Current Research in Pharmacology and Drug Discovery 3 (2022) 100124

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Erythropoietin administration exerted neuroprotective effects against cardiac ischemia/reperfusion injury

Titikorn Chunchai [a,b], Nattayaporn Apaijai [a,b,c], Juthipong Benjanuwattra [a,b], Hiranya Pintana [a,b], Kodchanan Singhanat [a,b,c], Busarin Arunsak [a,b], Nipon Chattipakorn [a,b,c], Siriporn C. Chattipakorn [a,b,d,*]

[a] Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand
[b] Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, 50200, Thailand
[c] Cardiac Electrophysiology Unit, Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand
[d] Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand

ARTICLE INFO

Keywords:
Apoptosis
Erythropoietin
Ischemic/reperfusion injury
Microglial function
Mitochondrial function
Necroptosis

ABSTRACT

Acute myocardial infarction (AMI) leads to cardiac dysfunction and also causes brain dysfunction and pathology. The neuroprotective effects of erythropoietin (EPO), the hormone controlling the production of red blood cells, have been shown in case of cerebral ischemic/reperfusion (I/R) injury. However, the effects of EPO on the brain pathologies induced by cardiac I/R injury have not been investigated. We hypothesized that the administration of EPO attenuates brain damage caused by cardiac I/R injury through decreasing peripheral and brain oxidative stress, preserving microglial morphology, attenuating hippocampal necroptosis, and decreasing hippocampal apoptosis, and hippocampal dysplasticity. Male Wistar rats (n = 38) were divided into two groups, sham (n = 6) and cardiac I/R (n = 32). All rats being subjected to the cardiac I/R operation were randomly divided into 4 subgroups (n = 8/group): vehicle, EPO pretreatment, EPO given during ischemia, and EPO given at the onset of reperfusion. The EPO was given at a dosage of 5000 units/kg via intravenous injection. Left ventricle function, oxidative stress, brain mitochondrial function, microglial morphology, hippocampal necroptosis, hippocampal apoptosis, and hippocampal plasticity were measured. EPO administration exerted beneficial anti-oxidative, anti-inflammatory, and anti-apoptotic effects on the brain against cardiac I/R. Giving EPO before cardiac ischemia conferred the greatest neuroprotection against cardiac I/R injury through the attenuation of LV dysfunction, decrease in peripheral and brain oxidative stress, and the attenuation of microglial activation, brain mitochondrial dysfunction, apoptosis, and necroptosis, leading to the improvement of hippocampal dysplasticity under cardiac I/R conditions. EPO pretreatment provided the greatest benefits on brain pathology induced by cardiac I/R.

1. Introduction

Acute myocardial infarction (AMI) is associated with ischemic injury as a consequence of oxygen and nutrient deprivation (Hausenloy and Yellon, 2013). However, reducing ischemic injury by reperfusion also caused reperfusion injury, a phenomenon known as cardiac ischemic–reperfusion (I/R) injury (Hausenloy and Yellon, 2013; Ekelof et al., 2016). In addition, our previous study demonstrated that cardiac I/R injury caused not only cardiac dysfunction, but also resulted in the development of brain dysfunction and pathologies. These included impairment of blood brain barrier (BBB) integration, increased brain oxidative stress, brain mitochondrial dysfunction, hippocampal apoptosis, dysfunction of glial cells, dendritic spine loss, decreased neurogenesis, and cognitive decline (Apaijai et al., 2019).

The BBB breakdown induced by cardiac I/R injury also allowed pro-inflammatory cytokines and reactive oxygen species (ROS) from the periphery into the brain. All the events following BBB breakdown led to increased brain oxidative stress and inflammation (Apaijai et al., 2019). It is widely known that the major producers of ROS following brain injury are the mitochondria (Saint-Georges-Chaumet and Edeas, 2016). A robust increase of ROS production or oxidative stress could cause microglial activation (Park et al., 2015). Both ROS and pro-inflammatory cytokines released from activated microglia are found to inhibit

* Corresponding author. Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand.
E-mail address: siriporn.c@cmu.ac.th (S.C. Chattipakorn).

https://doi.org/10.1016/j.crphar.2022.100124
Received 24 November 2021; Received in revised form 30 May 2022; Accepted 9 August 2022

Abbreviations

Akt	protein kinase B
AMI	acute myocardial infarction
AUC	area under the curve
BBB	blood brain barrier
DCF	dichlorofluorescein
DCFH-DA	2-μM 2′-7′dichlorofluorescin diacetate
DiI	1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate
EF	ejection fraction; eNOS, endothelial nitric oxide synthase
EPO	Erythropoietin
EPORs	Erythropoietin receptors
ERK	extracellular signal-regulated kinase
HIF-1	hypoxia inducible factor 1
HPLC	high performance liquid chromatography
I/R	ischemic–reperfusion
LAD	left anterior descending artery
LV	left ventricular
MDA	malondialdehyde
MEK	mitogen-activated protein kinase
NPCs	neural progenitor cells
PI3K	phosphoinositide 3-kinases
RBCs	red blood cells
ROS	reactive oxygen species
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TCA	trichloroacetic acid
TPR	tissue-protective receptor

long-term potentiation, resulting in cognitive impairment (Lee et al., 2010). Our previous study demonstrated that both inflammatory cytokines and oxidative stress also caused hippocampal dysplasticity including decreasing dendritic spine density and synaptic protein expression (Chunchai et al., 2018a,b). In addition, pro-inflammatory cytokines could trigger the intrinsic apoptotic pathway, leading to brain apoptosis (Grunnet et al., 2009).

Erythropoietin (EPO) is a hormone important in the production of red blood cells (RBCs). EPO is produced by the kidneys in response to low oxygen levels (Khaksari et al., 2017). During ischemic conditions an increase in serum EPO has been observed, leading to an increase in circulating reticulocytes and oxygen carrying capacity (Souvenir et al., 2015). There is growing evidence to demonstrate the neuroprotective effects of EPO following cerebral ischemic-reperfusion injury (Khaksari et al., 2017; Zhang et al., 2019). Although previous studies demonstrated that EPO administration either prior to cerebral ischemia (Bahcekapili et al., 2007; Liu et al., 2013) or at the onset of reperfusion (R. Wang et al., 2017) was associated with smaller brain infarct size, attenuated neurological deficit, and increased survival rate in a cerebral I/R injury model, there are still controversies existing regarding its efficacy (Calapai et al., 2000).

To date, the impact of EPO administration on brain pathology in response to cardiac I/R injury has never been investigated. Our hypothesis was that the administration of EPO attenuates brain damage resulting from cardiac I/R injury by causing a decrease in peripheral and brain oxidative stress, the preservation of microglia morphology, and the attenuation of brain necroptosis, apoptosis, and hippocampal dysplasticity. In addition, we investigated whether the administration of EPO before cardiac ischemia, during cardiac ischemia, or at the onset of reperfusion provides similar beneficial effects to the brain in the setting of cardiac I/R injury.

2. Materials and Methods

2.1. Animal model

The experimental protocol of the study was conducted following the ARRIVE guidelines and was approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, Chiang Mai University, Thailand (permit no. 04/2563).

Male Wistar rats (n = 38) were purchased from Nomura Siam International, Thailand. All rats were housed in temperature and environmental control with a 12:12 h light-dark cycle. All rats were fed with a standard diet (Mouse Feed Food No. 082, C.P. Company, Bangkok, Thailand) throughout the experiment protocol (Chunchai et al., 2018a, b). All rats received reverse osmosis drinking water *ad libitum*. After a week of acclimatization, all rats were randomly divided into two groups, sham (n = 6) and cardiac I/R (n = 32). All rats subjected to the cardiac I/R operation were randomly divided into 4 subgroups (n = 8/group). Specifically: cardiac I/R operated rats receiving 0.9% normal saline solution as the vehicle group; cardiac I/R operated rats receiving EPO pretreatment (15 min prior to ischemia), the PEPO group; cardiac I/R operated rats receiving EPO during ischemia (15 min after ischemia), the DEPO group; and cardiac I/R operated rats receiving EPO at the onset of reperfusion, the REPO group. The EPO (Recormon, Roche, Switzerland) was diluted in normal saline solution, and it was given at a dosage of 5000 unit/kg via intravenous injection. A summary of the experimental protocol is shown in.

2.2. Cardiac I/R injury protocol

The protocol of cardiac ischemia and the reperfusion were performed as previously described (Benjanuwattra et al., 2020). In cardiac I/R operated group, all rats were anesthetized by the combination of zoletil (50 mg/kg, Virbac, France) and xylazine (3 mg/kg, LBS labs, Thailand). Then, the left femoral vein was identified and inserted the catheter for drug delivery. After tracheostomy, the ventilator (SAR-830 Series, CWE Inc, USA) was used for room air ventilation. Next, skin was shaved, and thoracotomy was performed. After the left anterior descending artery (LAD) was identified, the LAD was ligated by 5–0 silk suture locating at 2 mm distal to its origin. After 30 min of LAD ligation, LAD was reperfused for 120 min. The ST-segment elevation was used to confirm the successful ligation. The sham operated group received the similar surgical procedure without LAD ligation.

