Biochemical and Biophysical Research Communications 423 (2012) 276–281

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Inhibition of poly(ADP-ribose) polymerase inhibits ischemia/reperfusion induced neurodegeneration in retina via suppression of endoplasmic reticulum stress

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ARTICLE INFO

Article history:
Received 16 May 2012
Available online 26 May 2012

Keywords:
PARP
ER stress
Neuroprotection
Ischemia and reperfusion

ABSTRACT

Poly(ADP-ribose) polymerase (PARP) inhibitors have neuroprotective effects after retinal ischemia and reperfusion (I/R) injury, but mechanisms of this action are not clear. A second generation PARP inhibitor, GPI 15427, was administrated to mice to investigate the possible mechanisms underlying its neuroprotective effects after retinal I/R injury. Ischemia was induced by increasing intraocular pressure to 80–90 mm Hg for 60 min followed by reperfusion, and mice were treated with GPI 15427 (40 mg/kg^{-1} day^{-1}, orally) 2 days before or 1 day after injury. Histopathology caused by the retinal I/R injury was estimated by TUNEL assay and histological analyses. Relative gene expressions were evaluated by RT-PCR, Western blotting and immunohistological studies. GPI 15427 inhibited the retinal I/R-induced PARP activation and glial cell activation. GPI 15427 also significantly inhibited the I/R-induced neurodegeneration, as well as increase in TUNEL-positive cells. I/R-induced PERK-eIF2α-CHOP activation and Bip over-expression were inhibited by GPI 15427, while it did not suppress I/R-induced CHOP over-expression and degeneration of retinal capillaries. Our results suggest that GPI 15427 inhibited retinal I/R-induced neurodegeneration and glial cell activation, and this was associated with an effect of the drug to suppress PERK-eIF2α-CHOP activation and Bip over-expression. These results provide evidence that GPI 15427 inhibits retinal I/R injury at least in part via inhibition of endoplasmic reticulum stress.

1. Introduction

Retinal ischemia and reperfusion (I/R) occurs in the development of multiple ocular diseases, including acute close-angle glaucoma [1] and diabetic retinopathy [2]. The retina consists of neurons, vasculature and glia, and each of these compartments can be affected in retinal I/R injury [3–6]. Cell death has been regarded as one of the major causes of the pathological changes after I/R injury, but the molecular mechanisms underlying the formation of these lesions have remained unclear.

Emerging evidences suggest that endoplasmic reticulum (ER) stress contributes to cell death in multiple retinal diseases, such as retinitis pigmentosa [7], glaucoma [8], and diabetic retinopathy [9]. Moreover, injection of ER stress inducers into the vitreous can cause retinal neurodegeneration [10,11]. Upon accumulation of unfolded or misfolded proteins in ER lumen, three canonical ER membrane-associated proteins, PERK (double-strand RNA activated protein kinase-like ER kinase), IRE1α (inositol requiring enzyme 1 isoform α), and ATF6 (activating transcription factor 6) are activated, accompanied by the elevation of ER chaperones. Upon prolonged ER stress, apoptosis is evoked by over-expression of CHOP (CCAAT enhancer-binding protein homologous protein) [12]. However, the reason why ER stress is elevated after retinal I/R remain unclear.

Poly(ADP-ribose) polymerase (PARP) plays critical roles in the regulation of cell death. On one hand, PARP mediates poly(ADP-ribosy)lation by transferring ADP-ribose units from NAD^+ to itself and other proteins. Thus, over-activation of PARP causes a rapid depletion of cellular NAD^+ and ATP, which leads to cell necrosis by energy depletion [13]. On the other hand, PARP activation mediates caspase-independent cell death involving AIF (apoptosis inducing factor) translocation from mitochondria to the nucleus,

Abbreviations: ATF6, activating transcription factor 6; Bip, immunoglobulin binding protein/glucose regulated protein; CHOP, CCAAT enhancer-binding protein homologous protein; eIF2α, eukaryotic translational initiation factor 2α; ER, endoplasmic reticulum; GCL, ganglion cell layer; GFAP, glial fibrillary acidic protein; I/R, ischemia and reperfusion; INL, inner nuclear layer; IPL, inner plexiform layer; IRE1α, inositol requiring ER-to-nucleus signal kinase 1 isoform α; ONL, outer nuclear layer; OPL, outer plexiform layer; PARP, poly(ADP-ribose) polymerase; PERK, double-strand RNA activated protein kinase-like ER kinase; PDI, protein disulfide isomerase.

