SPANDIDOS PUBLICATIONS

MOLECULAR MEDICINE REPORTS 16: 2179-2184, 2017

Epoxyeicosatrienoic acid ameliorates cerebral ischemia-reperfusion injury by inhibiting inflammatory factors and pannexin-1

ZHIGANG LIU[1*], YONGFANG LIU[1*], HAIXIAO ZHOU[2*], XIANGYUN FU[1] and GANG HU[1]

Departments of [1]Anesthesiology and [2]Plastic Surgery, Renmin Hospital of Wuhan University, Wuhan, Hubei 430060, P.R. China

Received February 23, 2016; Accepted February 22, 2017

DOI: 10.3892/mmr.2017.6831

Abstract. Epoxyeicosatrienoic acid (EET) has wide applications due to the unique biological effects of anti-hyperlipidemia, inhibition of platelet aggregation, anti-inflammation, anti-cancer, anti-lipid oxidation and the promotion of brain tissue development. The present study investigated whether EET ameliorates cerebral ischemia-reperfusion injury (CIRI) by inhibiting inflammatory factors and pannexin. Specific pathogen-free 7-week-old male Sprague-Dawley rats were randomly divided into three groups: Sham, CIRI and EET. Neurological deficit scores, cerebral infarct volume and cerebral edema were assessed in CIRI rats. Enzyme-linked immunosorbent assays were performed to detect tumor necrosis factor-α, interleukin-6, nuclear factor-κB and inducible nitric oxide synthase (iNOS) levels, and western blot analysis was performed also used to assess cleaved caspase-3, phospholipase A2 (PLA2), cyclooxygenase-2 and prostaglandin E2 (PGE2) protein expression levels. EET ameliorated cerebral injury and CIRI-induced cleaved caspase-3 protein expression levels in rats. EET additionally suppressed CIRI-induced inflammation reactions and iNOS protein expression in rats. Furthermore, the protein expression levels of PLA2, PGE2 and pannexin-1 in CIRI rats were inhibited by treatment with EET. These results indicated that EET reduces CIRI by inhibiting inflammation and levels of cleaved caspase-3, PLA2, PGE2 and pannexin-1.

Correspondence to: Dr Yongfang Liu, Department of Anesthesiology, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan, Hubei 430060, P.R. China
E-mail: liuyangfangffl@163.com

*Contributed equally

Key words: epoxyeicosatrienoic acid, cerebral ischemia-reperfusion injury, inflammation, pannexin

Introduction

Cerebrovascular disease is the third most common cause of mortality in the world (1). It has three major characteristics: High morbidity, high disability rate and high mortality rate (1). The morbidity of cerebral ischemia-reperfusion injury (CIRI) accounts for ~70% of cerebrovascular diseases, and this trend is increasing (1). Many patients lose the ability to work and function normally, which causes heavy economic and psychological burdens for the patients, families and society (2). Therefore, it is important to prevent and control the incidence and development of CIRI.

The therapeutic principle of ischemic stroke is to restore blood perfusion in ischemia, improve the blood circulation of the brain tissue, increase blood flow and oxygen supply at ischemic penumbra, control encephaledema and to prevent and cure further complications (3,4). However, reperfusion further aggravates brain dysfunction, and structural failure results from ischemia. Brain tissue injuries cause irreversible injury of brain cells (5); this is known as ischemic CIRI. Consequently, investigations are focused on developing methods to reduce reperfusion injury, save brain cells and restore brain functions (6), potentially by detecting markers of the degree of CIRI (7). The development of novel psychotherapeutic drugs for the prevention and treatment of cerebrovascular diseases therefore has great significance.

Epoxyeicosatrienoic acid (EET; Fig. 1) was initially identified from investigations into the low incidence of cardiovascular disease in eskimos (8). Docosahexaenoic acid, eicosapentaenoic acid and other long-chain unsaturated fatty acids have the efficacy to prevent cholesterol deposition on the arterial vascular wall, and to prevent the incidence of cardiovascular disease (9). EET has been demonstrated to exert anti-inflammatory effects through nuclear factor (NF)-κB, adipocyte protein 2 and other signaling pathways (10). The present study used a rat CIRI model to investigate the inhibition effects of EET, and the potential underlying mechanisms.

