampi of the HG animals. This result is consistent with our recent publications showing that rapamycin decreases neuronal death in ischemic animals under either euglycemic or hyperglycemic conditions [14,15,19].

mTOR is an evolutionarily conserved serine/threonine kinase which mediates apoptosis and autophagy through phosphorylating its downstream effectors including ribosomal protein S6 kinase beta-1 (p70S6K) and 4E Binding Protein 1 (4EBP1). mTOR has a pernicious role in a large number of neurological disorders, especially in epilepsy [8]. As it is known, mTOR activation plays a critical pathogenic role in tuberous sclerosis (TSC) [21]. At the same time, mTOR pathway activation in the hippocampus is also one of the main mechanisms for many acquired epilepsies [22,23]. In this study, we have demonstrated that the protein level of mTOR and its downstream protein p-S6 increased in the cortex and the hippocampi of the hyperglycemia rats after 3 h of I/R, especially in the hippocampi. We hypothesized that mTOR activation may mediate post-stroke seizure in the rats. To test this hypothesis, we pretreated the animals with rapamycin and subjected the animals to 10 min transient forebrain ischemia. The results showed that rapamycin suppressed p-mTOR and p-S6 in the HG animals after I/R at 3 h and 16 h. The inhibitory effect of rapamycin on mTOR signaling was more profound in the hippocampi. This inhibition of mTOR signaling may account for the significant reduction of post-ischemic seizure incidence in the HG ischemic animals. Only 1/8 animals developed post-ischemic seizure with much milder convulsion intensity in HG + RAPA animals compared to 8/8 that developed post-ischemic seizures in non-rapamycin-treated HG rats. These findings support our hypothesis that activation of mTOR signaling mediates the development of post-ischemic seizures in HG animals.

It is controversial whether mTOR signaling activation plays a detrimental or beneficial role in ischemic brain injury [24]. Previous studies have shown that activation of mTOR signaling mitigated ischemic brain damage [25,26]. However, our own studies have demostrated that suppression of mTOR offered protection to brain tissue against ischemic injury [19]. The current experiments have further confirmed our previous findings that ischemia activates mTOR signaling and repression of mTOR through rapamycin remarkably decreased ischemic brain injure in HG rats.

We have observed that p-ERK1/2 moderately elevated in both the cortex and hippocampi in EG animals experienced cerebral I/R injury. HG markedly augmented the increase in the two structures after I/R comparing to the EG counterparts. Activation of ERK1/2

has been previously observed in cerebral ischemic animals. It was found that HG amplified the increases of ERK1/2 after brain ischemia, especially in brain structures where damage was more severe with hyperglycemia, such as the cingulate cortex, hippocampal CA3 and dentate gyrus [27]. Inhibition of ERK1/2 by an ERK inhibitor was previously shown to alleviated HG-aggravated ischemic brain damage [27] and it is likely that activation of ERK1/2 in this present work plays a detrimental role in mediating HG-aggravated ischemic brain damage.

Activation of ERK1/2 has also been observed in the brains of humans and animals with epilepsy [10,28] with particularly high expression in hippocampal CA3 and dentate gyrus neurons and dentate neural progenitor cells, and is considered an important molecular pathway underlying the epileptogenesis in various seizure-associated disorders and animal models [29–31]. Activation of ERK1/2 may lead to neuronal hyperexcitability and synchronous firing through regulating mRNA binding proteins [30]. This is supported by the fact that transgenic upregulation of MEK1, an upstream positive regulator of ERK1/2, results in activation of ERK1/2 and spontaneous development of clinical epileptic seizures in mice by stimulating *N*-methyl-D-aspartate (NMDA) receptor activity [32]. Therefore, the inhibitory effect of rapamycin on ERK1/2 signaling observed in the present model may contribute to its anti-epileptic effect.

It is interesting that rapamycin inhibited the activations of both mTOR and ERK1/2 in the present study. This finding is consistent with our observations in diabetic ischemic animals treated with rapamycin [14]. ERK has been known to be an upstream factor that activates the mTOR pathway [33]; however, it is not known whether mTOR could regulate ERK phosphorylation. Rapamycin inhibiting both mTOR and ERK1/2 signaling could be explained by 1) rapamycin possesses an undiscovered inhibitory effect on ERK1/2, or 2) mTOR also serves as an upstream regulator for ERK1/2, or 3) there is a cross-inhibition between the mTOR and ERK pathways, which means that inhibition of downstream mTOR will negatively regulate the upstream ERK pathway, thereby inhibiting the ERK signaling.

