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ORIGINAL ARTICLE

Propionic Acid Abrogates the Deleterious Effects of Cerebral Ischemic Reperfusion Injury through Nuclear Factor-κb Signaling in Mice

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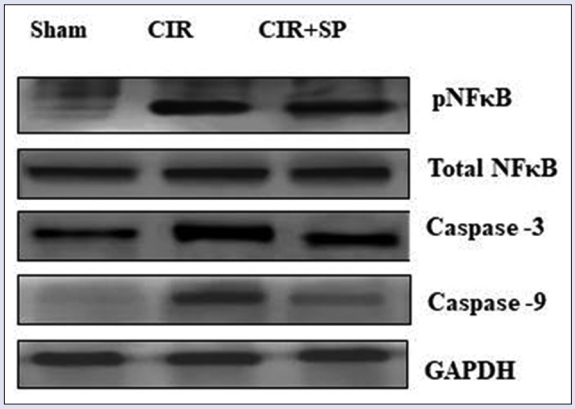
ABSTRACT

Background: Cerebral ischemia is caused due to insufficient blood flow to brain cells. This study evaluates the therapeutic effects of propionic acid (PA) on the abnormalities induced during the cerebral ischemic-reperfusion (CIR) injury in mice. **Materials and Methods:** CIR was induced by complete occlusion of the middle artery of the cerebrum for 15 min and reperfusion for 24 h in the experimental C57BL/6 mice. The analysis of mRNA levels of neuronal nitric oxide synthase (nNOS) was performed by reverse transcriptase polymerase chain reaction (RT-PCR). Inflammatory cytokines such as interleukin-6 (IL-6), IL-1 β, and tumor necrosis factor-alpha were quantitated by enzyme-linked immuno sorbent assay. Apoptosis-related proteins and nuclear factor-κB (NF-κB) were analyzed using Western blot technique. **Results:** PA demonstrated strong therapeutic effects on controlling the expression of nNOS that was analyzed using RT-PCR. Elevated levels of inflammatory cytokines, caspase-3, caspase-9, and phosphorylated NF-κB during cerebral ischemia were significantly controlled during PA treatment. **Conclusion:** Our findings demonstrated that PA through oral gavage exhibited healthier effects on the damage caused due to CIR injury.

Key words: Apoptosis, caspase-3, caspase-9, cerebral ischemia, inflammatory cytokines, neuronal nitric oxide synthase, nuclear factor-κB, reperfusion injury

SUMMARY

- PA effectively improved the neurological deficit in mice during cerebral ischemic reperfusion injury
- PA inhibited the abnormal elevation of cytokines after cerebral ischemic reperfusion injury and controlled the cerebral impairments.



Abbreviations used: PA: Propionic acid sodium salt; NFκB: Nuclear factor-κB; CIR: Cerebral ischemic-reperfusion; ELISA: Enzyme-linked immunosorbent assay; nNOS: Neuronal nitric oxide synthase; RT-PCR: Reverse transcription-polymerase chain reaction; TNF: Tumor necrosis factor-alpha; IL: Interleukin; ROS: Reactive oxygen species.

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INTRODUCTION

Cerebral ischemic reperfusion (CIR) injury causes brain damage, an irreversible cascade of biological events, leading to brain cells death. Further, it involves in many consequences in the brain, such as deficit volume of blood flow, inflammation, and oxidative stress, leading to apoptosis[1,2] which subsequently leads to functional impairment and neuronal death in the subjects.[3] The inflammatory response has also been proved to cause ischemic stroke and elevation of various inflammatory mediators such as interleukin-8 (IL-8), IL-1 β, and tumor necrosis factor-alpha (TNF-α), which can lead to neuronal damage.[4,5] Enhancement of the inflammatory response, by the produced cytokines, could initiate tissue damage through the diagnostic markers such as TNF-α and IL-6.[6] Many studies have proved that signaling pathways could have been involved in anti-apoptosis and phosphoinositide-3-kinase signaling pathways that are important for the survival and growth of cells in ischemic brain injury.[7] Postischemic inflammation can lead to secondary damage in CIR injury by inducing the coagulation cascade due to hypoxia, inflammatory cytokines production, and release of reactive oxygen species.[8]