Materials and methods

Animals and experimental groups. Specific pathogen-free male Sprague-Dawley rats (age, 7 weeks; weight, 200-240 g; n=50)

were purchased from the Center for Animal Experiments, Renmin Hospital of Wuhan University (Wuhan, China) and housed at 23±2˚C in a 12 h light/dark cycle in 50±10% humidity with free access to food and water. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Bethesda, MD, USA) and the experiments were approved by the Animal Care and Use Committee of Wuhan University. The rats were randomly divided into three groups: Sham, (n=10), CIRI (n=20) and EET (n=20). In the EET group, rats were administered $6.24x10^{-7}$ mol/l 10 ml/kg 11,12-EET via the jugular vein for 20 min. Rats in the other groups were administered with saline. All rats were intraperitoneally anesthetized with 30 mg/kg pentobarbital sodium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and were fixed in a supine position at 37-38˚C. A 4/0 surgical nylon filament with a silicone-beaded tip was inserted into the right internal carotid artery via the external carotid artery to occlude the origin of the middle cerebral artery. The occlusion was released for 24 h following ischemia at 2 h.

Neurological deficit score. Rats were suspended by the tail and neurological testing was conducted independently by 3 researchers. The neurological deficit scores were as follows: 0, forelimb flexion and body twisting; 1, inability to extend the left forepaw; 2, rat circled to the left; 3, inability to move the left forepaw; 4, inability to walk.

Cerebral infarct volume and cerebral edema. To assess cerebral infarct volume, the rats were sacrificed and brain tissue samples were obtained and weighed as wet weight (WW). Subsequently, tissue samples were sectioned into 4 mm-thick pieces and stained using 2% 2,3,5-triphenyltetrazolium chloride (Amresco, LLC; Solon, OH, USA) at room temperature for 30 min. The infarct volume is expressed as the percentage of the contralateral hemisphere. Tissue samples were dried at 110˚C overnight in an electric oven and weighed again to obtain the dry weight (DW). Water content (%)=[(WW-DW)/WW] x100% was calculated as brain water content.

Enzyme-linked immunosorbent assay (ELISA). Cells were lysed using a radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) at 4˚C for 15-20 min, the homogenate was centrifuged for 10 min at 30,000 x g at 4˚C and the supernatant was collected. Protein concentrations were detected with a bicinchoninic acid (BCA) assay kit (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The levels of TNF-α (cat no. ab46070), interleukin (IL)-6 (cat no. ab100772), NF-κB (cat no. ab176647) and inducible nitric oxide synthase (iNOS; cat no. ab196266) were determined using commercial ELISA kits (Abcam, Shanghai, China).

Western blot analysis. Cells were lysed using a radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology) at 4˚C for 15-20 min, the homogenate was centrifuged for 10 min at 30,000 x g at 4˚C and the supernatant was collected. Protein concentrations were detected with a BCA kit. Proteins (50 μg) were separated by 10-12% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). Membranes were blocked using 5% skim milk powder in TBS containing 0.05% Tween-20 (TBST) for 1 h at 37˚C and incubated overnight with the following primary antibodies at 4˚C: Anti-cleaved caspase-3 (1:3,000; cat no. 9664), anti-phospholipase A2 (PLA2; 1:3,000; cat no. 2832), anti-cyclooxygenase-2 (COX-2; 1:3,000; cat no. 12282), anti-pannexin-1 (1:3,000; cat no. 91137), purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA); anti-prostaglandin E2 (PGE2; 1:300; cat no. D262741) and anti-β-actin (1:3,000; cat no. D110007), obtained from Shanghai Sangong Pharmaceutical Co., Ltd. (Shanghai, China). Membranes were washed with TBST, and subsequently probed with horseradish peroxidase-labeled secondary antibodies (1:5,000; cat no. BM2006; Wuhan Boster Biological Technology, Ltd., Wuhan, China) at 37˚C for 1 h. Blots were visualized by enhanced chemiluminescence using the Pierce™ Fast Western Blot kit (cat no. 35050; Thermo Fisher Scientific, Inc.) and semi-quantified using Quantity One software version 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

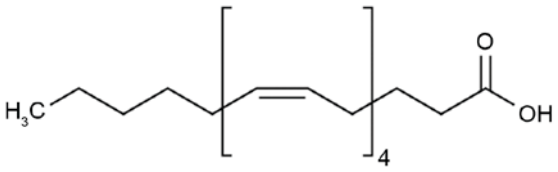

Figure 1. Constitutional formula of epoxyeicosatrienoic acid.

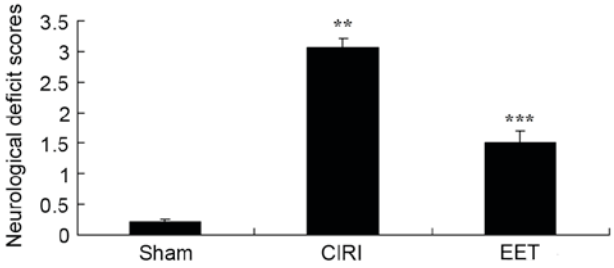

Figure 2. EET ameliorates CIRI-induced neurological deficit score. Neurological deficit scores in the sham, CIRI and EET groups. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid.