2.3. Determination of left ventricular (LV) function

The pressure-volume catheter (Transonic Scisense, Ontario, Canada) was used to investigate the LV function. The catheter was inserted via right common carotid artery. Then, the percentage of LV ejection fraction (%EF) was measured at the end of reperfusion using a pressure-volume admittance system (Transonic Scisense, Ontario, Canada). Data were analyzed with an analytical software program (Labscribe, Dover, New Hampshire, USA) (Benjanuwattra et al., 2020).

2.4. Brain mitochondrial isolation

The brain mitochondrial isolation protocol was performed as previously described (Pintana et al., 2012). Shortly, the brain was rapidly removed to into ice-cold MSE solution containing bacterial proteinase (Sigma, USA) after sacrificed. The brain mitochondria were isolated using the differential centrifugation method as described previously

(Pintana et al., 2012). Next, the bicinchoninic acid assay (Sigma-Aldrich, Missouri, USA) was used to measure brain mitochondrial protein concentration (Pintana et al., 2012).

2.5. Brain mitochondrial function

To investigate brain mitochondrial function, brain ROS production, brain mitochondrial membrane potential change, and brain mitochondrial swelling were measured as previously described (Pintana et al., 2012). Briefly, the 2-μM 2′-7′dichlorofluorescin diacetate (DCFH-DA) fluorescent dye was added into isolated brain mitochondrial fraction. The dichlorofluorescein (DCF) fluorescent intensity was measured. The increase of DCF fluorescence represents the increase ROS level. Next, to measure brain mitochondrial membrane potential change, the JC-1 fluorescent dye was added into another set of isolated brain mitochondrial fraction. The decrease of red to green (JC-1 polymer/JC-1 monomer) fluorescence ratio represents mitochondrial membrane depolarization. To further measure brain mitochondrial swelling, the change of absorbance at 540 nm of brain mitochondria incubating in respiration buffer. The decrease of absorbance value represents brain mitochondrial swelling.

2.6. Determination of malondialdehyde (MDA) level

The product of lipid oxidation, the MDA levels, were measured to investigate the oxidative stress in both serum and brain using High Performance Liquid Chromatography (HPLC) as described previously (Pintana et al., 2012). In brief, serum or homogenate brain tissue were incubated in 10% trichloroacetic acid (TCA) and subsequently incubated with M H3PO4 and thiobarbituric acid (TBA) solution to generate the thiobarbituric acid reactive substances (TBARS). Then, the TBARS was detected usin HPLC system (Thermo Fisher Scientific, USA).

2.7. Western blot analysis

The non-ionizing lysis buffer containing a protease inhibitor was used for tissue homogenization. Then, the Bradford protein assay (Bio-Rad Laboratories, Hercules, California, USA) was performed to investigate proteins. Next, the 10% or 12.5% gels were used for electrophoresis, then the proteins were transferred to a nitrocellulose membrane. In this study, WB analysis were carried out on hippocampal lysates. The membranes were probed with the primary antibodies (1:1000 dilution). The membranes were immersed in appropriate blocking solution for 1 h, subsequently probed with the primary antibodies, including anti-p-Akt^{ser473}, anti-Akt, anti-Claudin-5, anti-Caspase 8, anti-Cleaved caspase 3, anti-eNOS, anti-PI3K, anti-Procaspase 3, anti-p-RIPK1, anti-RIPK1, anti-p-RIPK3, anti-RIPK3, anti-p-MLKL, anti-MLKL, and anti-Actin. After being washed, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. Lastly, membranes were incubated with ClarityTM Western ECL Blotting Substrate (Bio-Rad Laboratories Ltd.) and protein bands were captured by the ChemiDoc™ Touch Gel Imaging System. To analyze the intensity of protein band, the Image J software (NIH image) was used.

2.8. Determination of microglial morphology

After decapitation, the brain tissue was removed and subsequently fixed by 4% paraformaldehyde for 24 h. Then, all brain samples were immersed in 30% sucrose in phosphate buffer saline (PBS) at 4 °C for 72 h for cryoprotection. Next, each brain was cut using cryotome (Leica CM1950, Leica Biosystem Nussloch GmbH, Nussloch, Germany) into 20 μm of thickness. Next, to permeabilize the sections, the 3% hydrogen peroxide was used, followed by incubated in blocking solution for an hour. Then, all sections were subsequently probed with the primary anti-ionization calcium-binding adapter molecule 1 (Iba1) (1:1000 dilution, goat, Abcam; ab5076) for 24 h at 4 °C (Chunchai et al., 2018a,b). All sections were washes in PBS 3 times and subsequently incubated with Alexa Fluor® 488 (1:1000 dilution, Abcam; ab150129), and DAPI (1:1000 dilution, Tocris Bioscience, #5748) for 1 h at room temperature. After being washed in PBS 3 times, all sections were mounted using anti-fading mounting medium Fluoromount (Sigma-Aldrich Chemie, Steinheim, Germany).

To illustrate the microglial morphology, we captured a series of z-stack images of microglia cells by confocal microscopy (Olympus fluoview FV3000, Tokyo, Japan). We captured 3 microglial cells per brain slice and 3 brain slices per animal from the CA1 region of the hippocampus. The stack images were used to render the 3-dimentinal microglial morphology using the Imaris software 7.0 (Bitplane, Oxford instrument company, AG, Zurich, Switzerland). We also determine the microglial cell complexity by Sholl analysis (Chunchai et al., 2018a,b).

2.9. Determination of dendritic spine density

Fresh brain samples were sectioned by vibratome at 400 nm of thickness for 2–3 slices. After being fixed by 4% paraformaldehyde for 1 h, all slices were washed by PBS and subsequently incubated carbocyanine dye 1,1′-dioctadecyl-3,3,3′,3′-Tetramethylindocarbocyanine Perchlorate (DiI; Invitrogen) for 7 days. Next, slices were mounted on slides and covered in a coverslip. To illustrate the dendritic spine, the neuronal branches of CA1 region of the hippocampus were captured using the same protocol of microglial capturing. The numbers of dendritic spine were calculated using Imaris software (Chunchai et al., 2018a,b).

2.10. Statistical analysis

All data were showed as mean ± S.D. The analysis was performed in the same animals. One-way ANOVA followed by Bonferroni's post-hoc test was used to compare the difference among groups. $p < 0.05$ was considered as statistically significant.

3. Results

3.1. The administration of EPO attenuated LV dysfunction, peripheral and brain lipid oxidation, but did not improve blood-brain barrier (BBB) integrity, against cardiac I/R injury

The LV function as indicated by %EF was significantly lower in the vehicle-treated cardiac I/R-operated rats, when compared to sham-operated rats (Fig. 1B). The EPO administration either prior or during ischemia, but not at the onset of reperfusion, increased %EF to an equal extent when compared to vehicle-treated cardiac I/R-operated rats (Fig. 1B). These findings indicated that giving EPO before or during ischemia, but not at the onset of reperfusion, effectively attenuated LV dysfunction against cardiac I/R injury. Next, the MDA levels in serum and the brain were measured. We found that both serum and brain MDA levels significantly increased in vehicle-treated cardiac I/R-operated rats, when compared to sham-operated rats (Fig. 1C and D). EPO given prior to ischemia decreased both serum and brain MDA levels, when compared to vehicle-treated cardiac I/R-operated rats, suggesting that EPO pretreatment ameliorated peripheral and brain oxidative stress in cardiac I/R injury (Fig. 1C and D). However, the administration of EPO either during ischemia or at the onset of reperfusion did not improve MDA levels (Fig. 1C and D). We also investigated the level of BBB integrity by the measurement of claudin-5 expression. The results showed that the expression of claudin-5 expression decreased equally in all cardiac I/R-operated groups, when compared to vehicle-treated cardiac I/R-operated rats (Fig. 1E). Overall, the administration of EPO either prior to or during ischemia demonstrated similar beneficial effects on LV function, whereas oxidative stress was only suppressed by EPO pretreatment.