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Microbial fermentation produced short-chain fatty acids, using dietary fibers, has been previously studied to prove the inhibition of intestinal pathogenic bacteria,[9] as an anticonvulsant and antipsychotic drugs.[10] Furthermore, previous reports had shown to have positive influence of short-chain fatty acids in extracellular signal-regulated protein kinases (ERK) pathways[11] which regulate transcription processes in different cell types by effecting phosphorylation of cAMP response element-binding protein (CREB).[12] Butyric acid was proved as a differentiation agent that acts through inhibiting histone deacetylase, alter gene expression, and mRNA stability.[13-15] CREB is an important transcription factor that involves in the transcriptional gene regulation that are required for neuronal cell survival. In the neuronal cells, the phosphorylation of CREB is mediated by ERK.[16] Signaling cascade of ERK 1/2 plays an important role for several forms of learning and deposition of memory in the hippocampus region of the brain.[17] Propionic acid (PA), a major metabolite produced in the human intestine by the action of anaerobic microbiota may contribute to the existence of the PA molecule in the ileum, cecum, and colon.[18] Small chain fatty acids show significant differences between the cell types involved in inflammatory processes and the suppression of pro-inflammatory cytokines production.[19] Few other research studies have also reported the pro-inflammatory and anti-inflammatory effects of short-chain fatty acids.[20,21]

Attenuation of elevated levels of cytokines such as TNF-α, IL-6, and nitric oxide (NO) in rat primary microglia cells had been proved in short-chain fatty acids administration.[22] Butyric acid was proved to attenuate brain infarct volume, activation of brain microglial cells, and enhancement of anti-inflammatory cytokine IL-10 production and its release.[23] However, a decrease in IL-10 (anti-inflammatory cytokine) production by monocytes was observed.[24] In a previous study, PA had been proved to stimulate neutrophil chemotaxis.[25] Jonsson *et al.* had showed that elevated plasma acetic acid level due to ethanol intake was associated with the prevention of the development of destructive arthritis.[26] With these information, the current study was designed to prove the efficiency of PA as a neuroprotective agent in experimental CIR injury in mice.

MATERIALS AND METHODS

Experimental animals

Animal experimentation procedures and protocols were approved by the Institutional Animal Care and Usage Committee of Heilongjiang University of Chinese Medicine (Approval # HUCM201902113409). Male C57BL/6 mice weighing 23–25 g and 8 weeks old were used for the study. Animals procured were acclimatized for 1 week in a sterile facility with water and feed *ad libitum*.[27] Light/dark cycles of 12 h each were maintained throughout the study.

Surgical and drug administration protocol

Animal model for CIR injury was done by a method previously described.[28] Precisely, middle artery occlusion was done for 15 min to induce cerebral ischemia using vascular clips.[29] Exposure of the ventral side common carotid artery of the neck was done by the surgical procedure under controlled ether anesthesia. After ischemia, vascular clips were removed and circulation was restored for 24 h before the sacrifice of the animals by cervical decapitation under anesthesia. A sham-operated group of mice also underwent same surgical procedure without arterial occlusion.

PA sodium salt with the highest purity ≥99% was obtained from Sigma Co. Ltd., St. Louis, USA. Random division of animals into three groups with 12 mice each was done. Group 1 served as sham-operated group with normal saline administration. Group 2 served as CIR injury group with normal saline. Group 3 animals were subjected to CIR injury protocol with PA administration at an optimal dose at an appropriate time. Intragastric administration of PA in a dose of 120 mg/kg (determined by dosage optimization protocol) was administered by oral gavage, 1 h after the initiation of reperfusion. A second dose of PA (same concentration) was administered after 12 h to Group 3 animals. At the end of experimental period, brain samples were dissected out and processed immediately appropriately for different assays. Protein assay was done using Beyotime assay kit from Beyotime Biotech Inc., Nanjing, China. All other reagents and chemicals used for this study were of analytical grade.

Neurological defects analysis

Evaluation of the neurological defects score on a five-point scale was done after 24 h of the CIR. A score of "0" indicates no defect and was appropriately increased up to "5" based on observed severity. Difficulty in full extension of contralateral forelimb scores "1," animals not being able to extend the contralateral forelimb scores "2," mild circling to the contralateral side scores "3", severe circling scores "4", and falling to the contralateral side scores "5." In each group, all the mice were evaluated and scores were recorded for the analyses.

Isolation of total RNA and neuronal nitric oxide synthase reverse transcription-polymerase chain reaction

Isolation of total RNA from the experimental animals' brain hippocampus region was done using the Axyprep multisource total RNA mini-prep kit (Axygen Biosciences). The isolation protocol followed was according to the manufacturer instructions given overleaf. Spectrophotometry based method was employed to find the yield and quality of total RNA isolated, by the ratio of absorbance at 260–280 nm (n = 3). The purity and quality of the isolated total RNA was confirmed by the value of ratio not <1.8 ± 0.1. To construct cDNA for the experiments, 2 μg of isolated total RNA was reverse-transcribed from each sample using the SYBR PrimeScript® reverse transcription-polymerase chain reaction (RT-PCR) kit and the manufacturer's protocol was followed (Takara Bio Inc., Japan).