Statistical analysis. Data were analyzed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation of 3 independent experiments. The statistical significance of the differences between groups was assessed using a one-way analysis of variance followed by a post hoc Tukey-Kramer test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

EET ameliorates CIRI-induced neurological deficit scores. Neurological deficit scores from each group are presented in Fig. 2. The neurological deficit score of the CIRI model group significantly increased compared with sham group. EET

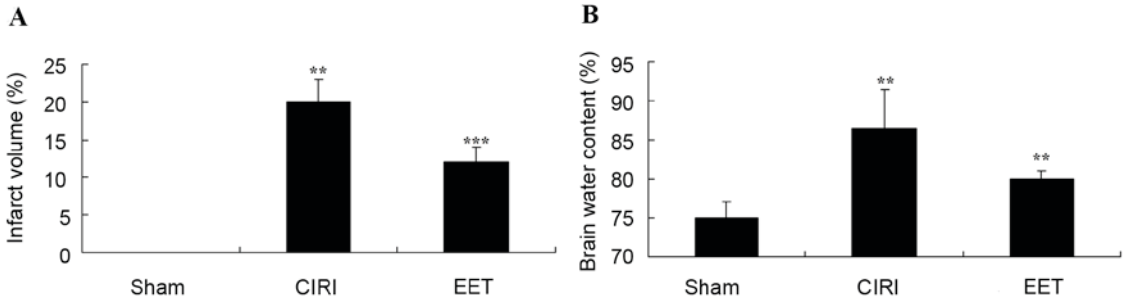

Figure 3. EET ameliorates CIRI-induced cerebral infarct volume and cerebral edema. (A) Cerebral infarct volume and (B) brain water content were assessed in the sham, CIRI and EET groups. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid.

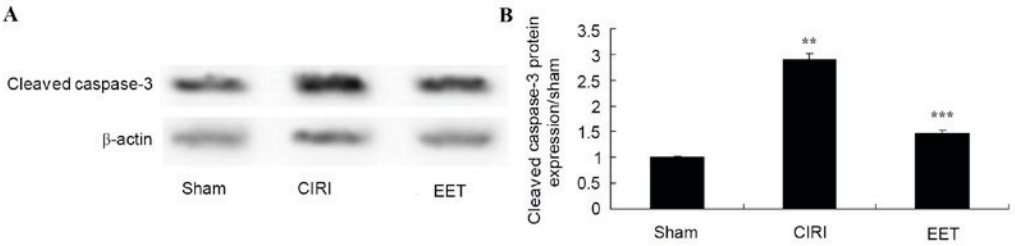

Figure 4. EET ameliorates CIRI-induced cleaved caspase-3. (A) Representative western blot images and (B) quantification of cleaved caspase-2 protein expression levels in sham, CIRI and EET-treated groups. β-actin served as an internal control. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid.

treatment caused a significant decrease in the neurological deficit score, when compared with the CIRI model group.

EET ameliorates CIRI-induced cerebral infarct volume and cerebral edema. CIRI-induced cerebral infarct volume and cerebral edema (brain water content) in all groups are presented in Fig. 3. The cerebral infarct volume in the sham group was negligible, and it was significantly increased in the CIRI group in comparison. There was a significant inhibition in cerebral infarct volume in the EET group compared with the CIRI model group (Fig. 3A). Cerebral edema was increased in the CIRI group compared with the sham group; however, treatment with EET significantly reduced this effect (Fig. 3B).

EET ameliorates CIRI-induced cleaved caspase-3. As assessed by western blot analysis (Fig. 4A), cleaved caspase-3 protein expression levels in CIRI tissue homogenates was significantly increased compared with the sham group. Pretreatment with EET significantly reduced CIRI-induced cleavedcaspase-3 protein expression levels (Fig. 4B).

EET ameliorates CIRI-induced inflammation. The levels of TNF-α (Fig. 5A), IL-6 (Fig. 5B) and NF-κB (Fig. 5C) measured in CIRI group samples were significantly increased compared with the sham group; however, treatment with EET significantly ameliorated this effect.

EET ameliorates CIRI-induced iNOS activity. CIRI-induced iNOS activity was markedly increased compared with the sham group, and EET treatment significantly attenuated this effect (Fig. 6).

EET ameliorates CIRI-induced PLA2 protein expression. PLA2 protein expression levels were determined by western blot analysis (Fig. 7A). CIRI rats exhibited significantly increased PLA2 expression levels compared with the sham group; however, EET pretreatment significantly inhibited this effect (Fig. 7B).