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http://dx.doi.org/10.1016/j.bbrc.2012.05.109

which results in cell death by initiating chromatin fragmentation [14]. Genetic deletion of PARP has neuroprotective effects in cerebral ischemic injury by inhibition of energy depletion [15]. PARP activation is elevated in retinas under I/R injury [3] and diabetes [16]. PARP inhibitors have protective effects against the development of diabetic retinopathy by regulating VEGF (vascular endothelial growth factor) expression [17] or the activity of the inflammatory transcription factor, NF-κB [16]. The PARP inhibitor, 3-aminobenzamide (3-ABA), inhibits retinal I/R injury-induced neuronal cell death when injected into the vitreous after the injury [18], but the mechanisms underlying the neuroprotective role of PARP inhibitor remains unclear.

We hypothesize that accumulation of PARP-mediated accumulation of poly(ADP-ribose) (PAR) modified proteins stimulates ER stress and causes retinal cell death after the I/R injury. To test this hypothesis, a specific PARP inhibitor (10-(4-methy-piperazin-1-yl-methyl)-2H-7-oxa-1,2-diaza-benzo[de]anthracen-3-1, GPI 15427) was administrated to mice subjected to I/R, and effects of the drug on the activation of ER stress signaling pathways, injury-induced glial cell activation, and neuronal and vascular degeneration were evaluated. Our results demonstrated that GPI 15427 inhibited retinal I/R-induced neurodegeneration and glial cell activation through inhibition of PERK-eIF2α-CHOP activation and Bip overexpression. Our study suggested that oral administration of GPI 15427 has neuroprotective effects on retinal I/R injury via inhibition of ER stress in the neural retina.

2. Materials and methods

2.1. Model of retinal ischemia and reperfusion

Male C57BL/6 mice were obtained from Wuhan University Animal Laboratory, and housed in ventilated microisolater cages with free access to water and food. Ocular ischemia was induced as previously reported [3,5,6] (60 min ischemia on one eye followed by perfusion). The other eye of the same animal was used as control. Mice were treated with GPI 15427 by gavage 2 days before I/R injury or 1 day post-injury, with a dosage of 40 mg/kg body weight per day. Retinas or eyes were collected at different times after the injury. All procedures involving the animals were conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and approved by the Committee on Ethics in the Care and Use of Laboratory Animals of Wuhan University.

2.2. Morphological examination of ganglion cell loss and retinal thickness

The enucleated eyes were routinely fixed, embedded, cut and stained with PASH (Periodic Acid Schiff and Hematoxylin). The nuclei in the ganglion cell layer (not including nuclei in the vessels) were counted, and thickness of different retinal layers were measured in each retina using the ImagePlus 6.0 software (Media Cybernetics, Bethesda, Maryland) as we previously reported [5].

2.3. TUNEL assay

TUNEL assay was performed as previously reported [3]. The TUNEL reaction was performed using a commercial in situ cell death detection kit (Roche, Mannheim, Germany). DNase1 treated section (5 U/section) was used as the positive control. Images were taken under an Olympus BX60 microscope (400× magnification). $TUNEL^+$ cells were counted and reported as the number of $TUNEL^+$ cells per mm of retinal length.

2.4. RNA isolation and RT-PCR

Total RNA isolation, cDNA reverse transcription and PCR were performed as previously reported [6]. The primer sequences were as follows: *Chop* forward primer, 5′-CCCTGCCTTTCACCTTGG-3′, *Chop* reverse primer, 5′-CCGCTCGTTCTCCTGCTC-3′; *Bip* forward primer, 5′-GGGAAAGAAGGTT ACCCATGC-3′, *Bip* reverse primer, 5′-CGAGTAGATCCACCAACCAGAG-3′; *18S rRNA* forward primer, 5′-TTAAGAGGGACGGCCGGGGG-3′, *18S rRNA* reverse primer, 5′-GCCGGGTGAGGTTTCCCGTG-3′. Band density was quantified using Quantity One 1-D Analysis Software (Bio-Rad). Transcription levels of *Chop* and *Bip* were quantitated against *18S rRNA* level in the same sample, and normalized to non-injured retinas, which was set as one fold.