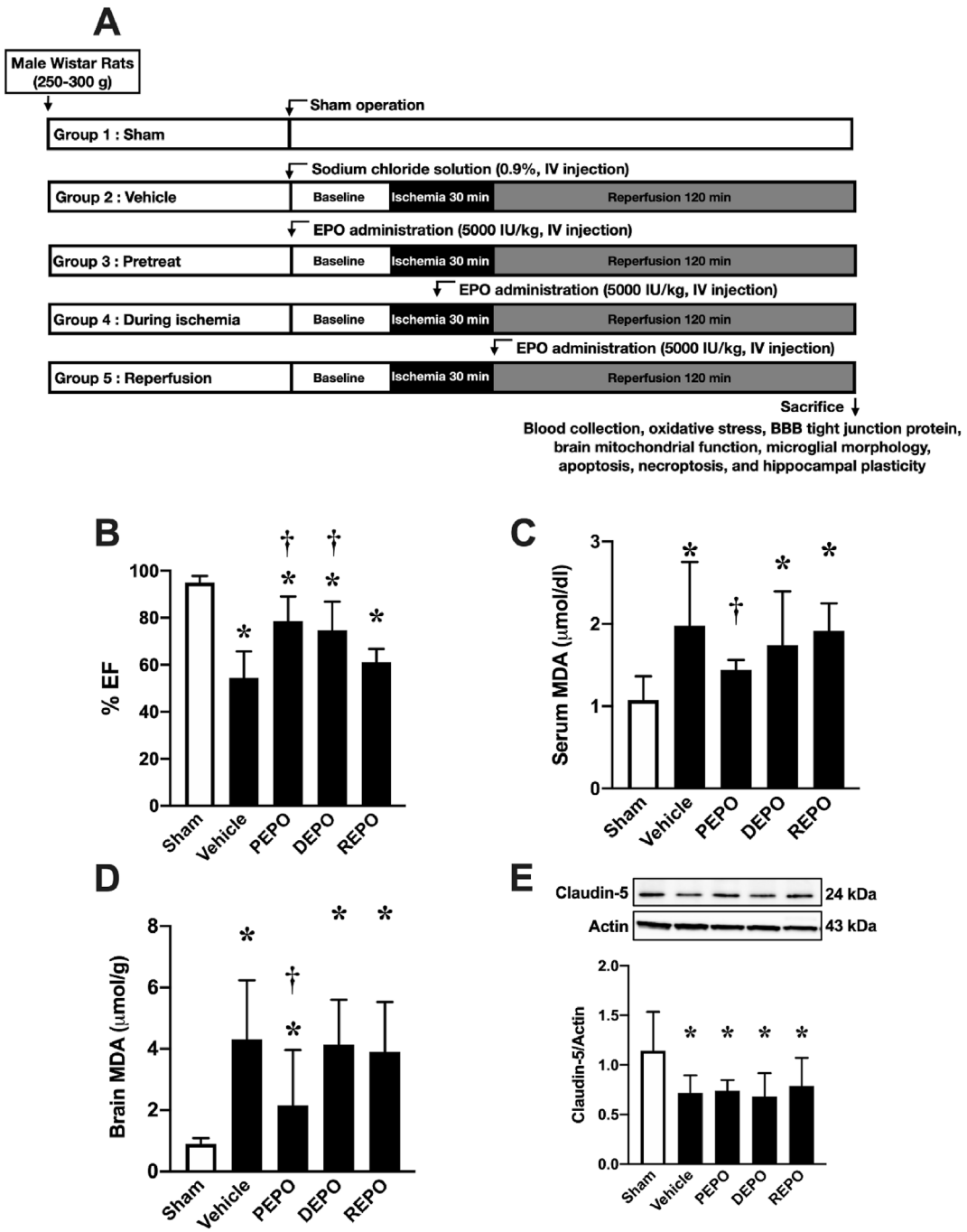

Fig. 1. The effects of EPO on LV function, peripheral and brain MDA levels, and BBB function against cardiac I/R injury. (A) Experimental protocol of the study. (B) % EF of left ventricle measured by PV-loop catheter at the end of reperfusion. (C) Serum MDA level measured by HPLC method. (D) Brain MDA level measured by HPLC method. (E) Hippocampal BBB function as indicated by the expression of tight junction proteins including claudin-5. (n = 5–8/group) *$p < 0.05$ vs. sham; †$p < 0.05$ vs. vehicle.

3.2. The administration of EPO prior to ischemia demonstrated the greatest effect on improvement of microglial morphology against cardiac I/R injury

The 3D structures were rendered from the series of z-stack fluorescent images using Imaris software (Fig. 2A). The results demonstrated that the mean fluorescent intensity of Iba1-positive cells, which was significantly increased in vehicle-treated cardiac I/R-operated rats, was decreased to an equal extent in all groups of EPO-treated cardiac-I/R operated rats (Fig. 2B). The microglial number also increased in vehicle-treated cardiac I/R-operated rats, when compared to sham-operated rats (Fig. 2C). Interestingly, the EPO administration prior to ischemia significantly suppressed microglial numbers to a lower frequency than those of sham-operated rats (Fig. 2C). Giving EPO either during ischemia or at the onset of reperfusion normalized microglial numbers to the same level as those of sham-operated rats (Fig. 2C). Next, we measured microglial cell volume. We found that microglial cell volume increased in vehicle-treated cardiac I/R-operated rats, however it decreased following administration of EPO at all time points (Fig. 2D). Microglial process length was investigated next. The vehicle-treated cardiac I/R-operated rats had significantly decreased microglial process length, which was normalized following administration of EPO given prior to ischemia (Fig. 2E). The EPO administration either during ischemia or at the onset of reperfusion

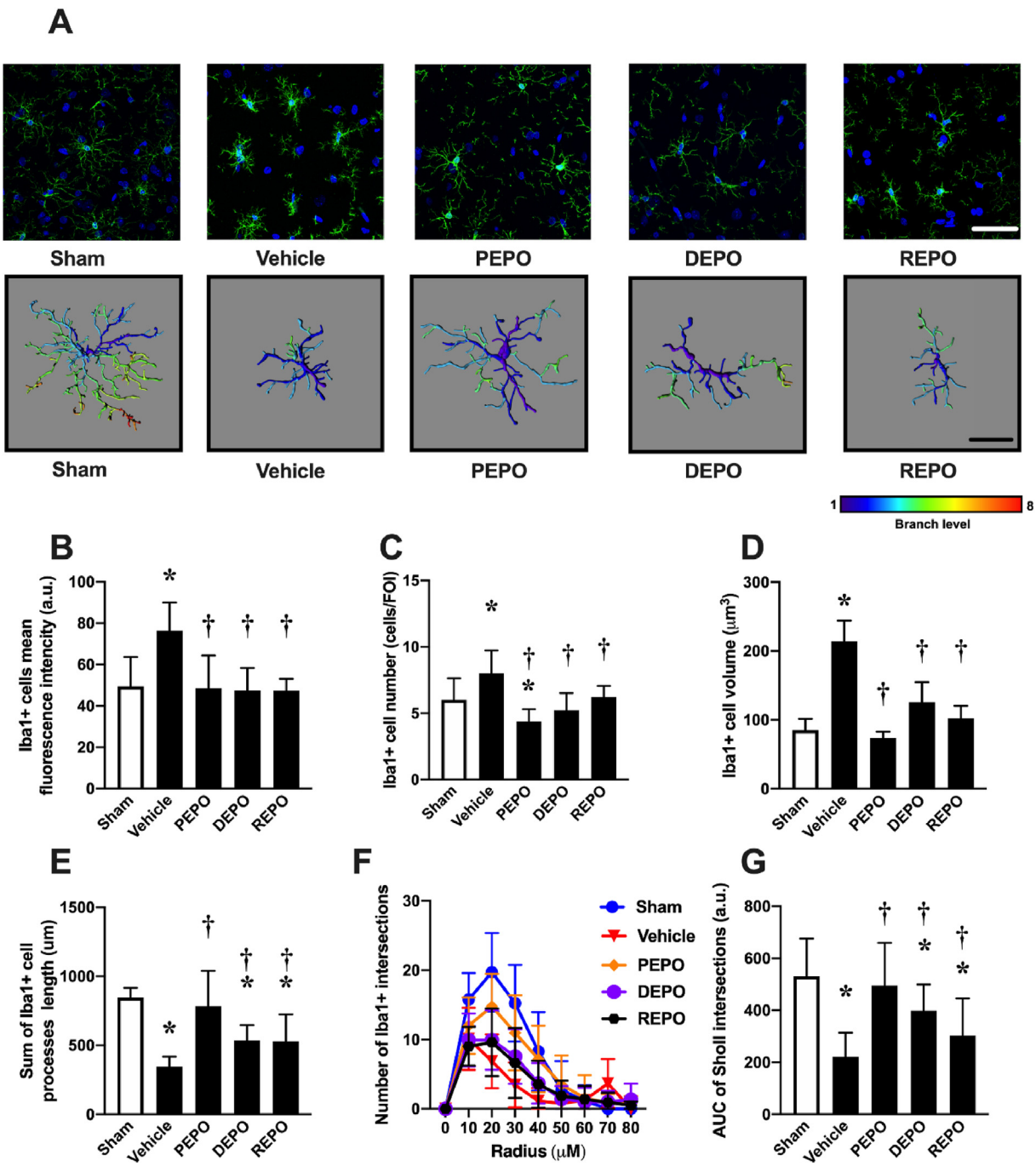

Fig. 2. The effects of EPO on microglial morphology against cardiac I/R injury. (A) *Upper row*: Representative images of microglial morphology under confocal microscopy at CA1 of the hippocampus by immunofluorescent staining of DAPI (blue) and Iba1 (green) (bar = 50 μm); *Lower row*: Representative images of the 3D structure rendering of microglial morphology by Imaris software (bar = 10 μm). The 3D structures were statistically colored by branch level. (B) Mean immunofluorescence intensity of Iba1 positive cells. (C) Number of Iba1 positive cells. (D) Mean cell volume of Iba1 positive cells. (E) Sum of Iba1 positive cell process length. (F–G) Microglial process complexity as indicated by Sholl analysis and area under the curve of Sholl intersections. (n = 5–8/group) *$p < 0.05$ vs. sham; †$p < 0.05$ vs. vehicle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

improved microglial process retraction to an equal extent (Fig. 2E). In addition, we determined the complexity of microglial morphology using the Sholl analysis (Fig. 2F and G). The results showed decreased area under the curve (AUC) of Sholl intersections in vehicle-treated cardiac I/R-operated rats, which was normalized by EPO given prior to ischemia (Fig. 2F and G). The EPO administration either during ischemia or at the onset of reperfusion resulted in equally improved microglial complexity (Fig. 2F and G). Taken together, all these findings demonstrated that EPO given at all time points attenuated microglial dysmorphology against cardiac I/R injury, however, the greatest beneficial effects were observed in rats pretreated with EPO.