Semi-quantitative RT-PCR was used to analyze the neuronal nitric oxide synthase (nNOS) mRNA expression in the samples. cDNAs constructed from the reverse transcription of isolated high-quality total RNA were amplified using an Applied Biosystems Thermal Cycler/RT-PCR System (USA) using an SYBR technology (PrimeScript®, Takara Bio Inc., Japan) PCR Kit. Hot-start protocol was followed for PCR amplification. Briefly, initial incubation at 94°C for 180s, followed by denaturation at 94°C for 15s, annealing for 50s at 63°C and extension for 60s at 68°C for 40 cycles. Amplification of a desired and single PCR product was confirmed by melt curve analyses. β-actin gene of mouse was used as an internal control gene. Primers used were as followed: For nNOS detection-Fwd-5'GTGGCCATCGTGTCCT ACCATAC3' and Rev-5'GTTTCGAGGCAGGTGGAAGCTA3'; for β-actin detection-Fwd-5'CCGTTTCTCCTGGCTCAGT TTA3' and Rev-5'CCCCAATACCACATCATCCAT3' were used. Amount of target transcript (mRNA level) was calculated as 2 ΔCT (ΔCT-differences in threshold cycles for nNOS gene), which was normalized by an endogenous reference gene (β-actin). 2 ΔCT was calculated as relative fold-change over the values of the reference gene in the analyses. A vial, only with water and without cDNA, was used as a negative control during the analysis. Results were reported as mean ± standard deviation (SD) of the samples in a group.

Inflammatory cytokines assay by enzyme linked immunosorbant assay

Inflammatory cytokines such as TNF-α, IL-1 β and IL-6 were quantified by enzyme-linked immuno-sorbent assay (ELISA). ELISA kits used were procured from Keygen biotech Co., Ltd., Nanjing, China, and analyses were performed according to the manufacturer instructions. Levels of inflammatory cytokines were expressed as pictogram (pg) per milligram (mg) of protein in the brain hippocampus.

Protein expression by Western blot analysis

Brain hippocampus tissue was dissected and homogenized in ice-cold Tris buffer and centrifuged briefly to remove cell debris. The supernatant was used to determine the total protein levels in the sample. After adjustment of samples for equal protein concentrations, separation was done by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After blocking with 5% fat-free milk for 2 h, samples separated in polyvinylidene fluoride membranes were incubated in the respective primary antibodies (antibodies specific for mouse caspase-3, caspase-9, NFκB, and phosphorylated NFκB (pNFκB) were procured from Abcam Shanghai Company Ltd., Shanghai, China) overnight at a temperature of 4°C. After development of the bands further using enhanced chemiluminescence reagent, visualization and documentation were done using automated software (Biorad Laboratories Inc., CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a control gene for the protein expression studies.

Statistical analysis

Collected data were summarized as mean ± SD. Statistical analyses were performed using the statistical analysis software package (SPSS 17.0, Chicago, IL, USA). Analyses were performed by one-way analysis of variance. *Post hoc* analysis and comparisons were made using Student's *t*-test. Statistical significance was considered when P value is ≤ 0.05.

RESULTS

Neurological defects analysis

CIR caused neurological deficit in the experimental mice that was confirmed by paralysis of left forelimbs. In the present study, neuroprotective effects of PA were evaluated based on neurological deficit scores. Figure 1 depicts the effects of PA on CIR in all the experimental groups. Sham-operated group showed no neurological deficit. CIR group showed severe neurological deficit ($P \leq 0.05$) when compared to the sham group. During treatment with PA, neurological deficit was partially recovered in the treatment group ($P \leq 0.05$). A significant neuroprotective effect of PA against CIR based on neurological deficit scores was observed.

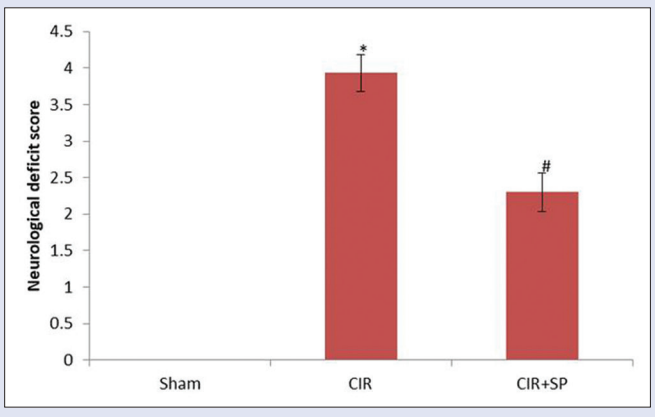

Figure 1: Protective effect of propionic acid on cerebral ischemic reperfusion injury based on neurological deficit score in experimental mice. Data were presented as mean ± standard deviation A $P \leq 0.05$ was considered statistically significant. Comparisons: *-with Sham; #-with cerebral ischemic reperfusion

Levels of neuronal nitric oxide synthase mRNA by reverse transcriptase-polymerase chain reaction

Expression levels of nNOS mRNA that was quantitated through RT-PCR is shown in Figure 2. Sham-operated group of mice showed normal level of expression during the experimental period. However, significantly higher level was observed in CIR group of mice. However, during the therapeutic administration, PA controlled the elevated level of nNOS in CIR + PA group of mice that was elevated due to cerebral ischemia. This significant decreased level of nNOS explains the possible therapeutic protective effects during cerebral ischemia.