EET ameliorates CIRI-induced COX-2 protein expression. COX-2 protein expression levels were examined by western blot analysis (Fig. 8A). CIRI led to a significant increase in COX-2 protein expression levels compared with sham-operated rats; however, EET treatment significantly ameliorated this effect (Fig. 8B).

EET ameliorates CIRI-induced PGE2 protein expression. The protein expression levels of PGE2 was evaluated in CIRI tissue (Fig. 9A). PGE2 was highly expressed in CIRI tissue but not in sham-operated rats. In addition, this activation of PGE2 protein expression was significantly inhibited by treatment with EET (Fig. 9B).

EET ameliorates CIRI-induced pannexin-1 protein expression. Pannexin-1 protein expression levels were examined by western blot analysis (Fig. 10A). These results demonstrated that, following CIRI, pannexin-1 protein expression levels were increased compared with the sham group. Treatment with EET significantly inhibited CIRI-induced pannexin-1 protein expression in rats (Fig. 10B).

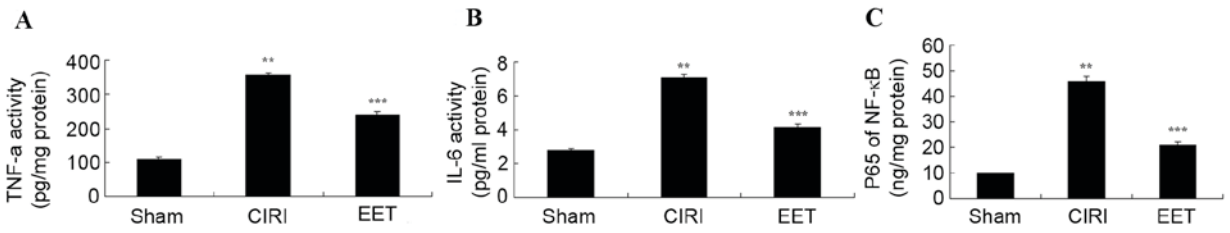

Figure 5. EET ameliorates CIRI-induced inflammation. (A) TNF-α, (B) IL-6 and (C) and NF-κB levels in the sham, CIRI and EET-treated groups, as determined by ELISA. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid; NF-κB, nuclear factor-κB; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

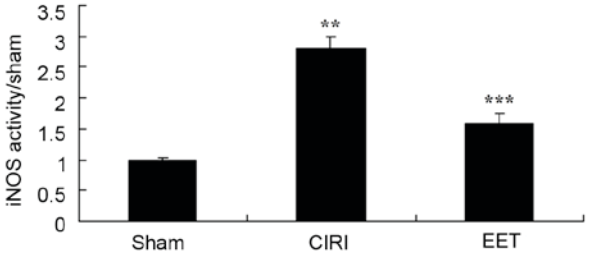

Figure 6. EET ameliorates CIRI-induced iNOS activity. iNOS activity in the sham, CIRI and EET-treated groups, as determined by enzyme-linked immunosorbent assay. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid; iNOS, inducible nitric oxide synthase.

Discussion

CIRI refers to cellular function metabolic disorders and organizational structure damage aggravation following blood reperfusion of ischemic damage (11). Although timely blood reperfusion may rescue certain cells close to the site of infarction, reperfusion causes a greater extent of tissue damage than pure ischemia injury (11). Previous studies have indicated that CIRI is primarily associated with inflammatory reactions, excitability amino acids, intracellular friction overload, oxygen free radicals and cell apoptosis (4,12). These various factors interact during cerebral ischemia reperfusion, and interlink to form a vicious circle. It eventually leads to ischemia reperfusion injury (13). Among these factors, the inflammatory reaction is considered to be one of the most important causes of CIBI (13). TNF is a cytokine with key effects in the network of inflammation. It is considered as an initiating medium for systemic inflammatory reaction (14). It may directly lead to reduced circulation resistance, increased vasopermeability and decreased numbers of vascular endothelial cells. In addition, it may induce a cascade release of IL-1, IL-6 IL-8 and additional cytokines, which forms amplification effects on inflammatory injury (14). The present study demonstrated that EET treatment significantly ameliorated CIRI-induced neurological deficit scores, cerebral infarct volume and cerebral edema, and inhibited the expression levels of cleaved caspase-3, TNF-α, IL-6 and NF-κB in CIRI rats. Previous studies have demonstrated that EET attenuates IL-8 production in bronchial epithelial cells (15) and inflammation in the cardiovascular system (16).