In conclusion, our findings have suggested that 1) Post-ischemic seizure development in hyperglycemic animals is related to activation of the mTOR and ERK1/2 signaling pathways; 2) Rapamycin is capable of preventing post-ischemic seizure development in hyperglycemic animals; and 3) the anti-epileptic effect of rapamycin involves inhibition of mTOR and ERK1/2 signaling pathways. Since rapamycin has been shown to reduce ischemic brain damage and prevent post-ischemic seizures, it is worthwhile to explore clinical interventions with rapamycin in stroke patients who have a high risk of post-ischemic seizure development.

Conflicts of interest

The authors have declared that no conflicts of interest.

Author contributions

XY, CH, PAL conceived and designed the experiments. CH, PL, XY, performed the experiments. CH, PL, XY, PAL analyzed the data. XY, CH, PAL wrote the manuscript.

Acknowledgements

The authors Thank Dr. Mary Zimmerman for editing this manuscript. This study is supported by National Natural Science Foundation of China (No.81560226 and No.81860250) to XY, Natural Science Foundation of Ningxia Hui Autonomous (2018AAC03134) to XY, National Natural Science Foundation of China (No.81660206) to PL. The Biomanufacturing Research Institute and Technology Enterprise (BRITE) is partially funded by the Golden Leaf Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.09.096

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.09.096.

References

[1] C.F. Bladin, A.V. Alexandrov, A. Bellavance, N. Bornstein, B. Chambers, R. Cote, L. Lebrun, A. Pirisi, J.W. Norris, Seizures after stroke: a prospective multicenter study, Arch. Neurol. 57 (2000) 1617–1622.
[2] J.L. De Reuck, Stroke-related seizures and epilepsy, Neurol. Neurochir. Pol. 41 (2007) 144–149.
[3] A. Shindo, H. Tomimoto, [Diabetes and ischemic stroke], Brain Nerve 66 (2014) 107–119.
[4] R. Roivainen, E. Haapaniemi, J. Putaala, M. Kaste, T. Tatlisumak, Young adult ischaemic stroke related acute symptomatic and late seizures: risk factors, Eur. J. Neurol. 20 (2013) 1247–1255.
[5] C.H. Chen, A. Manaenko, Y. Zhan, W.W. Liu, R.P. Ostrowki, J. Tang, J.H. Zhang, Hydrogen gas reduced acute hyperglycemia-enhanced hemorrhagic transformation in a focal ischemia rat model, Neuroscience 169 (2010) 402–414.
[6] P. Lipton, Ischemic cell death in brain neurons, Physiol. Rev. 79 (1999) 1431–1568.
[7] Z. Lei, H. Zhang, Y. Liang, Q. Cui, Z. Xu, Z.C. Xu, Reduced expression of IA channels is associated with postischemic seizures in hyperglycemic rats, J. Neurosci. Res. 92 (2014) 1775–1784.
[8] A.P. Ostendorf, M. Wong, mTOR inhibition in epilepsy: rationale and clinical perspectives, CNS Drugs 29 (2015) 91–99.
[9] E. Russo, R. Citraro, G. Donato, C. Camastra, R. Iuliano, S. Cuzzocrea, A. Constanti, G. De Sarro, mTOR inhibition modulates epileptogenesis, seizures and depressive behavior in a genetic rat model of absence epilepsy, Neuropharmacology 69 (2013) 25–36.
[10] G. Gangarossa, S. Sakkaki, P. Lory, E. Valjent, Mouse hippocampal phosphorylation footprint induced by generalized seizures: focus on ERK, mTORC1 and Akt/GSK-3 pathways, Neuroscience 311 (2015) 474–483.
[11] Y. Li, Z. Peng, B. Xiao, C.R. Houser, Activation of ERK by spontaneous seizures in neural progenitors of the dentate gyrus in a mouse model of epilepsy, Exp. Neurol. 224 (2010) 133–145.
[12] P.A. Li, Q.P. He, O. Yi-Bing, B.R. Hu, B.K. Siesjo, Phosphorylation of extracellular signal-regulated kinase after transient cerebral ischemia in hyperglycemic rats, Neurobiol. Dis. 8 (2001) 127–135.
[13] J.Z. Zhang, L. Jing, Y. Ma, F.Y. Guo, Y. Chang, P.A. Li, Monosialotetrahexosy-1 ganglioside attenuates diabetes-enhanced brain damage after transient forebrain ischemia and suppresses phosphorylation of ERK1/2 in the rat brain, Brain Res. 1344 (2010) 200–208.
[14] P. Liu, X. Yang, C. Hei, Y. Meli, J. Niu, T. Sun, P.A. Li, Rapamycin reduced ischemic brain damage in diabetic animals is associated with suppressions of mTOR and ERK1/2 signaling, Int. J. Biol. Sci. 12 (2016) 1032–1040.
[15] X. Yang, C. Hei, P. Liu, Y. Song, T. Thomas, S. Tshimanga, F. Wang, J. Niu, T. Sun, P.A. Li, Inhibition of mTOR pathway by rapamycin reduces brain damage in rats subjected to transient forebrain ischemia, Int. J. Biol. Sci. 11 (2015) 1424–1435.
[16] R.J. Racine, Modification of seizure activity by electrical stimulation. I. After-discharge threshold, Electroencephalogr. Clin. Neurophysiol. 32 (1972) 269–279.
[17] C.L. Lu, Y.H. Chang, Y. Sun, C.Y. Li, A population-based study of epilepsy incidence in association with type 2 diabetes and severe hypoglycaemia, Diabetes Res. Clin. Pract. 140 (2018) 97–106.
[18] P.A. Li, M. Shamloo, K. Katsura, M.L. Smith, B.K. Siesjo, Critical values for plasma glucose in aggravating ischaemic brain damage: correlation to extracellular pH, Neurobiol. Dis. 2 (1995) 97–108.
[19] C. Hei, P. Liu, X. Yang, J. Niu, P.A. Li, Inhibition of mTOR signaling confers protection against cerebral ischemic injury in acute hyperglycemic rats, Int. J. Biol. Sci. 13 (2017) 878–887.
[20] B.C. Kalita, M.S. Chaliha, K. Ambardekar, Comparison of patients with diabetes & those without diabetes presenting with ischemic stroke, J. Assoc. Phys. India 64 (2016) 95.
[21] F. Palavra, C. Robalo, F. Reis, Recent advances and challenges of mTOR inhibitors use in the treatment of patients with tuberous sclerosis complex,