Levels of inflammatory cytokines

Levels of inflammatory cytokines in the hippocampus region of the brain, assayed by ELISA are presented in Figures 3-5. As shown in the Figure, significantly elevated levels of TNF-α, IL-1 β, and IL-6 were observed in the CIR group of animals when compared to sham group. PA treatment attenuated these abnormalities by a significant decrease in the abnormally elevated cytokines levels of the treatment group when compared to CIR group. Thus, significantly decreased levels of the inflammatory cytokines were observed in the hippocampus of the brain during PA treatment.

Protein expression levels by western blot analysis

Expression levels of apoptotic proteins such as NfκB, caspase-3, and caspase-9 that were involved in the apoptosis-related pathways were analyzed using Western blot technique. Figure 6a-c lists the Western blot images of the proteins under investigation and its relative expression levels to GAPDH. Expression levels of pNfκB, caspase-3, and caspase-9

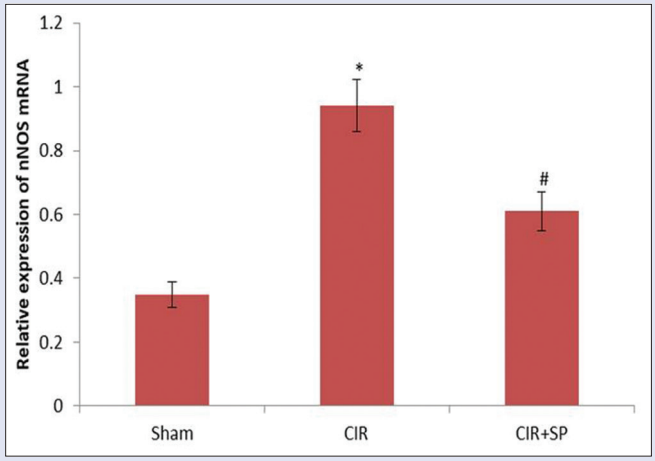

Figure 2: Expression levels of neuronal nitric oxide synthase mRNA in experimental groups of mice. ΔCt = Ct (nNOS)–Ct (β-actin), where Ct = cycle threshold, β-actin is the endogenous reference gene. Relative neuronal nitric oxide synthase mRNA expression: PΔCt = 2 ΔCt, representing neuronal nitric oxide synthase mRNA levels. Final data in the graph were magnified to 1000 × 2 ΔCt. Data were reported as mean ± standard deviation. A $P \leq 0.05$ (two-tailed Student "*t*" test). Comparisons: *-with Sham; #-with cerebral ischemic reperfusion

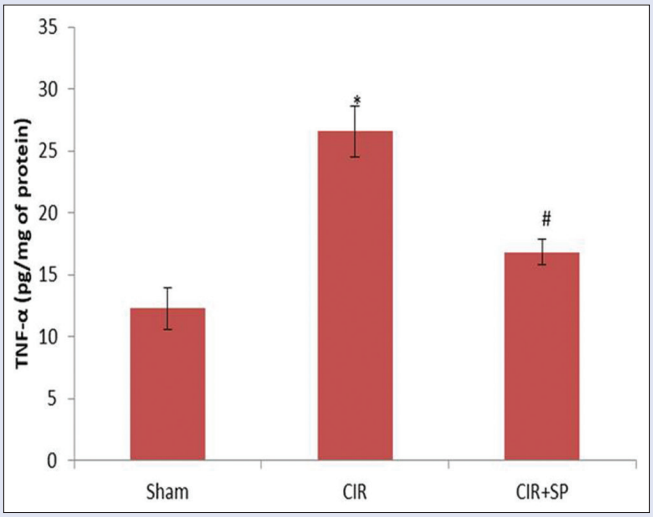

Figure 3: Levels of inflammatory cytokine tumor necrosis factor-α on cerebral ischemic reperfusion injury and treatment by propionic acid in experimental mice. Data were presented as mean ± standard deviation. A $P \leq 0.05$ was considered statistically significant. Comparisons: *-with Sham; #-with cerebral ischemic reperfusion