PLA2 has been suggested as an inflammatory marker closely associated with atherosclerosis (17). A combination of PLA2 and low-density lipoproteins in the blood generates a great amount of blood transportation, lecithin and free fatty acid oxidation, promoting atherosclerosis (18). The primary pathological basis of ischemic stroke, particularly cerebral thrombosis, is atherosclerosis (19). Consequently, it is a predictive index for atherosclerosis cerebral infarction. The present study revealed that EET treatment significantly ameliorates CIRI-induced PLA2 protein expression in rats. Jiang *et al* (20) reported that the release of EETs from red blood cells may be via cytosolic PLA2.

Increased expression levels of PLA2 may induce genetic expression of COX-2 for numerous inflammation-stimulating factors, including lipopolysaccharide, IL-1, TNF, epidermal growth factor α and platelet activating factor (21), and increase levels of PGE2, prostacyclin and PGE1 at inflammatory sites. A positive association between COX-2 mRNA and PGE2 levels at inflammatory sites and severity of inflammation has been verified in numerous experiments (22). At present, PGE2 is considered as an important mediator of inflammation. It has been demonstrated that the expression of COX-2 in the brain is the highest among all visceral organs, and is primarily distributed in the hippocampus and temporal lobe cortex (23,24). This may be associated with the high density of excitatory amino acid receptors in these regions. Overexpression of COX-2 in nervous tissue may directly damage nerve cells (25). In the present study, COX-2 protein expression levels were significantly reduced by treatment with EET. Michaelis *et al* (26) indicated that EET induces COX-2 protein expression in endothelial cells.

The gap-junction connected gel networks of astrocytes have been suggested to be involved in ischemia; therefore, have attracted increasing attention (27). Pannexin, the primary protein composition for close connection of astrocytes, is involved in transmission of information within astrocyte networks via communication between gap junctions (27). Gap junction intercellular communication may disperse antioxidative, anti-apoptotic and growth factors around the ischemia area via tight junctions to alleviate ischemic injury. However, dying nerve cells in the ischemic core area may disperse excitatory amino acids, free radical products, apoptosis signals and other injurious factors to ischemia area to induce cell injury and apoptosis in the peripheral zone, further expanding the cerebral infarction volume (28,29). The present study demonstrated that EET ameliorates CIRI-induced PGE2 and pannexin-1 protein expression levels in rats. Nüsing *et al* (30) suggested that EET may affect renal tubular epithelial cells via PGE2 and COX-2.

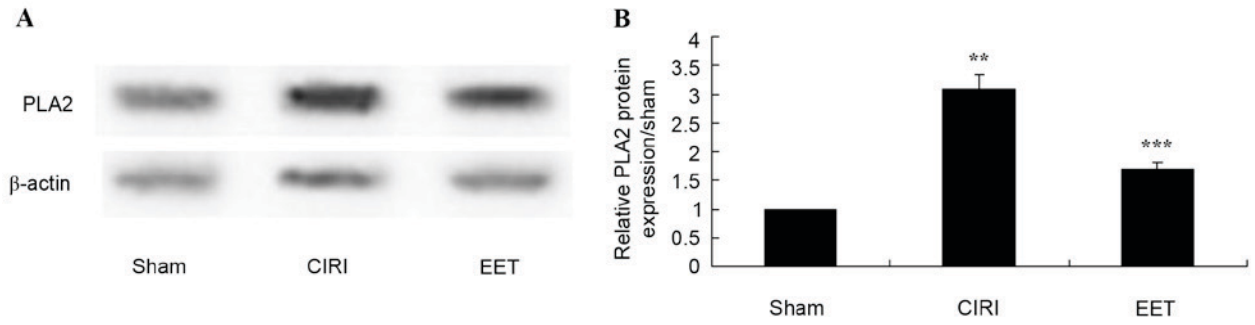

Figure 7. EET ameliorates CIRI-induced PLA2 protein expression. (A) Representative western blot images and (B) quantification of PLA2 protein expression levels in sham, CIRI and EET-treated groups. β-actin served as an internal control. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid; PLA2, phospholipase A2.