Oxid Med Cell Longev 2017 (2017), 9820181.
[22] S.S. McDaniel, M. Wong, Therapeutic role of mammalian target of rapamycin (mTOR) inhibition in preventing epileptogenesis, Neurosci. Lett. 497 (2011) 231–239.
[23] K. Maiese, Z.Z. Chong, Y.C. Shang, S. Wang, mTOR: on target for novel therapeutic strategies in the nervous system, Trends Mol. Med. 19 (2013) 51–60.
[24] K. Maiese, Cutting through the complexities of mTOR for the treatment of stroke, Curr. Neurovascular Res. 11 (2014) 177–186.
[25] J.S. Xi, Y.F. Wang, X.X. Long, Y. Ma, Mangiferin potentiates neuroprotection by isoflurane in neonatal hypoxic brain injury by reducing oxidative stress and activation of phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling, Med. Sci. Monit. 24 (2018) 7459–7468.
[26] H. You, T. Li, J. Zhang, Q. Lei, X. Tao, P. Xie, W. Lu, Reduction in ischemic cerebral infarction is mediated through golgi phosphoprotein 3 and Akt/mTOR signaling following salvianolate administration, Curr. Neurovascular Res. 11 (2014) 107–113.
[27] J.Z. Zhang, L. Jing, A.L. Ma, F. Wang, X. Yu, Y.L. Wang, Hyperglycemia increased brain ischemia injury through extracellular signal-regulated protein kinase, Pathol. Res. Pract. 202 (2006) 31–36.
[28] Z.Q. Xi, X.F. Wang, R.Q. He, M.W. Li, X.Z. Liu, L.Y. Wang, X. Zhu, F. Xiao, J.J. Sun, J.M. Li, Y. Gong, L.F. Guan, Extracellular signal-regulated protein kinase in human intractable epilepsy, Eur. J. Neurol. 14 (2007) 865–872.
[29] G. Curia, F. Gualtieri, R. Bartolomeo, R. Vezzali, G. Biagini, Resilience to audiogenic seizures is associated with p-ERK1/2 dephosphorylation in the subiculum of Fmr1 knockout mice, Front. Cell. Neurosci. 7 (2013) 46.
[30] H.F. Pernice, R. Schieweck, M.A. Kiebler, B. Popper, mTOR and MAPK: from localized translation control to epilepsy, BMC Neurosci. 17 (2016) 73.
[31] K. Sawicka, A. Pyronneau, M. Chao, M.V. Bennett, R.S. Zukin, Elevated ERK/p90 ribosomal S6 kinase activity underlies audiogenic seizure susceptibility in fragile X mice, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E6290–E6297.
[32] A.S. Nateri, G. Raivich, C. Gebhardt, C. Da Costa, H. Naumann, M. Vreugdenhil, M. Makwana, S. Brandner, R.H. Adams, J.G. Jefferys, O. Kann, A. Behrens, ERK activation causes epilepsy by stimulating NMDA receptor activity, EMBO J. 26 (2007) 4891–4901.
[33] M.C. Mendoza, E.E. Er, J. Blenis, The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation, Trends Biochem. Sci. 36 (2011) 320–328.