3.3. *The administration of EPO ameliorated the brain mitochondrial dysfunction induced by cardiac I/R injury*

The results demonstrated that brain ROS production was significantly increased in vehicle-treated IR-operated rats, when compared to sham-operated rats (Fig. 3A). Giving EPO either prior or during ischemia

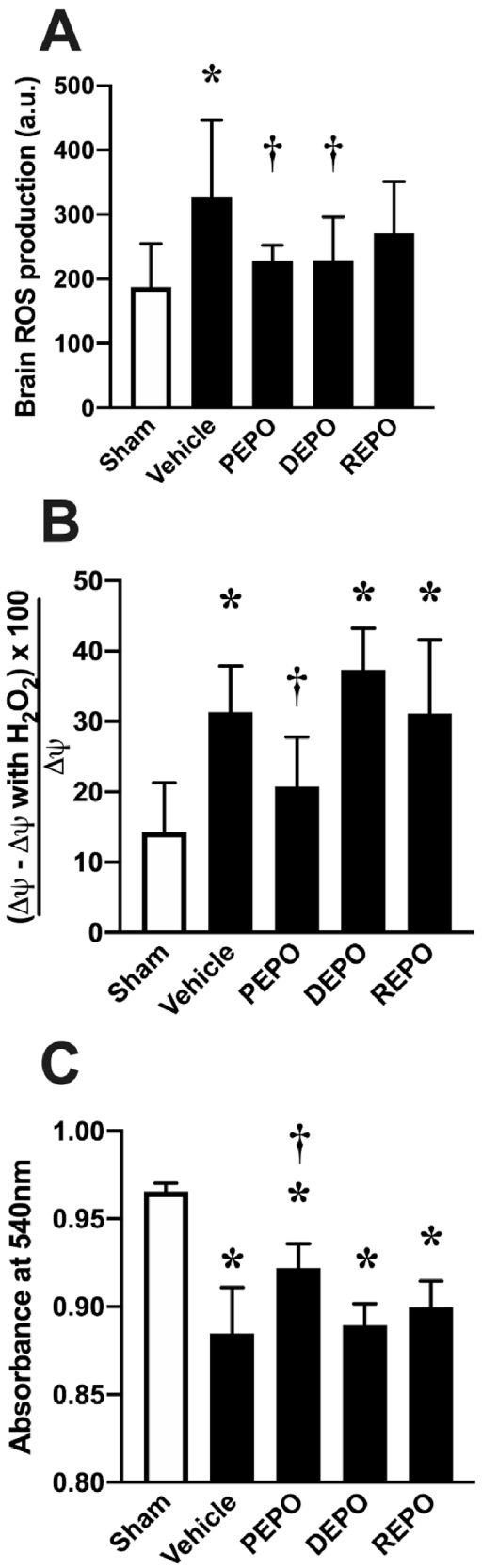

Fig. 3. The effects of EPO on brain mitochondrial function against cardiac I/R injury. (A) Brain ROS production. (B) Percentage change of brain mitochondrial membrane potential depolarization after H_2O_2 stimulation. (C) Mitochondrial swelling as indicated by absorbance at a wavelength of 540 nm. (n = 5–8/group) *$p < 0.05$ vs. sham; †$p < 0.05$ vs. vehicle.

equally suppressed brain mitochondrial ROS production, when compared to vehicle-treated cardiac I/R-operated rats however this was not the case when it was given at the onset of reperfusion (Fig. 3A). The percentage changes of brain mitochondrial membrane potential after H_2O_2 stimulation were increased in vehicle-treated cardiac I/R-operated rats and were only decreased in the group given EPO prior to ischemia (Fig. 3B). Giving EPO either during ischemia or at the onset of reperfusion did not attenuate percentage changes of brain mitochondrial membrane potential after H_2O_2 stimulation (Fig. 3B). Similarly, brain mitochondrial swelling was increased in vehicle-treated cardiac I/R-operated rats, while only EPO given prior to ischemia significantly attenuated the swelling (Fig. 3C). All findings demonstrated that EPO given prior to ischemia provided the greatest beneficial effects on the attenuation of brain mitochondrial dysfunction induced by cardiac I/R injury.

3.4. The administration of EPO attenuated the hippocampal apoptosis and necroptosis induced by cardiac I/R injury

The results demonstrated that caspase 8 expression, which was significantly increased in vehicle-treated cardiac I/R-operated rats, was decreased to an equal extent in all EPO-treated cardiac I/R-operated rats (Fig. 4A and B). Similarly, the expression of cleaved caspase 3, which was increased in vehicle-treated cardiac I/R-operated rats, was also found to have decreased equally in all EPO-treated cardiac I/R-operated rats (Fig. 4A and C). There was no difference in procaspase 3 expression among all groups (Fig. 4A and D). These findings demonstrated that EPO given at any of these time points attenuated hippocampal apoptosis induced by cardiac I/R injury. Next, to investigate hippocampal necroptosis, the necroptotic proteins including p-RIPK1, RIPK1, p-RIPK3, RIPK3, p-MLKL, and MLKL were measured (Fig. 4A and 4E-J). We found that the expression of p-RIPK1 and p-RIPK3 were increased in vehicle-treated cardiac I/R-operated rats and were decreased equally in all EPO-treated cardiac I/R-operated rats (Fig. 4E and G). There was no difference in RIPK1 or RIPK3 expression among all groups (Fig. 4F and H). The expression of both p-MLKL and MLKL did not different across all groups. These findings suggested that EPO given at any of these time points ameliorated the hippocampal necroptosis induced by cardiac I/R injury.

3.5. The administration of EPO alleviated the hippocampal dysplasticity induced by cardiac I/R injury

To investigate hippocampal plasticity, the dendritic spines were stained with DiI and measured using Imaris software. A significant spine loss was observed in vehicle-treated cardiac I/R-operated rats, when compared to sham-operated rats (Fig. 5A and B). The EPO administration prior to ischemia restored dendritic spine density back to the same level of sham-operated rats (Fig. 5A and B), while the administration of EPO either during ischemia or at the onset of reperfusion equally attenuated dendritic spine loss, in comparison to vehicle-treated cardiac I/R-operated rats (Fig. 5A and B). These findings indicated that EPO given prior to ischemia provided the greatest beneficial effect on the reduction of hippocampal dysplasticity.

3.6. The administration of EPO exerted neuroprotective effects following cardiac I/R injury by enhancing the phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt)/endothelial nitric oxide synthase (eNOS) pathway in the hippocampus

To validate the signal transduction of EPO receptor (EPOR) in the hippocampus, PI3K/Akt/eNOS, a direct downstream signaling of EPOR has been measured. The results demonstrated that vehicle-treated cardiac I/R-operated rats had significantly decreased PI3K, p-Akt^{ser473}/Akt ratio, and eNOS expression in the hippocampus when compared to sham-operated rats (Fig. 6A–D). EPO given at all time points equally increased