2.5. Western blot analysis

Retinas were isolated and sonicated in RIPA buffer (Beyotime Biotech, China). Western blots were performed as we reported previously [19,20] with antibodies for poly(ADP-ribose) (Alexis Biochemicals), GFAP (Millipore), p-PERK (CST, Danvers, MA), p-eIF2α (CST), eIF2α (CST), CHOP (Santa Cruz Biotechnology), Bip (BD transduction Laboratories), PDI (CST) and β-actin (Sigma, St. Louis, MO). Most protein levels were quantitated relative to β-actin level in the same sample except for the phosphorylated eIF2α which was quantitated relative to the corresponding total eIF2α level. The expressions of target proteins in different groups were normalized to non-injured group, which was set as one fold.

2.6. Immunohistochemical studies

Retinal sections were deparaffinated and rehydrated, and immunohistochemical studies were performed as we previously reported [5,20] Briefly, sections were incubated with 3% H_2O_2 for 5 min. After blocking with 2% normal goat serum, sections were incubated with primary antibodies against poly(ADP-ribose) (Alexis Biochemicals) and CHOP (Santa Cruz Biotechnology) overnight at 4 °C, and then incubated with biotinylated anti-rabbit or anti-mouse IgG (Vector laboratories, Burlingame, CA). Staining was visualized by DAB substrate (Vector laboratories) reaction following the ABC kit (Vector laboratories). Negative controls were set up parallelly without applying primary antibodies.

2.7. Examination of capillary degeneration

Retinal vasculatures were isolated and quantitated as we previously reported [5,16]. Briefly, seven days after retinal I/R injury, pure retinal vasculatures were isolated. Isolated vascular trees were stained with PASH for light microscopy examination (200× magnification). Acellular (degenerate) capillaries were counted and reported as number per mm^2 of retinal area.

2.8. CHOP immunofluorescence staining on retinal vasculatures

One day after retinal I/R injury, retinal vasculatures were isolated, and CHOP immunofluorescence staining on retinal vessel tress were performed as we previously reported [6]. Images were taken under a fluorescence microscope. Negative control was set up parallelly without applying primary antibody.

2.9. Statistical analysis

All results were expressed as the mean ± SD. Data was analyzed by the nonparametric Kruskal–Wallis test followed by the Mann–Whitney test. Differences were considered statistically

significant when the $P < 0.05$. The changes were considered as trends when $0.05 < P \leqslant 0.08$.

3. Results

3.1. GPI 15427 inhibits retinal I/R injury-induced PARP activation

PARP activation was estimated by measuring the levels of poly(ADP-ribosy)lated proteins. Consistent with our previous report [3], there was 8.8-fold increase in the level of poly(ADP-ribosy)lated proteins in the retinas one day after I/R injury compared to that in the non-injured retinas ($P < 0.05$; Fig. 1A and B), and this activation lasted at least 4 days after the injury (data not shown). Immunohistochemical study demonstrated that the levels of poly(ADP-ribosy)lated proteins were significantly increased in GCL (ganglion cell layer), INL (inner nuclear layer) and ONL (outer nuclear layer) one day after I/R injury. GPI 15427 pre-treatment (initiated before injury) significantly inhibited the injury-induced PARP activation ($P < 0.05$) in all three of these layers (Fig. 1C).

3.2. GPI 15427 inhibits retinal I/R-induced glial cell activation and neuronal cell death

The upregulation of GFAP is regarded as one of the earliest hallmarks of reactive gliosis [21]. I/R induced a 1.7-fold increase in GFAP expression in injured retinas compared to non-injured retinas one day after the injury ($P < 0.05$), while GPI 15427 pre-treatment significantly inhibited this injury-induced glial cell activation ($P < 0.05$, Fig. 2A).

Besides glial cell activation, I/R injury also caused a 51% cell loss in GCL in the injured retinas compared to non-injured retinas (measured 4 days after the injury). Treatment with GPI 15427 after the injury (post-treatment) significantly inhibited the I/R-induced cell loss in GCL ($P < 0.01$), and treatment with GPI 15427 before the injury (pre-treatment) likewise showed a trend to inhibit I/R-induced cell loss in GCL ($P = 0.08$; Fig. 2B). TUNEL assay was performed to investigate apoptotic cell death one day after retinal I/R injury (Fig. 2C). I/R-injured retinas had abundant TUNEL-positive cells that were located in GCL, INL and ONL. GPI 15427 pre-treatment resulted in significantly less TUNEL-positive cells in all layers, with 79% reduction of total cell number compared to that in I/R injured retinas ($P < 0.05$, Fig. 2D).