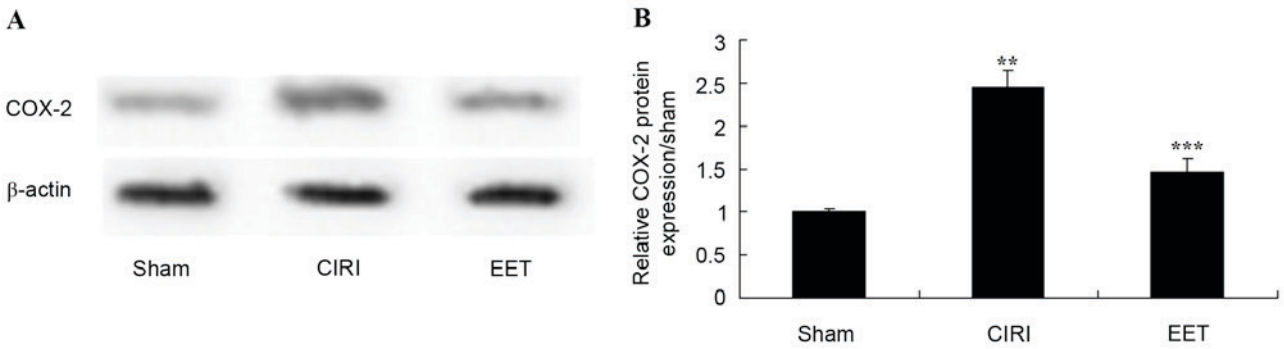

Figure 8. EET ameliorates CIRI-induced COX-2 protein expression. (A) Representative western blot images and (B) quantification of COX-2 protein expression levels in sham, CIRI and EET-treated groups. β-actin served as an internal control. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid; COX-2, cyclooxygenase 2.

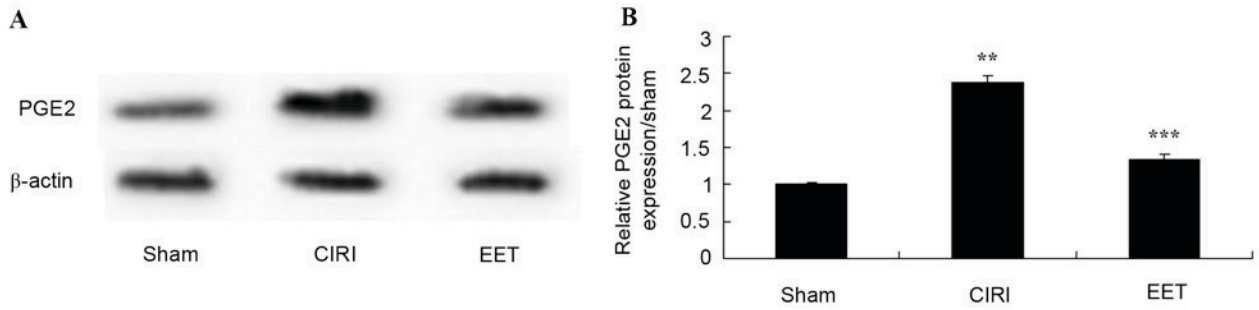

Figure 9. EET ameliorates CIRI-induced PGE2 protein expression. (A) Representative western blot images and (B) quantification of PGE2 protein expression levels in sham, CIRI and EET-treated groups. β-actin served as an internal control. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid; PGE2, prostaglandin E2.

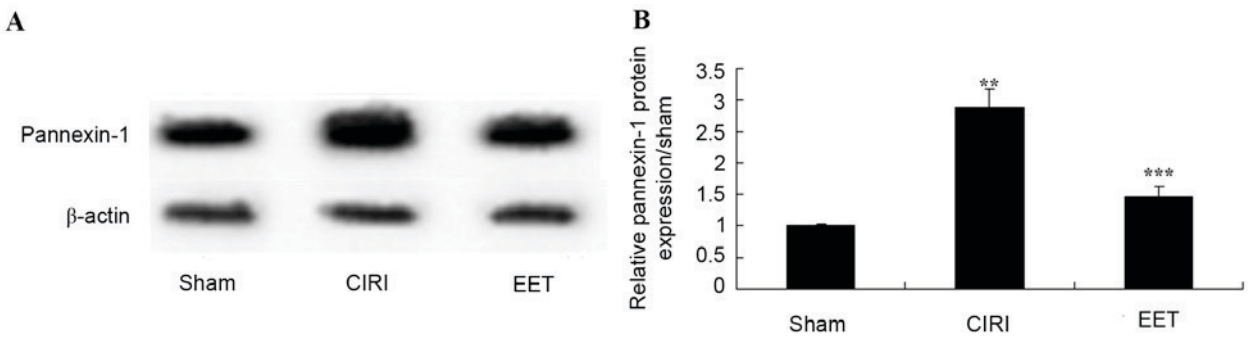

Figure 10. EET ameliorates CIRI-induced pannexin-1 protein expression. (A) Representative western blot images and (B) quantification of pannexin-1 protein expression levels in sham, CIRI and EET-treated groups. β-actin served as an internal control. Data are expressed as the mean ± standard deviation. **P<0.01 vs. sham; ***P<0.01 vs. CIRI. CIRI, cerebral ischemia-reperfusion injury; EET, epoxyeicosatrienoic acid.