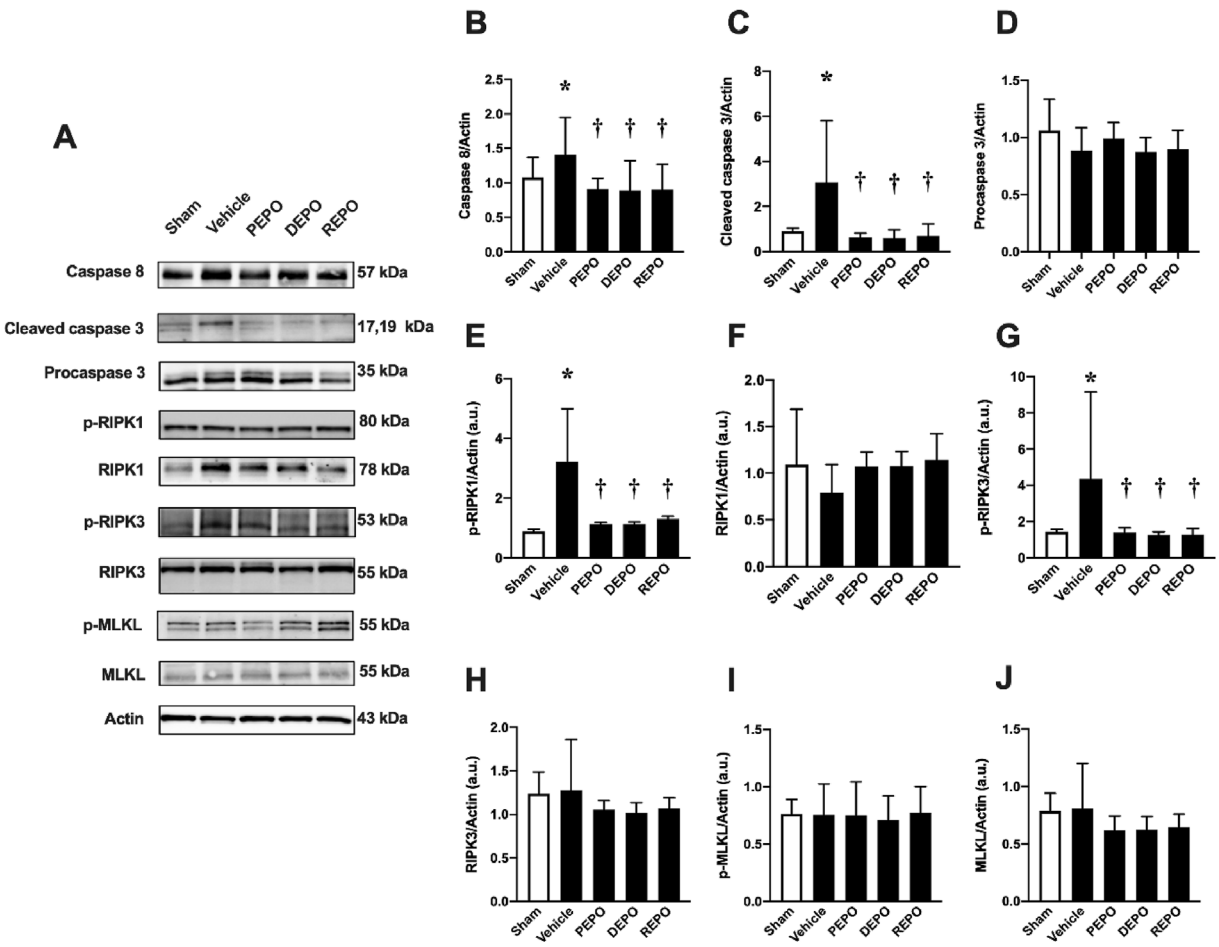

Fig. 4. The effects of EPO on hippocampal apoptosis and hippocampal necroptosis against cardiac I/R injury. (A) Representative Western blot bands of apoptosis and necroptosis proteins. (B–D) Expression of hippocampal apoptotic proteins including caspase 8, cleaved caspase 3, and procaspase 3, respectively. (E–J) Expression of hippocampal necroptotic proteins including p-RIPK1, RIPK1, p-RIPK3, RIPK3, p-MLKL, and MLKL, respectively. (n = 5–8/group) *$p < 0.05$ vs. sham; †$p < 0.05$ vs. vehicle.

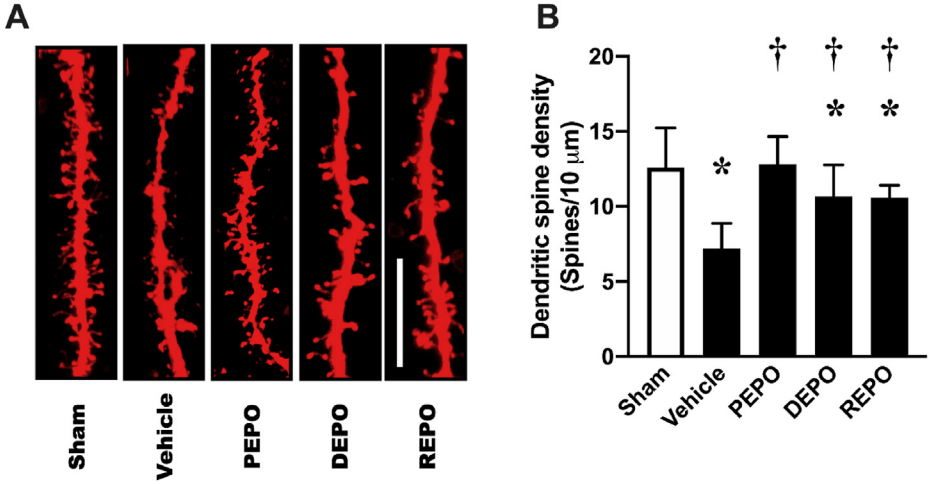

Fig. 5. The effects of EPO on hippocampal plasticity as indicated by dendritic spine density against cardiac I/R injury. (A) Representative images of dendritic spine density under confocal microscopy at CA1 of the hippocampus by DiI staining (red) (bar = 10 μm). (B) Dendritic spine density. (n = 5–8/group) *$p < 0.05$ vs. sham; †$p < 0.05$ vs. vehicle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

all parameters, when compared to vehicle-treated cardiac I/R-operated rats (Fig. 6A–D). Taken together, the administration of EPO exerted neuroprotective effects following cardiac I/R injury by enhancing the PI3K/Akt/eNOS pathway in the hippocampus.

4. Discussion

The major findings of our study are as follows: (1) Cardiac I/R caused not only LV dysfunction and systemic oxidative stress, but also impaired

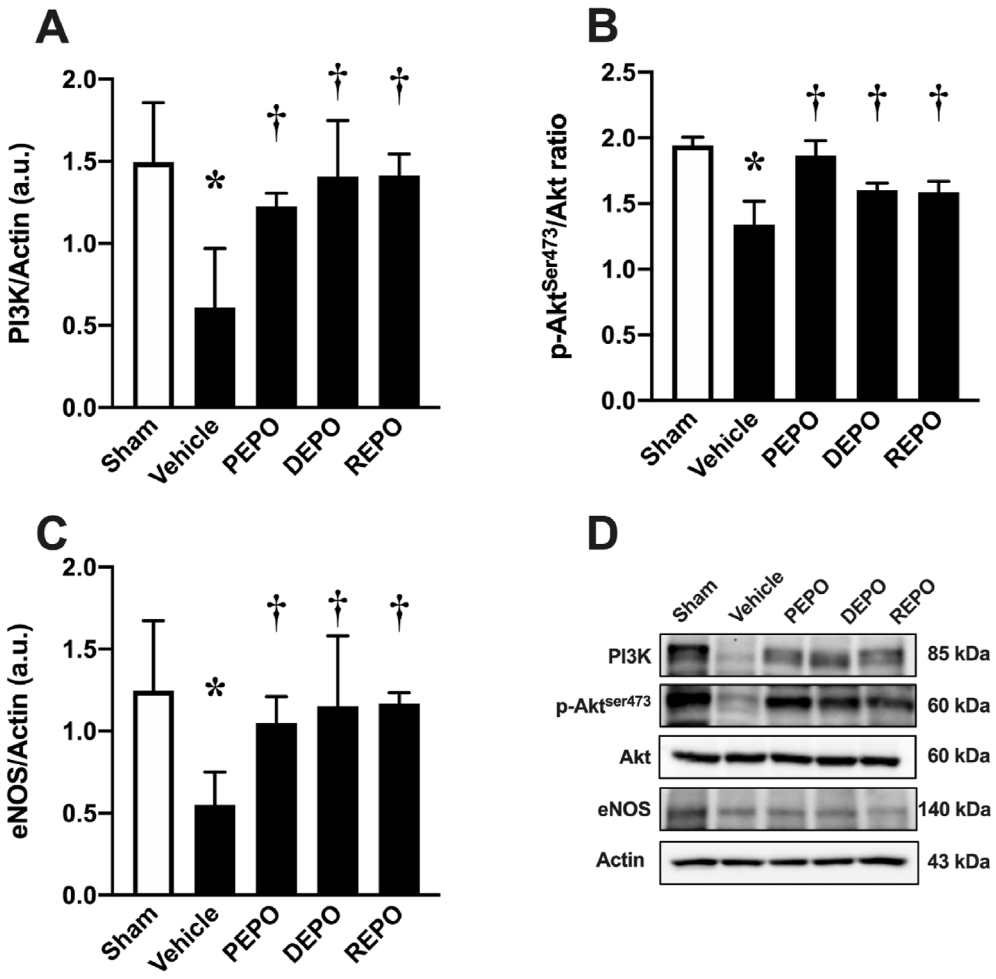

Fig. 6. The changes of molecular signaling proteins underlying the administration of EPO. (A) Expression of PI3K protein. (B) Expression of p-Akt^{ser473}/Akt ratio. (C) Expression of eNOS protein. (D) Representative Western blot bands of PI3K, p-Akt^{ser473}, Akt, and eNOS, respectively. (n = 5–8/group) *p < 0.05 vs. sham; †p < 0.05 vs. vehicle.

BBB integrity, brain oxidative stress, microglial dysmorphology, brain mitochondrial dysfunction, and increased hippocampal apoptosis, and necroptosis, resulting in hippocampal dysplasticity. (2) EPO given prior to ischemia alleviated LV dysfunction, systemic and brain oxidative stress, microglial dysmorphology, brain mitochondrial dysfunction, hippocampal apoptosis, and hippocampal necroptosis, leading to the greatest improvement in hippocampal dysplasticity associated with cardiac I/R injury. (3) Giving EPO during ischemia attenuated LV dysfunction, microglial dysmorphology, brain ROS production, hippocampal apoptosis and necroptosis, and improved hippocampal dysplasticity. (4) Giving EPO at the onset of reperfusion showed beneficial effects regarding the preservation of microglial morphology, and hippocampal apoptosis and necroptosis, leading to improved hippocampal dysplasticity resulting from cardiac I/R injury.