3.3. GPI 15427 inhibits retinal I/R-induced reduction in retinal thickness

Reduction of retinal thickness is another parameter of neurodegeneration [3,22]. Effects of pre- and post-treatment with GPI 15427 on retinal I/R-induced reduction in retinal thicknesses were evaluated 4 days after the injury. As shown in Table 1, retinal I/R caused significant reduction in thickness for the GCL and IPL (39% and 43% reduction compared to the non-injured ones, respectively; $P < 0.01$). GPI 15427 pre-treatment significantly inhibited the I/R-induced reduction in retinal GCL thickness, and tended to have a beneficial effect also on IPL thickness, but the result did not achieve statistical significance. Surprisingly, pre-treatment with GPI 15427 significantly increased the thickness of INL, OPL and ONL. Intervention with drug after injury also showed inhibition effects on retinal I/R-induced reduction in the thickness of GCL and IPL ($P < 0.05$).

3.4. GPI 15427 inhibits retinal I/R-induced PERK-eIF2α-CHOP activation and Bip over-expression

To explore the possible mechanisms that contribute to the neuroprotective effects of GPI 15427, the ER stress-associated PERK-eIF2α-CHOP signaling pathway, and expression level of Bip (a protein chaperone) were investigated one day after I/R injury. As shown in Fig. 3A and B, mRNA levels of *Chop* and *Bip* were sig-

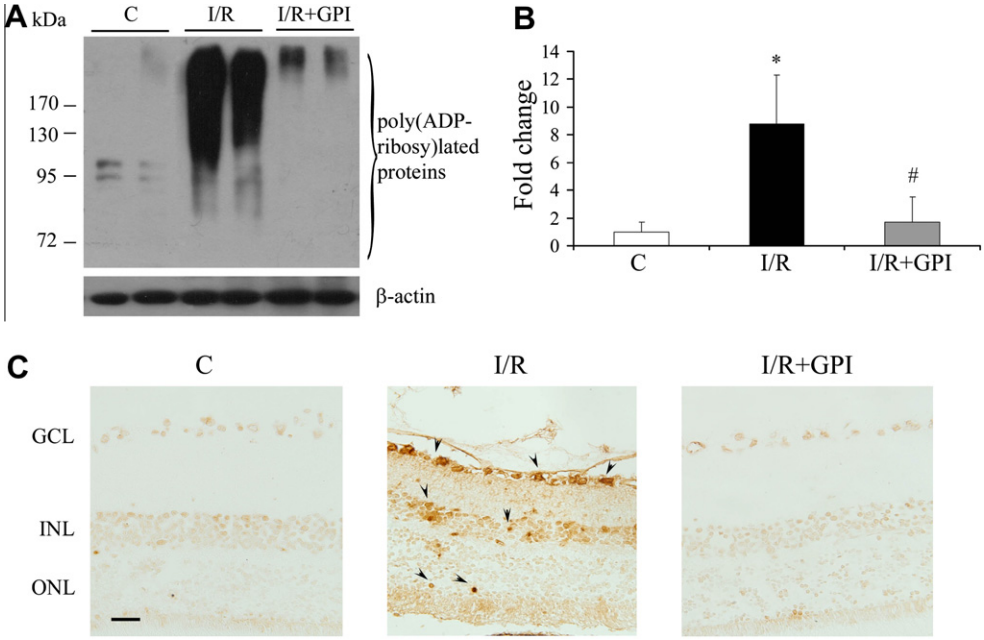

Fig. 1. GPI 15427 inhibited retinal I/R-induced PARP activation one day after retinal I/R injury. (A) Representative Western blot of poly(ADP-ribosy)lated proteins; (B) densitometric quantitative results of PARP activation; (C) immunohistochemical detection of poly(ADP-ribosy)lated protein on retinal sections. Magnification, 400×, scale bar represents 20 μm. Positive-stained cells (brown color) are indicated by arrowheads. ($n = 4$ per group; C, non-injured retinas; I/R, I/R-injured retinas; I/R + GPI, I/R-injured retinas with GPI 15427 pre-injury treatment; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; $^{*}P < 0.05$ compared with non-injured retinas; $^{\#}P < 0.05$ compared with I/R-injured retinas.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