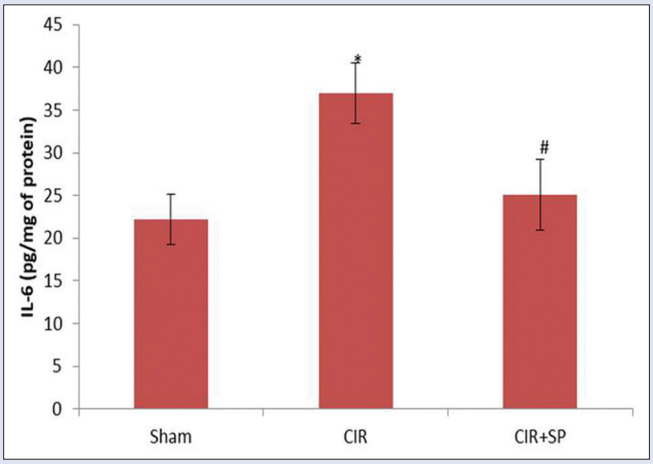

Figure 5: Levels of inflammatory cytokine interleukin-6 on cerebral ischemic reperfusion injury and treatment by propionic acid in experimental mice. Data were presented as mean ± standard deviation. A $P \leq 0.05$ was considered statistically significant. Comparisons: *-with Sham; #-with cerebral ischemic reperfusion

were increased in the CIR group of mice. Interestingly, elevated levels of caspases (3 and 9) were controlled during PA treatment. Furthermore, a relatively decreased level of pNfκB was observed during PA administration.

DISCUSSION

Complex carbohydrates of dietary sources after bacterial fermentation produces short-chain fatty acids such as acetic acid, butyric acid, and PA[30] and is readily absorbed by intestinal epithelium.[31] Previous studies proved that inflammatory response, apoptosis, and oxidative stress were the important reasons for the pathogenesis of stroke.[32] Short-chain fatty acids were proved to inhibit inflammation and neuronal apoptosis that could lead to pathogenesis of brain in

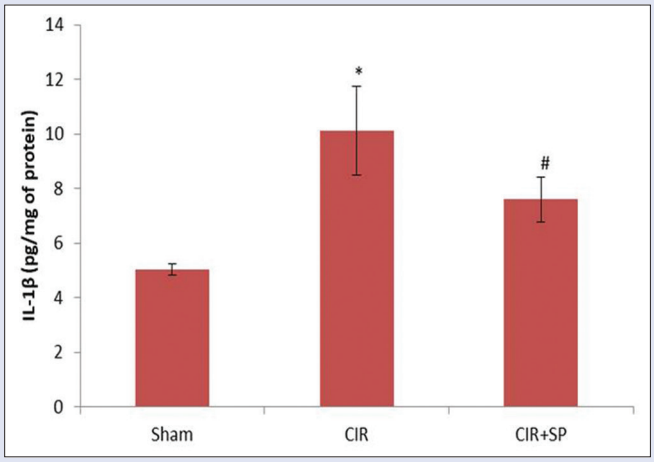

Figure 4: Levels of inflammatory cytokine interleukin-1 β on cerebral ischemic reperfusion injury and treatment by PA in experimental mice. Data were presented as mean ± standard deviation. A $P \leq 0.05$ was considered statistically significant. Comparisons: *-with Sham; #-with cerebral ischemic reperfusion

the experimental animals,[33] and also have reported to improve memory and spatial learning in Alzheimer's disease experimental mice.[34] Significant decrease in the neuropathological alterations in the treatment group showed that PA effectively improved the neurological deficit in mice during CIR injury.

NOS-1 or nNOS is one among the three types of NOS that have been studied well in stroke research.[35] In CIR group, elevated mRNA level could possibly due to ischemic stress during carotid artery occlusion. Expression of neuronal NOS could lead to the synthesis of NO that helps in the long-term synaptic plasticity and memory formation[36,37] and differentiation of axon to form multi-innervated spines.[38] Although we have not assayed NO levels, elevated level of nNOS mRNA in CIR group might be due to the ischemic response that stimulate elevated production of NO for repairing the damage due to arterial occlusion during the experimentation. Attenuation of abnormal levels of NO in bronchoalveolar lavage fluid that was elevated due to acute lung injury was observed in short-chain fatty acid administration.[39] Probably, the administration of PA in the treatment (CIR + PA) group could have controlled indirectly the expression of nNOS by controlling NO in the hippocampus region.

Brain cells are more susceptible to neuronal damage due to over-activated astrocytes and microglia in brain cells that secrete higher levels of inflammatory cytokines.[40,41] Cytokines are the important signaling molecules that could trigger the pathogenesis of ischemic damage in the brain cells.[42] Cytokine signaling could trigger a crucial inflammatory signaling cascade by the activation of mitogen-activated protein kinase pathways.[43] IL-6, TNF-α, IL-10, and IL-1 β are the important inflammatory cytokines related to ischemia in brain cells and are the therapeutic targets and biomarkers for prognosis.[44] Furthermore, elevated levels of IL-1 β could increase calcium influx through N-methyl-D-aspartate-receptor ionophores leading to neuronal damage[45] and elevated IL-6 level after stroke could lead to brain damage.[46] In this study, elevated levels of cytokines clearly demonstrated the deterioration of hippocampus region through abnormal neuronal deficit and nNOS levels during CIR injury. In our study, restoration of abnormalities in the levels of cytokines such as TNF-α, IL-1 β, and IL-6 during PA treatment could explain the protective effect in CIR injury. Dietary supplementation of fibers or short-chain fatty acids was proved