It has been shown that AMI caused not only cardiac dysfunction, but also brain dysfunction and pathologies. Previous studies demonstrated that cardiac I/R injury induced oxidative stress and systemic circulatory inflammation, which consequently disrupted the assembly of the BBB tight junction proteins including claudin-5, resulting in increased permeability and hence passage of systemic ROS and pro-inflammatory cytokines into the brain (Kumfu et al., 2018; Apaijai et al., 2019). These could contribute to brain oxidative stress, neuroinflammation, and microglial activation. Several studies have also found alterations in microglial function under cardiac I/R conditions (Frick et al., 2016; Taguchi et al., 2016). Following cardiac I/R, there was an increase in microglial activity and number, as indicated by increased numbers of Iba1-positive cells (Frick et al., 2016). The level of microglia activation reached its peak 3 days after cardiac I/R, suggesting a time-dependent response of microglial activation following cardiac I/R (Yuan et al., 2014). In addition to those previous studies, the present study also found changes in microglial complexity after cardiac I/R, transformations occurring into ameboid-like shapes. All these events worsened brain inflammation and subsequently impaired brain mitochondrial function, leading to neuronal apoptosis (Kumfu et al., 2018; Apaijai et al., 2019). A previous study reported that neuronal apoptosis was observed following cardiac I/R, which is possibly associated with neuronal degeneration in the hippocampus (Frick et al., 2016). Consistent with these findings, we found that cardiac I/R triggered hippocampal neuronal cell death via increased neuronal apoptosis, and also increased necroptosis through the RIPK1-RIPK3 signaling pathway. The activation of RIPK1 also stimulated the release of pro-inflammatory cytokines by the microglia, and subsequently promoted neuronal death (Chan et al., 2019). It has been widely accepted that oxidative stress, inflammation, apoptosis, and necroptosis in the brain affected hippocampal plasticity (Benjanuwattra et al., 2020; Ongnok et al., 2021). Taken together, cardiac I/R caused peripheral and brain oxidative stress, both of which activated microglia and impaired brain mitochondrial function, resulting in hippocampal apoptosis, necroptosis, and hippocampal dysplasticity.

In the physiological condition, peritubular capillary endothelial cells in the renal cortex are responsible for the production of EPO in response to hypoxia (Sirén et al., 2001). During hypoxic conditions, serum EPO level is raised via the hypoxia inducible factor 1 (HIF-1) pathway, which

is the enzymatic activity response during conditions of insufficient oxygen (Sirén et al., 2001), suggesting a vital role of EPO under cardiac I/R conditions. The role of EPO in RBC production has been known for some time however it is now becoming clear that it has many more crucial functions in the body. In addition, it has been shown that Erythropoietin receptors (EPORs) are expressed in several areas including cardiovascular (Wu et al., 1999) and nervous organs (Tsai et al., 2006). Endogenous EPO signaling is necessary for the development of the central nervous system and neuroprotection (Wang et al., 2006). Previous studies demonstrated that EPO can be produced by astrocytes, suggesting an important role of EPO in the brain (Masuda et al., 1994; Marti et al., 1996).

Due to its role with oxygen sensors, the effects of EPO under cerebral I/R conditions have been investigated. Previous studies demonstrated that pretreatment with EPO in cerebral I/R injury attenuated BBB breakdown, decreased pro-inflammatory cytokines (Liu et al., 2013), ameliorated lipid peroxidation (Bahcekapili et al., 2007), increased neuronal proliferation and maturation (Zhang et al., 2019), and decreased neuronal apoptosis (Khaksari et al., 2017), resulting in the reduction of brain infarct size, and the improvement of cognitive function. Taken together, all findings suggested the neuroprotective effects on the brain in cerebral I/R condition. Previous studies reported the anti-oxidative effect of EPO (Li et al., 2021; Menger et al., 2021). For instance, EPO nanoparticles greatly reduced ionizing radiation-induced ROS production in the bone marrow cells (Li et al., 2021). Co-incubation of isolated pancreatic islets with hydrogen peroxide and EPO showed no change in pancreatic cell viability, suggesting that EPO scavenged the oxidative products induced by hydrogen peroxide (Menger et al., 2021). Therefore, we speculated that EPO directly attenuated oxidative stress by scavenging the brain mitochondrial ROS. Moreover, EPO has been recognized as a multifunctional cytokine with anti-inflammatory properties, providing significant effects in both acute and chronic inflammatory models (Silva et al., 2021). Previous studies showed that the binding of EPO to the tissue-protective receptor (TPR) on macrophages inhibited the activation of NF-κB p65 via the EPOR/-Jak2/PI3K pathway (Nairz et al., 2011, 2017; Watanabe et al., 2016; Peng et al., 2020). In addition, our findings demonstrated that EPO indirectly alleviated inflammation in the hippocampus by preserving M2 microglial morphology and promoting M2 polarization. It has also been found that the number of newly generated astrocytes and microglia were suppressed in EPO-pretreated rats with cerebral I/R injury (Zhang et al., 2019), leading to the decrease of pro-inflammatory cytokines (Liu et al., 2013) and lipid peroxidation (Bahcekapili et al., 2007). It has been shown, consistently, that EPO activated JAK/STAT and Akt signaling, inhibited GSK-3β activity, modulated NF-κB p65, and decreased levels of pro-inflammatory cytokines and iNOS (Bond and Rex, 2014). In addition, EPO administration also promoted phagocytosis and M2 activation state polarization in macrophages, whereas EPO redirected microglia back to or maintained microglia in a normal state and prevented microglial proliferation (Bond and Rex, 2014).

In addition to the anti-oxidative and anti-inflammatory effects of EPO, several studies also demonstrated the anti-apoptotic effect of EPO (Yu et al., 2001; Fisher, 2003; Khaksari et al., 2017). EPO was found to inhibit the apoptosis of bone marrow erythroid progenitor cells (Fisher, 2003). Neuronal apoptosis and decreased numbers of neural progenitor cells were observed in EPO-null and EPOR-null mice, suggesting EPO has a role in neuronal survival and apoptosis (Yu et al., 2001). Moreover, an increase in hippocampal neuronal density, together with decreasing apoptotic index, were noted in EPO-treated rats with cerebral I/R injury (Khaksari et al., 2017). In addition to those previous studies, we showed that EPO inhibited not only neuronal apoptosis, but also neuronal necroptosis including the RIPK1-RIPK3 signaling pathway, resulting in the improvement of hippocampal dysplasticity.

It has been shown that if EPO is given at doses appropriate for erythropoiesis (200–400 U/kg) it does not cross the BBB in a detectable amount (Juul et al., 1999). Although the dose of EPO used in the present study is higher than the range used for anemia treatment, it did not improve BBB integrity. This can probably be explained by the realization that the impairment of BBB integrity was indirectly affected by the cardiac I/R injury. Despite %EF being improved in the EPO pretreated and during ischemia groups, it was not restored back to within normal levels in the present study. Adding weight to this finding a previous study showed that peripheral administration of EPO (5000 IU/kg BW) did not improve survival rate in cerebral I/R injury (Calapai et al., 2000). All of these pieces of evidence would imply that other factors from the periphery such as cardiac dysfunction might influence the effect of EPO on BBB integrity. In addition, the dose of EPO administration might not be high enough to improve BBB integrity since previous studies reported that EPO exerted neuroprotective effects against cerebral I/R injury in a dose-dependent manner (Calapai et al., 2000; Weber et al., 2005; Kellert et al., 2007). A previous study demonstrated that administration of EPO at a dose of 5000 U/kg BW exerted a greater benefit on the brain in brain-injured neonatal rats than at the dose of 2500 U/kg BW, suggesting EPO provides beneficial effects in dose-dependent manner (Kellert et al., 2007). Notably, the toxicity of very high dose of EPO was also reported. Giving EPO at the dose of 20,000 IU/kg BW increased neuronal death rate in rat pups subjected to moderate hypoxia (Kellert et al., 2007). Although a high dose of EPO could not be used due to its toxicity, the administration of EPO at a dose of 5000 IU/kg BW could be used together with the guideline-therapy for the greatest protective effects on the brain against cardiac I/R injury.

In this study, we demonstrated that administration of EPO prior to cardiac I/R has the greatest brain benefit and could theoretically be effectively used as a potential prevention regimen. The greatest efficacy of EPO pre-treatment in cardiac I/R could possibly be due to the fact that EPO passed through the BBB and accumulated in the brain in a time-dependent manner (Yoo et al., 2017). A previous study in rats showed that brain EPO levels were increased significantly within 3 h of EPO injection via the tail vein and reached their maximum at 6 h after injection (Yoo et al., 2017). Although administration of EPO before cardiac I/R showed the greatest brain benefit, it cannot be translated into clinical use because patients with cardiac ischemia are treated after the ischemia has already occurred. However, there is the potential for the use of EPO as a preventive therapy, as we have also shown that giving EPO during or at the onset of reperfusion has neuroprotective effects, particularly in terms of preserving microglial morphology, reducing neuronal cell death in the hippocampus. Collectively, administration of EPO during ischemia or at the onset of reperfusion could be used as a combination therapy with gold-standard drugs in clinical use and could provide the greatest benefit to the brain of patients with cardiac I/R injury.