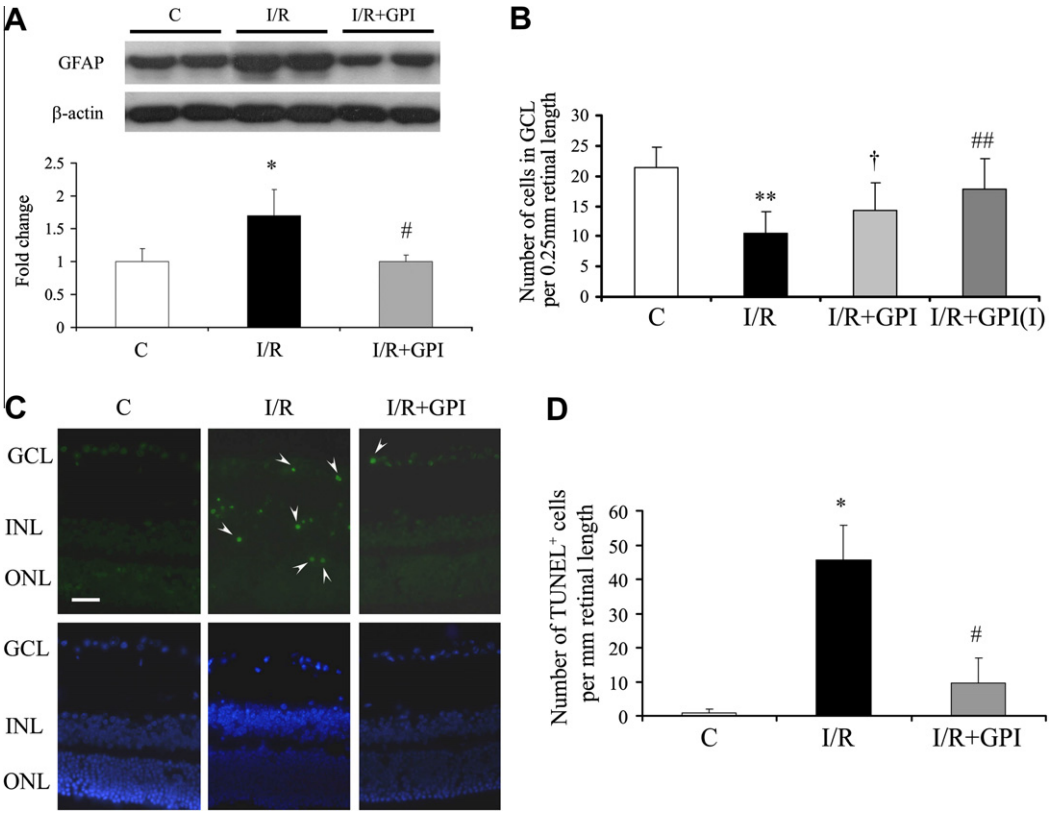

Fig. 2. GPI 15427 prevented retinal glial cell activation and neuronal cell death induced by retinal I/R injury. (A) Representative Western blot of retinal GFAP expression one day after retinal I/R injury was shown in the up, with densitometric quantitative result shown in the bottom (n = 4–5 in each group). (B) Quantitative results of cell number in retinal GCL four days after retinal I/R injury. (C) Representative images of TUNEL$^+$ cells (green, indicated by arrowheads) on retinal sections were shown in up panels, with DAPI staining (blue) of the same area on the bottom panels. Magnification, 400×, scale bar represents 20 μm. (D) Quantitative results of TUNEL assay (n = 3–4 in each group). C, non-injured retinas; I/R, I/R-injured retinas; I/R + GPI, I/R-injured retinas with GPI 15427 pre-injury treatment; I/R + GPI (I), I/R-injured retinas with GPI 15427 post-injury treatment. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; $^*P < 0.05$ and $^{**}P < 0.01$ compared with non-injured retinas; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with I/R-injured retinas; $^{\dagger}P = 0.08$ compared with I/R-injured retinas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
GPI 15427 inhibited I/R injury-induced reduction in retinal thickness.