In conclusion, the present study demonstrated that EET inhibits CIRI-induced neurological deficit scores, cerebral infarct volume and cerebral edema, and inhibited the levels of cleaved caspase-3, TNF-α, IL-6 and NF-κB in CIRI rats via inhibiting the activation of PLA2/COX-2/PGE2. Therefore, EET may represent a potential therapeutic agent for the prevention and treatment of CIRI.

Acknowledgements

The present study was supported by the Natural Science Foundation of Hubei Province (grant no. 2013BKB014).

References

1. Nasri H: Renal cell protection of erythropoietin beyond correcting the anemia in chronic kidney disease patients. Cell J 15: 378-380, 2014.
2. Zager RA, Johnson AC and Becker K: Acute unilateral ischemic renal injury induces progressive renal inflammation, lipid accumulation, histone modification and 'end-stage' kidney disease. Am J Physiol Renal Physiol 301: F1334-F1345, 2011.
3. Liu YF, Zhang Y, Dai D and Xu Z: Expression of NF-κB, MCP-1 and MMP-9 in a cerebral aneurysm rabbit model. Can J Neurol Sci 41: 200-205, 2014.
4. Wang S, Liu K, Seneviratne CJ, Li X, Cheung GS, Jin L, Chu CH and Zhang C: Lipoteichoic acid from an clinical strain promotes TNF-a expression through the NF-kB and p38 MAPK signaling pathways in differentiated THP-1 macrophages. Biomed Rep 3: 697-702, 2015.
5. Ma XF, Zhang J, Shuai HL, Guan BZ, Luo X and Yan RL: IKKa/NF-kB mediated the low doses of bisphenol A induced migration of cervical cancer cells. Arch Biochem Biophys 573: 52-58, 2015.
6. Li J, Deng Z, Wang Z, Wang D, Zhang L, Su Q, Lai Y, Li B, Luo Z, Chen X, *et al*: Zipper-interacting protein kinase promotes epithelial-mesenchymal transition, invasion and metastasis through AKT and NF-kB signaling and is associated with metastasis and poor prognosis in gastric cancer patients. Oncotarget 6: 8323-8338, 2015.
7. Kuper C, Beck FX and Neuhofer W: NFAT5 contributes to osmolality-induced MCP-1 expression in mesothelial cells. Mediators Inflamm 2012: 513015, 2012.
8. Lakkappa N, Krishnamurthy PT, Hammock BD, Velmurugan D and Bharath MM: Possible role of Epoxyeicosatrienoic acid in prevention of oxidative stress mediated neuroinflammation in Parkinson disorders. Med Hypotheses 93: 161-165, 2016.
9. Zhang X, Liang D, Guo L, Liang W, Jiang Y, Li H, Zhao Y, Lu S and Chi ZH: Curcumin protects renal tubular epithelial cells from high glucose-induced epithelial-to-mesenchymal transition through Nrf2-mediated upregulation of heme oxygenase-1. Mol Med Rep 12: 1347-1355, 2015.
10. Mawdsley JE, Jenkins DG, Macey MG, Langmead L and Rampton DS: The effect of hypnosis on systemic and rectal mucosal measures of inflammation in ulcerative colitis. Am J Gastroenterol 103: 1460-1469, 2008.
11. Inui Y, Mochida H, Yamairi F, Okada M, Ishida J, Fukamizu A and Arakawa K: Effects of aging and uninephrectomy on renal changes in Tsukuba hypertensive mice. Biomed Rep 1: 359-364, 2013.
12. Yao L, Lu P, Li Y, Yang L, Feng H, Huang Y, Zhang D, Chen J and Zhu D: Osthole relaxes pulmonary arteries through endothelial phosphatidylinositol 3-kinase/Akt-eNOS-NO signaling pathway in rats. Eur J Pharmacol 699: 23-32, 2013.
13. Hua KF, Yang SM, Kao TY, Chang JM, Chen HL, Tsai YJ, Chen A, Yang SS, Chao LK and Ka SM: Osthole mitigates progressive IgA nephropathy by inhibiting reactive oxygen species generation and NF-kB/NLRP3 pathway. PLoS One 8: e77794, 2013.
14. Tsai YF, Yu HP, Chung PJ, Leu YL, Kuo LM, Chen CY and Hwang TL: Osthol attenuates neutrophilic oxidative stress and hemorrhagic shock-induced lung injury via inhibition of phosphodiesterase 4. Free Radic Biol Med 89: 387-400, 2015.