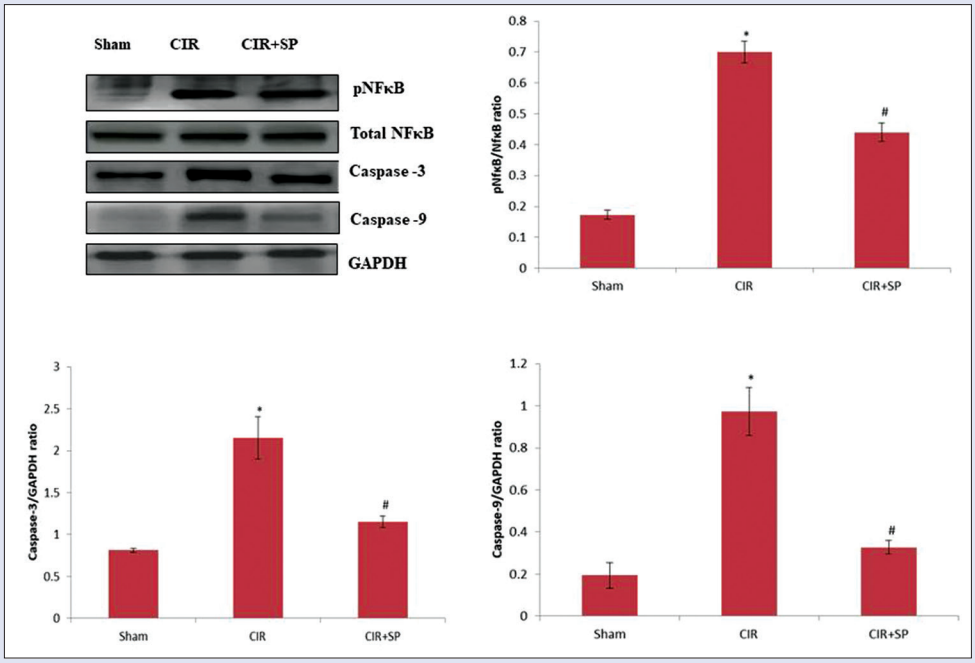

Figure 6: (a-c) Western blot analyses of apoptotic proteins in cerebral ischemic reperfusion injury and propionic acid treatment in experimental mice. Data were presented as mean ± standard deviation. A $P \leq 0.05$ was considered statistically significant. Comparisons: *-with Sham; #-with cerebral ischemic reperfusion

to reduce the levels of inflammatory cytokines in inflammatory bowel diseases[47] and the levels of NOS and TNF-α activities.[48] It could be due to the protective effect of bacterial digested and released fatty acids that circulate to the brain cells that include PA.[18] These were consistent with the data obtained in the present study, in which supplemented PA could have beneficial effects in decreasing the levels of inflammatory cytokines. Administration of PA after CIR injury attenuated the elevated levels of inflammatory cytokines and controlled the cerebral impairments.

NfκB, caspase-3, and caspase-9 are the major important proteins involved in the inflammatory processes. Of those, NfκB has been reported to involve in the inflammatory processes by its translocation into the nucleus after phosphorylation.[49] In line with this report, our study showed an elevated level of pNfκB to NfκB during CIR inflammation and was reverted during treatment with PA. Concomitantly, this could have decreased the expression levels of inflammatory cytokines, leading to beneficial effects in PA treatment. Furthermore, we showed that PA inhibited the activation of NF-κB pathway, mediated by TNF-α, which benefits to treat the CIR injury. Inflammatory signaling network involves Rho-kinase (RhoK), and NfκB-mediated caspase activation leading to apoptosis in the injured tissues.[50] In line with these research, our study demonstrated decreased level of caspases (3 and 9) that were elevated during CIR injury.