Group	GCL (μm)	IPL (μm)	INL (μm)	OPL (μm)	ONL (μm)	Total (μm)
C	17.4 ± 1.7	54.7 ± 6.7	26.0 ± 3.4	11.3 ± 2.1	44.1 ± 5.9	153.4 ± 19.1
I/R	10.7 ± 2.2**	31.0 ± 11.3**	23.6 ± 2.0	11.0 ± 0.9	39.7 ± 1.8	116.0 ± 16.6**
I/R + GPI	14.6 ± 2.0$^{\#}$	38.7 ± 13.4	29.7 ± 3.5$^{\#\#}$	13.8 ± 1.9$^{\#\#}$	49.3 ± 8.5$^{\#\#}$	146.1 ± 25.7$^{\#}$
I/R + GPI (I)	13.6 ± 1.8$^{\#}$	40.8 ± 6.9$^{\#}$	25.8 ± 2.7§	12.8 ± 1.9$^{\#}$	44.7 ± 7.7	137.6 ± 16.5$^{\#}$

C, non-injured group; I/R, I/R-injured group; I/R + GPI, I/R-injured group with GPI 15427 pre-injury treatment; I/R + GPI (I), I/R-injured group with GPI 15427 post-injury treatment. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; Total, whole retina containing all layers.
n = 7–8 in each group;
$^{**}P < 0.01$ compared to the non-injured group;
$^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to the I/R injured group;
$^{\S}P < 0.05$, compared to the I/R + GPI group.

nificantly elevated 2.1-fold and 3-fold, respectively, in the injured retinas compared to those in the non-injured retinas ($P < 0.01$). Protein levels of CHOP and Bip were also significantly elevated by 5.7-fold and 2.2-fold, respectively, in the injured retinas compared to those in the non-injured retinas ($P < 0.05$; Fig. 3C and D). The phosphorylation levels of PERK and eIF2α were increased 7.9-fold and 6.3-fold, respectively, in the injured retinas compared to non-injured retinas ($P < 0.05$; Fig. 3C and D). All these I/R-induced elevations of ER stress markers were significantly blocked by GPI 15427 pre-treatment. Immunohistochemical studies of CHOP were also performed, and showed that cells immunostained for CHOP were detected in GCL, INL and ONL one day after I/R injury. GPI 15427 pre-treatment significantly blocked CHOP up-regulation induced by I/R injury in all of these three layers (Fig. 3E). Protein levels of another protein chaperone, PDI, were not altered in three different groups (Fig. 3C and D).

3.5. GPI 15427 does not inhibit retinal I/R-induced capillary degeneration and CHOP over-expression in the retinal vasculature

We also assessed effects of GPI 15427 on the retinal I/R-induced vascular degeneration seven days after the injury. Quantitative results of acellular capillaries formation are shown in Fig. 4A. Although I/R injury caused significant capillary degeneration