Several cell types in the brain express EPORs, including neural progenitor cells (NPCs), astrocytes, neurons, and oligodendrocytes. EPO was shown to promote proliferation and differentiation of NPCs into astrocytes or oligodendrocytes (Iwai et al., 2010). The general mechanisms responsible for the neuroprotective effects of EPO are suppressing apoptosis, inflammation, and neurotoxicity, as well as promoting of neuronal regeneration and repair (Cantarelli et al., 2019). A previous study showed that EPO activated downstream signaling molecules such as JAK2, IRS-1, PI3K, Akt, and eNOS in skeletal muscle of db/db diabetic mice (Pan et al., 2017). Furthermore, the administration of EPO resulted in cardioprotection by stimulating PI3K/Akt and mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) in coronary artery endothelial cells to produce NO in cardiac I/R in mice (Teng et al., 2011). In addition to those previous studies, we showed that the PI3K/Akt/eNOS pathway in the brain was also activated by EPO. The activation of these pathways might explain the attenuation of brain damage following cardiac I/R.

Moreover, it has been shown that EPO and its derivatives inhibited inflammation by activating EPOR/Jak2/PI3K pathway on macrophages (Nairz et al., 2011, 2017; Watanabe et al., 2016; Peng et al., 2020) and facilitated macrophages/microglia M2 polarization (S. Wang et al., 2017; Peng et al., 2020), leading to suppression of inflammation and promotion

of tissue repair. However, in the present study, microglial cells could not be isolated because of the limited tissue. Further studies are encouraged to examine the effects of EPO on these signaling molecules on microglial cells.

5. Conclusions

The EPO administration has a neuroprotective effect against cardiac I/R injury through antioxidant, anti-inflammatory and anti-apoptotic/necroptotic effects. EPO administered before cardiac ischemia conferred the greatest neuroprotection by attenuating LV dysfunction, oxidative stress in the periphery and the brain, microglial activation, brain mitochondrial dysfunction, and hippocampal apoptosis and necroptosis, resulting in an improvement in hippocampal dysplasticity under conditions of cardiac I/R.

Funding

This work was supported by The Endowment fund from Faculty of Medicine, Chiang Mai University (Grant number 153/2563 to TC); the Research Grant for Talented Young Researchers from the National Research Council of Thailand (N41A640111 to TC, N42A650207 to HP); Thailand Science Research and Innovation-Chiang Mai University (FF 2565 to SCC and TC); the Senior Research Scholar Grant from the National Research Council of Thailand (SCC); the Chiang Mai University Center of Excellence Award, Thailand (NC); and the NSTDA Research Chair grant from the National Science and Technology Development Agency Thailand (NC).

CRediT authorship contribution statement

Titikorn Chunchai: Methodology, Software, Formal analysis, Investigation, Writing – original draft, Visualization, Funding acquisition. **Nattayaporn Apaijai:** Methodology, Formal analysis, Investigation. **Juthipong Benjanuwattra:** Methodology, Formal analysis, Investigation. **Hiranya Pintana:** Writing – original draft, Visualization. **Kodchanan Singhanat:** Methodology, Formal analysis, Investigation. **Busarin Arunsak:** Methodology, Formal analysis, Investigation. **Nipon Chattipakorn:** Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Siriporn C. Chattipakorn:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Aysha Mohamed Rafik Patel, Sasiwan Kerdphoo, and Thidarat Jaiwongkam for their technical assistance in the laboratory. Our thanks also go to Thura Tun Oo for his editorial assistance.

References

Apaijai, N., Moisescu, D.M., Palee, S., McSweeney, C.M., Saiyasit, N., Maneechote, C., Boonnag, C., Chattipakorn, N., Chattipakorn, S.C., 2019. Pretreatment with pcsk9 inhibitor protects the brain against cardiac ischemia/reperfusion injury through a reduction of neuronal inflammation and amyloid beta aggregation. J. Am. Heart Assoc. 8 (2) e010838-e010838.

Bahcekapili, N., Uzüm, G., Gökkusu, C., Kuru, A., Ziylan, Y.Z., 2007. The relationship between erythropoietin pretreatment with blood-brain barrier and lipid peroxidation after ischemia/reperfusion in rats. Life Sci. 80 (14), 1245–1251.

Benjanuwattra, J., Apaijai, N., Chunchai, T., Kerdphoo, S., Jaiwongkam, T., Arunsak, B., Wongsuchai, S., Chattipakorn, N., Chattipakorn, S.C., 2020. Metformin preferentially provides neuroprotection following cardiac ischemia/reperfusion in non-diabetic rats. Biochim. Biophys. Acta, Mol. Basis Dis. 1866 (10), 165893.

Bond, W.S., Rex, T.S., 2014. Evidence that erythropoietin modulates neuroinflammation through differential action on neurons, astrocytes, and microglia. Front. Immunol. 5, 523.

Calapai, G., Marciano, M.C., Corica, F., Allegra, A., Parisi, A., Frisina, N., Caputi, A.P., Buemi, M., 2000. Erythropoietin protects against brain ischemic injury by inhibition of nitric oxide formation. Eur. J. Pharmacol. 401 (3), 349–356.

Cantarelli, C., Angeletti, A., Cravedi, P., 2019. Erythropoietin, a multifaceted protein with innate and adaptive immune modulatory activity. Am. J. Transplant. 19 (9), 2407–2414.

Chan, H.H., Koh, R.Y., Lim, C.L., Leong, C.O., 2019. Receptor-interacting protein kinase 1 (ripk1) as a potential therapeutic target: an overview of its possible role in the pathogenesis of alzheimer's disease. Curr. Alzheimer Res. 16 (10), 907–918.

Chunchai, T., Apaijai, N., Keawtep, P., Mantor, D., Arinno, A., Pratchayasakul, W., Chattipakorn, N., Chattipakorn, S.C., 2018a. Testosterone deprivation intensifies cognitive decline in obese male rats via glial hyperactivity, increased oxidative stress, and apoptosis in both hippocampus and cortex. Acta Physiol., e13229

Chunchai, T., Thunapong, W., Yasom, S., Wanchai, K., Eaimworawuthikul, S., Metzler, G., Lungkaphin, A., Pongchaidecha, A., Sirilun, S., Chaiyasut, C., Pratchayasakul, W., Thiennimitr, P., Chattipakorn, N., Chattipakorn, S.C., 2018b. Decreased microglial activation through gut-brain axis by prebiotics, probiotics, or synbiotics effectively restored cognitive function in obese-insulin resistant rats. J. Neuroinflammation 15 (1), 11.

Ekelof, S.V., Halladin, N.L., Jensen, S.E., Zaremba, T., Aaroe, J., Kjaergaard, B., Simonsen, C.W., Rosenberg, J., Gogenur, I., 2016. Effects of intracoronary melatonin on ischemia-reperfusion injury in st-elevation myocardial infarction. Heart Ves. 31 (1), 88–95.

Fisher, J.W., 2003. Erythropoietin: physiology and pharmacology update. Exp. Biol. Med. 228 (1), 1–14.

Frick, T., Springe, D., Grandgirard, D., Leib, S.L., Haenggi, M., 2016. An improved simple rat model for global cerebral ischaemia by induced cardiac arrest. Neurol. Res. 38 (4), 373–380.

Grunnet, L.G., Aikin, R., Tonnesen, M.F., Paraskevas, S., Blaabjerg, L., Storling, J., Rosenberg, L., Billestrup, N., Maysinger, D., Mandrup-Poulsen, T., 2009. Proinflammatory cytokines activate the intrinsic apoptotic pathway in beta-cells. Diabetes 58 (8), 1807–1815.

Hausenloy, D.J., Yellon, D.M., 2013. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J. Clin. Invest. 123 (1), 92–100.

Iwai, M., Stetler, R.A., Xing, J., Hu, X., Gao, Y., Zhang, W., Chen, J., Cao, G., 2010. Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. Stroke 41 (5), 1032–1037.

Juul, S.E., Stallings, S.A., Christensen, R.D., 1999. Erythropoietin in the cerebrospinal fluid of neonates who sustained cns injury. Pediatr. Res. 46 (5), 543–547.

Kellert, B.A., McPherson, R.J., Juul, S.E., 2007. A comparison of high-dose recombinant erythropoietin treatment regimens in brain-injured neonatal rats. Pediatr. Res. 61 (4), 451–455.

Khaksari, M., Mehrjerdi, F.Z., Rezvani, M.E., Safari, F., Mirgalili, A., Niknazar, S., 2017. The role of erythropoietin in remote renal preconditioning on hippocampus ischemia/reperfusion injury. J. Physiol. Sci. 67 (1), 163–171.