CONCLUSION

Overall the findings reflect the healthier effect of PA toward brain cells. Furthermore, as seen together, decreased apoptotic protein levels augmented the control of cytokines level during PA administration, in the inflammatory processes in CIR injury. In summary, we found that PA treatment exhibited a healthier effect in CIR injury by interacting with RhoK/NfκB pathways signaling in brain hippocampus of mice.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Danton GH, Dietrich WD. Inflammatory mechanisms after ischemia and stroke. J Neuropathol Exp Neurol 2003;62:127-36.
2. Kleinig TJ, Vink R. Suppression of inflammation in ischemic and hemorrhagic stroke: Therapeutic options. Curr Opin Neurol 2009;22:294-301.
3. Ashafaq M, Khan MM, Shadab Raza S, Ahmad A, Khuwaja G, Javed H, *et al*. S-allyl cysteine mitigates oxidative damage and improves neurologic deficit in a rat model of focal cerebral ischemia. Nutr Res 2012;32:133-43.
4. Pignataro G, Scorziello A, Di Renzo G, Annunziato L. Post-ischemic brain damage: Effect of ischemic preconditioning and postconditioning and identification of potential candidates for stroke therapy. FEBS J 2009;276:46-57.
5. Xia W, Han J, Huang G, Ying W. Inflammation in ischaemic brain injury: Current advances and future perspectives. Clin Exp Pharmacol Physiol 2010;37:253-8.
6. Czepiel J, Biesiada G, Brzozowski T, Ptak-Belowska A, Perucki W, Birczynska M, *et al*. The role of local and systemic cytokines in patients infected with *Clostridium difficile*. J Physiol Pharmacol 2014;65:695-703.
7. Mahajan SK, Kashyap R, Sood BR, Jaret P, Mokta J, Kaushik NK, *et al*. Stroke at moderate altitude. J Assoc Physicians India 2004;52:699-702.
8. Yan T, Chopp M, Chen J. Experimental animal models and inflammatory cellular changes in

cerebral ischemic and hemorrhagic stroke. Neurosci Bull 2015;31:717-34.