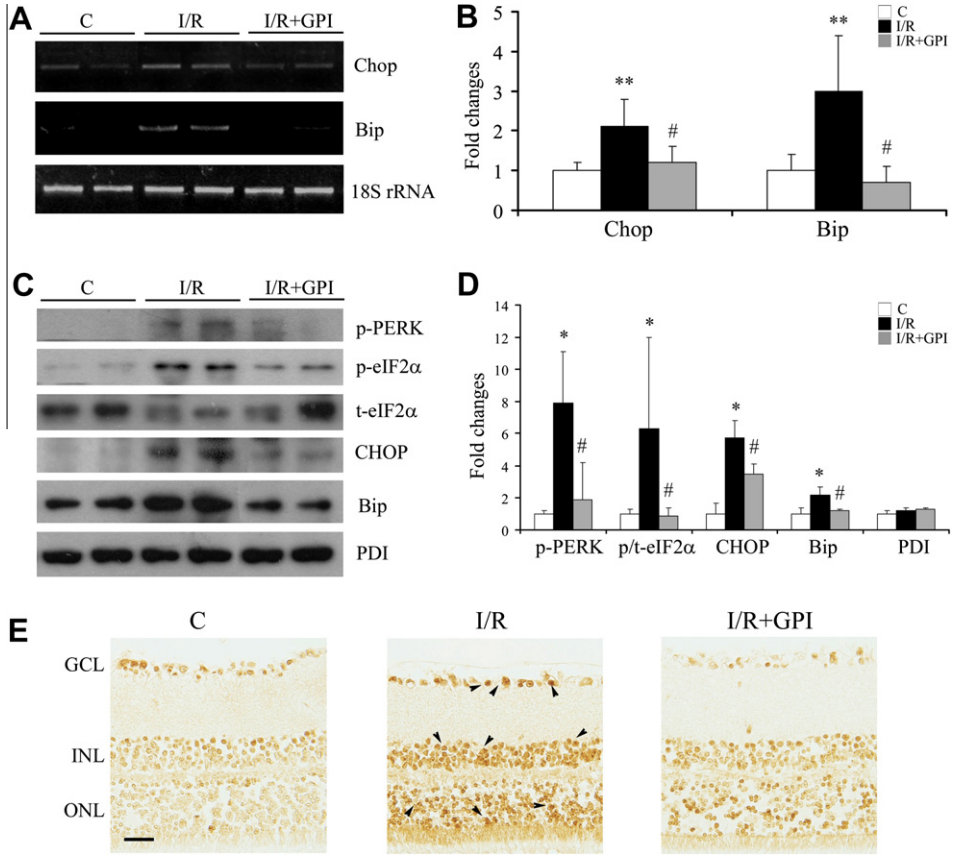

Fig. 3. GPI 15427 inhibited retinal I/R-induced PERK-eIF2α-CHOP activation and Bip over-expression one day after the injury. (A) Representative images of mRNA levels of *Chop*, *Bip* and *18S rRNA*. (B) Densitometric quantitative results of mRNA levels. (C) Representative Western blots of p-PERK and p-eIF2α, total eIF2α, CHOP, Bip and PDI. (D) Densitometric quantitative results of protein levels. (E) Representative images of CHOP expression on retinal sections. Magnification, 400×, scale bar represents 20 μm. Representative CHOP positive stained cells (brown color) are indicated by arrowheads. (n = 4–5 in each group; C, non-injured retinas; I/R, I/R-injured retinas; I/R + GPI, I/R-injured retinas with GPI 15427 pre-injury treatment; GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with non-injured retinas; $^{\#}P < 0.05$ compared with I/R-injured retinas.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

($P < 0.05$) as we previously reported [5,6], GPI 15427 pre-treatment had no effect on the I/R-induced vascular degeneration.

To further investigate why GPI 15427 had no effect on retinal I/R-induced vascular degeneration, CHOP levels in the retinal vasculatures were evaluated one day after the injury. As shown in Fig. 4B, CHOP expression was dramatically increased in I/R injured retinal vasculatures as we previously reported [6]. In contrast to its inhibitory effect on retinal I/R-induced CHOP elevation on neurons, GPI 15427 pre-treatment showed no effects on I/R-induced over-expression of CHOP in the retinal vasculature.

4. Discussion

Inhibition of PARP has beneficial effects on multiple organs after I/R injury, which makes it a promising drug target [23]. The first generation of PARP inhibitors, nicotinamide and 3-ABA, were able to protect against retinal I/R injury-induced cell damages [18,24]. GPI 15427 is a second generation PARP inhibitor [23]. In the present study, GPI 15427 showed efficient inhibition of PARP activity (Fig. 1), and exhibited significant neuroprotective effects against retinal I/R injury in both pre- and post-treatments (Table 1 and Fig. 2). Compared to post-treatment of 3-ABA after I/R injury in which neuroprotective effects of which were seen only 12 and 18 h after injury, our results indicate that GPI 15427 has significant neuroprotective effects even when administered 24 h after the injury. Moreover, effects of post-injury administration of GPI 15427 on retinal I/R-induced neurodegeneration were similar to those of GPI 15427 pre-injury treatment.

Emerging evidences indicate the involvement of ER stress in development of retinal neurodegeneration under multiple insults including NMDA and ER stress inducers [10,11,25]. Injection of ER stress inducers such as tunicamycin into the vitreous induce rapid retinal neurodegeneration including ganglion cell loss and reduction of retinal thicknesses [10,11], suggesting that ER stress is an important causative factor of retinal neurodegeneration. Bip inducer (BIX) inhibits the over-expressions of CHOP induced by NMDA and tunicamycin, and thus attenuates retinal neurodegenerations [11]. Consistently, CHOP deficiency is protective for NMDA-induced retinal neurodegeneration [25].