Kumfu, S., Charununtakorn, S.T., Jaiwongkam, T., Chattipakorn, N., Chattipakorn, S.C., 2018. Humanin exerts neuroprotection during cardiac ischemia-reperfusion injury. J Alzheimers Dis 61 (4), 1343–1353.

Lee, K.Y., Chung, K., Chung, J.M., 2010. Involvement of reactive oxygen species in long-term potentiation in the spinal cord dorsal horn. J. Neurophysiol. 103 (1), 382–391.

Li, H., Liang, X., Duan, J., Chen, Y., Tian, X., Wang, J., Zhang, H., Liu, Q., Yang, J., 2021. Ros-responsive epo nanoparticles ameliorate ionizing radiation-induced hematopoietic injury. Biomater. Sci. 9 (19), 6474–6485.

Liu, K., Sun, T., Wang, P., Liu, Y.-h., Zhang, L.-w., Xue, Y.-x., 2013. Effects of erythropoietin on blood-brain barrier tight junctions in ischemia-reperfusion rats. J. Mol. Neurosci. 49 (2), 369–379.

Marti, H.H., Wenger, R.H., Rivas, L.A., Straumann, U., Digicaylioglu, M., Henn, V., Yonekawa, Y., Bauer, C., Gassmann, M., 1996. Erythropoietin gene expression in human, monkey and murine brain. Eur. J. Neurosci. 8 (4), 666–676.

Masuda, S., Okano, M., Yamagishi, K., Nagao, M., Ueda, M., Sasaki, R., 1994. A novel site of erythropoietin production. Oxygen-dependent production in cultured rat astrocytes. J. Biol. Chem. 269 (30), 19488–19493.

Menger, M.M., Nalbach, L., Roma, L.P., Laschke, M.W., Menger, M.D., Ampofo, E., 2021. Erythropoietin exposure of isolated pancreatic islets accelerates their revascularization after transplantation. Acta Diabetol. 58 (12), 1637–1647.

Nairz, M., Haschka, D., Dichtl, S., Sonnweber, T., Schroll, A., Asshoff, M., Mindur, J.E., Moser, P.L., Wolf, D., Swirski, F.K., Theurl, I., Cerami, A., Brines, M., Weiss, G., 2017. Cibinetide dampens innate immune cell functions thus ameliorating the course of experimental colitis. Sci. Rep. 7 (1), 13012.

Nairz, M., Schroll, A., Moschen, A.R., Sonnweber, T., Theurl, M., Theurl, I., Taub, N., Jamnig, C., Neurauter, D., Huber, L.A., Tilg, H., Moser, P.L., Weiss, G., 2011. Erythropoietin contrastingly affects bacterial infection and experimental colitis by inhibiting nuclear factor-kappab-inducible immune pathways. Immunity 34 (1), 61–74.

Ongnok, B., Khuanjing, T., Chunchai, T., Kerdphoo, S., Jaiwongkam, T., Chattipakorn, N., Chattipakorn, S.C., 2021. Donepezil provides neuroprotective effects against brain injury and alzheimer's pathology under conditions of cardiac ischemia/reperfusion injury. Biochim. Biophys. Acta, Mol. Basis Dis. 1867 (1), 165975.

Pan, Y., Yang, X.H., Guo, L.L., Gu, Y.H., Qiao, Q.Y., Jin, H.M., 2017. Erythropoietin reduces insulin resistance via regulation of its receptor-mediated signaling pathways in db/db mice skeletal muscle. Int. J. Biol. Sci. 13 (10), 1329–1340.
Park, J., Min, J.S., Kim, B., Chae, U.B., Yun, J.W., Choi, M.S., Kong, I.K., Chang, K.T., Lee, D.S., 2015. Mitochondrial ros govern the lps-induced pro-inflammatory response in microglia cells by regulating mapk and nf-kappab pathways. Neurosci. Lett. 584, 191–196.
Peng, B., Kong, G., Yang, C., Ming, Y., 2020. Erythropoietin and its derivatives: from tissue protection to immune regulation. Cell Death Dis. 11 (2), 79.
Pintana, H., Apaijai, N., Pratchayasakul, W., Chattipakorn, N., Chattipakorn, S.C., 2012. Effects of metformin on learning and memory behaviors and brain mitochondrial functions in high fat diet induced insulin resistant rats. Life Sci. 91 (11–12), 409–414.
Saint-Georges-Chaumet, Y., Edeas, M., 2016. Microbiota-mitochondria inter-talk: consequence for microbiota-host interaction. Pathog Dis 74 (1), ftv096.
Silva, I., Alipio, C., Pinto, R., Mateus, V., 2021. Potential anti-inflammatory effect of erythropoietin in non-clinical studies in vivo: a systematic review. Biomed. Pharmacother. 139, 111558.
Sirén, A.L., Knerlich, F., Poser, W., Gleiter, C.H., Brück, W., Ehrenreich, H., 2001. Erythropoietin and erythropoietin receptor in human ischemic/hypoxic brain. Acta Neuropathol. 101 (3), 271–276.
Souvenir, R., Doycheva, D., Zhang, J.H., Tang, J., 2015. Erythropoietin in stroke therapy: friend or foe. Curr. Med. Chem. 22 (10), 1205–1213.
Taguchi, N., Nakayama, S., Tanaka, M., 2016. Single administration of soluble epoxide hydrolase inhibitor suppresses neuroinflammation and improves neuronal damage after cardiac arrest in mice. Neurosci. Res. 111, 56–63.
Teng, R., Calvert, J.W., Sibmooh, N., Piknova, B., Suzuki, N., Sun, J., Martinez, K., Yamamoto, M., Schechter, A.N., Lefer, D.J., Noguchi, C.T., 2011. Acute erythropoietin cardioprotection is mediated by endothelial response. Basic Res. Cardiol. 106 (3), 343–354.
Tsai, P.T., Ohab, J.J., Kertesz, N., Groszer, M., Matter, C., Gao, J., Liu, X., Wu, H., Carmichael, S.T., 2006. A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. J. Neurosci. 26 (4), 1269–1274.
Wang, L., Zhang, Z.G., Zhang, R.L., Jiao, Z.X., Wang, Y., Pourabdollah-Nejad, D.S., LeTourneau, Y., Gregg, S.R., Chopp, M., 2006. Neurogenin 1 mediates erythropoietin enhanced differentiation of adult neural progenitor cells. J. Cerebr. Blood Flow Metabol. 26 (4), 556–564.
Wang, R., Zhao, H., Li, J., Duan, Y., Fan, Z., Tao, Z., Ju, F., Yan, F., Luo, Y., 2017. Erythropoietin attenuates axonal injury after middle cerebral artery occlusion in mice. Neurol. Res. 39 (6), 545–551.
Wang, S., Zhang, C., Li, J., Niyazi, S., Zheng, L., Xu, M., Rong, R., Yang, C., Zhu, T., 2017. Erythropoietin protects against rhabdomyolysis-induced acute kidney injury by modulating macrophage polarization. Cell Death Dis. 8 (4), e2725.
Watanabe, M., Lundgren, T., Saito, Y., Cerami, A., Brines, M., Ostenson, C.G., Kumagai-Braesch, M., 2016. A nonhematopoietic erythropoietin analogue, ara 290, inhibits macrophage activation and prevents damage to transplanted islets. Transplantation 100 (3), 554–562.
Weber, A., Dzietko, M., Berns, M., Felderhoff-Mueser, U., Heinemann, U., Maier, R.F., Obladen, M., Ikonomidou, C., Bührer, C., 2005. Neuronal damage after moderate hypoxia and erythropoietin. Neurobiol. Dis. 20 (2), 594–600.
Wu, X.C., Johns, E.J., Richards, N.T., 1999. Relationship between erythropoietin and nitric oxide in the contraction of rat renal arcuate arteries and human umbilical vein endothelial cells. Clin. Sci. (Lond.) 97 (4), 413–419.
Yoo, S.J., Cho, B., Lee, D., Son, G., Lee, Y.B., Soo Han, H., Kim, E., Moon, C., Moon, C., 2017. The erythropoietin-derived peptide mk-x and erythropoietin have neuroprotective effects against ischemic brain damage. Cell Death Dis. 8 (8), e3003.
Yu, X., Lin, C.S., Costantini, F., Noguchi, C.T., 2001. The human erythropoietin receptor gene rescues erythropoiesis and developmental defects in the erythropoietin receptor null mouse. Blood 98 (2), 475–477.
Yuan, S., Zhang, X., Bo, Y., Li, W., Zhang, H., Jiang, Q., 2014. The effects of electroacupuncture treatment on the postoperative cognitive function in aged rats with acute myocardial ischemia-reperfusion. Brain Res. 1593, 19–29.
Zhang, S.-J., Wang, R.-L., Zhao, H.-P., Tao, Z., Li, J.-C., Ju, F., Han, Z.-P., Ma, Q.-F., Liu, P., Ma, S.-B., Cao, G.-D., Luo, Y.-M., 2019. Mepo promotes neurogenesis and angiogenesis but suppresses gliogenesis in mice with acute ischemic stroke. Eur. J. Pharmacol. 849, 1–10.