15. Mannon PJ, Hornung RL, Yang Z, Yi C, Groden C, Friend J, Yao M, Strober W and Fuss IJ: Suppression of inflammation in ulcerative colitis by interferon-b-1a is accompanied by inhibition of IL-13 production. Gut 60: 449-455, 2011.
16. Yang SK, Jung HY, Kang GH, Kim YM, Myung SJ, Shim KN, Hong WS and Min YI: Appendiceal orifice inflammation as a skip lesion in ulcerative colitis: An analysis in relation to medical therapy and disease extent. Gastrointest Endosc 49: 743-747, 1999.
17. Xia Y, Kong L, Yao Y, Jiao Y, Song J, Tao Z, You Z and Yang J: Osthole confers neuroprotection against cortical stab wound injury and attenuates secondary brain injury. J Neuroinflammation 12: 155, 2015.
18. Lin VC, Chou CH, Lin YC, Lin JN, Yu CC, Tang CH, Lin HY and Way TD: Osthole suppresses fatty acid synthase expression in HER2-overexpressing breast cancer cells through modulating Akt/mTOR pathway. J Agric Food Chem 58: 4786-4793, 2010.
19. Yang YZ, Tang YZ and Liu YH: Wogonoside displays anti-inflammatory effects through modulating inflammatory mediator expression using RAW264.7 cells. J Ethnopharmacol 148: 271-276, 2013.
20. Jiang H, McGiff JC, Quilley J, Sacerdoti D, Reddy LM, Falck JR, Zhang F, Lerea KM and Wong PY: Identification of 5,6-trans-epoxyeicosatrienoic acid in the phospholipids of red blood cells. J Biol Chem 279: 36412-36418, 2004.
21. Yang SM, Chan YL, Hua KF, Chang JM, Chen HL, Tsai YJ, Hsu YJ, Chao LK, Feng-Ling Y, Tsai YL, *et al*: Osthole improves an accelerated focal segmental glomerulosclerosis model in the early stage by activating the Nrf2 antioxidant pathway and subsequently inhibiting NF-kB-mediated COX-2 expression and apoptosis. Free Radic Biol Med 73: 260-269, 2014.
22. Lucas AL, Ouellette SP, Kabeiseman EJ, Cichos KH and Rucks EA: The trans-Golgi SNARE syntaxin 10 is required for optimal development of Chlamydia trachomatis. Front Cell Infect Microbiol 5: 68, 2015.
23. Finocchietti S, Cappagli G and Gori M: Encoding audio motion: Spatial impairment in early blind individuals. Front Psychol 6: 1357, 2015.
24. Nordahl H, Osler M, Frederiksen BL, Andersen I, Prescott E, Overvad K, Diderichsen F and Rod NH: Combined effects of socioeconomic position, smoking, and hypertension on risk of ischemic and hemorrhagic stroke. Stroke 45: 2582-2587, 2014.
25. Venturino A, Oda A and Perin P: Hair cell-type dependent expression of basolateral ion channels shapes response dynamics in the frog utricle. Front Cell Neurosci 9: 338, 2015.
26. Michaelis UR and Fleming I: From endothelium-derived hyperpolarizing factor (EDHF) to angiogenesis: Epoxyeicosatrienoic acids (EETs) and cell signaling. Pharmacol Ther 111: 584-595, 2006.
27. de Groote RP, Budinčević I, Billowes J, Bissell ML, Cocolios TE, Farooq-Smith GJ, Fedosseev VN, Flanagan KT, Franchoo S, Garcia Ruiz RF, *et al*: Use of a continuous wave laser and pockels cell for sensitive high-resolution collinear resonance ionization spectroscopy. Phys Rev Lett 115: 132501, 2015.
28. Shen Y, Qiao H, Fan Q, Zhang S and Tang T: Potentiated osteoinductivity via cotransfection with BMP-2 and VEGF genes in microencapsulated C2C12 cells. Biomed Res Int 2015: 435253, 2015.
29. Rodriguez DA, de Lima RF, Campos MS, Costa JR, Biancardi MF, Marques MR, Taboga SR and Santos FC: Intrauterine exposure to bisphenol A promotes different effects in both neonatal and adult prostate of male and female gerbils (Meriones unguiculatus). Environ Toxicol, Oct 7, 2015 (Epub ahead of print) doi: 10.1002/tox.22176.
30. Nusing RM, Schweer H, Fleming I, Zeldin DC and Wegmann M: Epoxyeicosatrienoic acids affect electrolyte transport in renal tubular epithelial cells: Dependence on cyclooxygenase and cell polarity. Am J Physiol Renal Physiol 293: F288-F298, 2007.