Besides its roles in NMDA- and tunicamycin-induced retinal neurodegeneration, ER stress activation also contributes to I/R injury-induced neurodegeneration. We recently reported that high intraocular pressure-induced retinal I/R injury in mouse caused activation of eIF2α-CHOP and IRE1α-XBP1 pathways [6]. Likewise, expression levels of CHOP and Bip are elevated in a similar model of rat, accompanied with significant loss of retinal ganglion cells (RGC) [26]. Consistent with these studies, here we demonstrate that retinal I/R stimulated activation of PERK-eIF2α-CHOP pathway and Bip over-expression, and administration of the PARP inhibitor significantly inhibited the I/R-induced ER stress activation. Treatment with the PARP inhibitor mediated important effects on I/R-induced elevated CHOP levels on the retinal neurons, suggesting for the first time that the neuroprotective effects of PARP inhi-

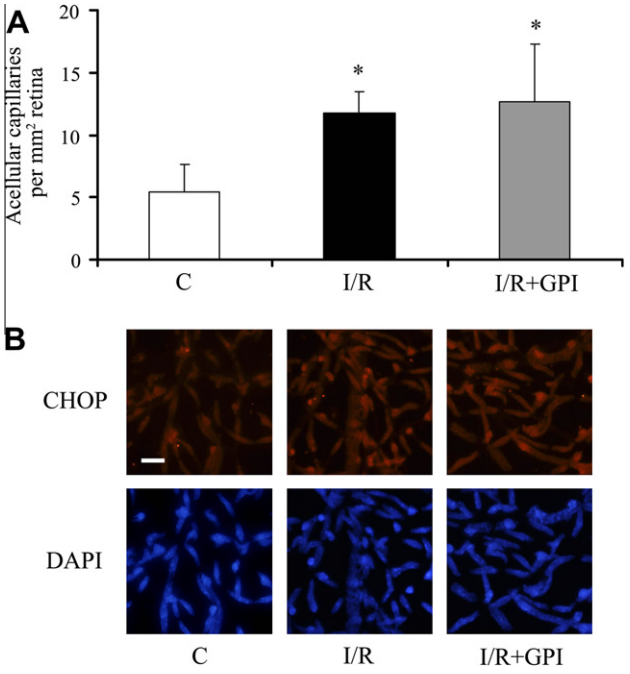

Fig. 4. GPI 15427 did not inhibit retinal I/R-induced capillary degeneration and CHOP over-expression in retinal vasculatures. (A) Quantitative results of acellular capillaries examined seven days after retinal I/R injury. (B) Representative images of CHOP expressions (red color) and DAPI stained nuclei (blue color) on retinal vasculatures of different groups one day after retinal I/R injury. Magnification, 400×, scale bar represents 20 μm. ($n = 4$ in each group; C, non-injured retinas; I/R, I/R-injured retinas; I/R + GPI, I/R-injured retinas with GPI 15427 pre-injury treatment; $^{*}P < 0.05$ compared with non-injured retinas.). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bition occur at least through the normalization of PERK-eIF2α-CHOP signaling pathway and Bip over-expression on retinal neurons.

Vascular degeneration is another pathological alternation resulted from retinal I/R injury [3,5]. Previously, we demonstrated that both intravitreal injection of tunicamycin and retinal I/R injury can induce ER stress in the retinal vasculature, which may contribute to retinal vascular degeneration [6]. In contrast to its beneficial effects in neurons after I/R, GPI 15427 had no effect on the I/R-induced vascular degeneration or on over-expression of CHOP. The underlying mechanism needs further investigation.

In summary, retinal I/R injury induces PARP activation, which leads to accumulation of poly(ADP-ribosy)lated proteins. The poly(ADP-ribosy)lated proteins initiate ER stress, thus leading to neurodegeneration in retina. The PARP inhibitor, GPI 15427, protects the retina from I/R-induced glial cell activation and neurodegeneration, and the neuroprotective effects are mediated through attenuation of ER stress, including inhibition of PERK-eIF2α-CHOP activation and Bip over-expression. These findings demonstrate a new relationship between PARP activation and ER stress signaling in vivo.

Acknowledgments

The authors acknowledge Eisai, Inc. (Woodcliff Lake, NJ, USA) for providing GPI 15427 used in the present study. This work was Supported by the National Basic Research Program of China (2009CB918304 and 2012CB524901), the Natural Science Foundation of China (No. 81100687), Program for New Century Excellent Talents in University (NECT-10-0623), and the Doctoral Independent Research Program of Wuhan University (No. 204276401).

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