9. Liu T, Li J, Liu Y, Xiao N, Suo H, Xie K, *et al.* Short-chain fatty acids suppress lipopolysaccharide-induced production of nitric oxide and proinflammatory cytokines through inhibition of NF-κB pathway in RAW264.7 cells. Inflammation 2012;35:1676-84.
10. Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS, *et al.* Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J Biol Chem 2001;276:36734-41.
11. Hao Y, Creson T, Zhang L, Li P, Du F, Yuan P, *et al.* Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis. J Neurosci 2004;24:6590-9.
12. Treisman R. Regulation of transcription by MAP kinase cascades. Curr Opin Cell Biol 1996;8:205-15.
13. Maclean KN, McKay IA, Bustin SA. Differential effects of sodium butyrate on the transcription of the human TIS11 family of early-response genes in colorectal cancer cells. Br J Biomed Sci 1998;55:184-91.
14. Marks PA, Richon VM, Rifkind RA. Histone deacetylase inhibitors: Inducers of differentiation or apoptosis of transformed cells. J Natl Cancer Inst 2000;92:1210-6.
15. Stoecklin G, Colombi M, Raineri I, Leuenberger S, Mallaun M, Schmidlin M, *et al.* Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. EMBO J 2002;21:4709-18.
16. Lai TW, Zhang S, Wang YT. Excitotoxicity and stroke: Identifying novel targets for neuroprotection. Prog Neurobiol 2014;115:157-88.
17. Sweatt JD. The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 2001;76:1-0.
18. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28:1221-7.
19. Park JS, Lee EJ, Lee JC, Kim WK, Kim HS. Anti-inflammatory effects of short chain fatty acids in IFN-gamma-stimulated RAW 264.7 murine macrophage cells: Involvement of NF-kappaB and ERK signaling pathways. Int Immunopharmacol 2007;7:70-7.
20. Bailón E, Cueto-Sola M, Utrilla P, Rodríguez-Cabezas ME, Garrido-Mesa N, Zarzuelo A, *et al.* Butyrate *in vitro* immune-modulatory effects might be mediated through a proliferation-related induction of apoptosis. Immunobiology 2010;215:863-73.
21. Halili MA, Andrews MR, Labzin LI, Schroder K, Matthias G, Cao C, *et al.* Differential effects of selective HDAC inhibitors on macrophage inflammatory responses to the toll-like receptor 4 agonist LPS. J Leukoc Biol 2010;87:1103-14.
22. Huuskonen J, Suuronen T, Nuutinen T, Kyrylenko S, Salminen A. Regulation of microglial inflammatory response by sodium butyrate and short-chain fatty acids. Br J Pharmacol 2004;141:874-80.
23. Kim HJ, Rowe M, Ren M, Hong JS, Chen PS, Chuang DM, *et al.* Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: Multiple mechanisms of action. J Pharmacol Exp Ther 2007;321:892-901.
24. Cox MA, Jackson J, Stanton M, Rojas-Triana A, Bober L, Laverty M, *et al.* Short-chain fatty acids act as antiinflammatory mediators by regulating prostaglandin E(2) and cytokines. World J Gastroenterol 2009;15:5549-57.
25. Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, *et al.* Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem 2003;278:25481-9.
26. Jonsson IM, Verdrengh M, Brisslert M, Lindblad S, Bokarewa M, Islander U, *et al.* Ethanol prevents development of destructive arthritis. Proc Natl Acad Sci U S A 2007;104:258-63.
27. Zhu J, Jiang Y, Wu L, Lu T, Xu G, Liu X, *et al.* Suppression of local inflammation contributes to the neuroprotective effect of ginsenoside rb1 in rats with cerebral ischemia. Neuroscience 2012;202:342-51.
28. Moskowitz MA, Kiwak KJ, Hekimian K, Levine L. Synthesis of compounds with properties of leukotrienes C4 and D4 in gerbil brains after ischemia and reperfusion. Science 1984;224:886-9.
29. Zhao X, Strong R, Zhang J, Sun G, Tsien JZ, Cui Z, *et al.* Neuronal PPARgamma deficiency increases susceptibility to brain damage after cerebral ischemia. J Neurosci 2009;29:6186-95.
30. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol 2006;40:235-43.
31. Cook SI, Sellin JH. Review article: Short chain fatty acids in health and disease. Aliment Pharmacol Ther 1998;12:499-507.
32. Li J, Qu Y, Chen D, Zhang L, Zhao F, Luo L, *et al.* The neuroprotective role and mechanisms of TERT in neurons with oxygen-glucose deprivation. Neuroscience 2013;252:346-58.
33. Chuang DM, Leng Y, Marinova Z, Kim HJ, Chiu CT. Multiple roles of HDAC inhibition in neurodegenerative conditions. Trends Neurosci 2009;32:591-601.
34. Ricobaraza A, Cuadrado-Tejedor M, Pérez-Mediavilla A, Frechilla D, Del Río J, García-Osta A, *et al.* Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. Neuropsychopharmacology 2009;34:1721-32.
35. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: Therapeutic approaches. J Transl Med 2009;7:97.
36. Chien WL, Liang KC, Teng CM, Kuo SC, Lee FY, Fu WM, *et al.* Enhancement of long-term potentiation by a potent nitric oxide-guanylyl cyclase activator, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole. Mol Pharmacol 2003;63:1322-8.
37. Taqatqeh F, Mergia E, Neitz A, Eysel UT, Koesling D, Mittmann T, *et al.* More than a retrograde messenger: Nitric oxide needs two cGMP pathways to induce hippocampal long-term potentiation. J Neurosci 2009;29:9344-50.
38. Nikonenko I, Jourdain P, Muller D. Presynaptic remodeling contributes to activity-dependent synaptogenesis. J Neurosci 2003;23:8498-505.
39. Ni YF, Wang J, Yan XL, Tian F, Zhao JB, Wang YJ, *et al.* Histone deacetylase inhibitor, butyrate, attenuates lipopolysaccharide-induced acute lung injury in mice. Respir Res 2010;11:33.
40. Li B, Zhong L, Yang X, Andersson T, Huang M, Tang SJ, *et al.* WNT5A signaling contributes to aβ-induced neuroinflammation and neurotoxicity. PLoS One 2011;6:e22920.
41. Ferrarese C, Mascarucci P, Zoia C, Cavarretta R, Frigo M, Begni B, *et al.* Increased cytokine release from peripheral blood cells after acute stroke. J Cereb Blood Flow Metab 1999;19:1004-9.
42. Liu T, Clark RK, McDonnell PC, Young PR, White RF, Barone FC, *et al.* Tumor necrosis factor-alpha expression in ischemic neurons. Stroke 1994;25:1481-8.
43. Waetzig GH, Seegert D, Rosenstiel P, Nikolaus S, Schreiber S. P38 mitogen-activated protein kinase is activated and linked to TNF-alpha signaling in inflammatory bowel disease. J Immunol 2002;168:5342-51.
44. Vila N, Castillo J, Dávalos A, Esteve A, Planas AM, Chamorro A, *et al.* Levels of anti-inflammatory cytokines and neurological worsening in acute ischemic stroke. Stroke 2003;34:671-5.
45. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, *et al.* Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci 2003;23:8692-700.
46. Azzimondi G, Bassein L, Nonino F, Fiorani L, Vignatelli L, Re G, *et al.* Fever in acute stroke worsens prognosis. A prospective study. Stroke 1995;26:2040-3.
47. Nishimura T, Andoh A, Hashimoto T, Kobori A, Tsujikawa T, Fujiyama Y, *et al.* Cellobiose prevents the development of dextran sulfate sodium (DSS)-induced experimental colitis. J Clin Biochem Nutr 2010;46:105-10.
48. Rodríguez-Cabezas ME, Gálvez J, Lorente MD, Concha A, Camuesco D, Azzouz S, *et al.* Dietary fiber down-regulates colonic tumor necrosis factor alpha and nitric oxide production in trinitrobenzenesulfonic acid-induced colitic rats. J Nutr 2002;132:3263-71.
49. Chen T, Wang R, Jiang W, Wang H, Xu A, Lu G, *et al.* Protective effect of astragaloside IV against paraquat-induced lung injury in mice by suppressing rho signaling. Inflammation 2016;39:483-92.
50. Hannan JL, Matsui H, Sopko NA, Liu X, Weyne E, Albersen M, *et al.* Caspase-3 dependent nitrergic neuronal apoptosis following cavernous nerve injury is mediated via RhoA and ROCK activation in major pelvic ganglion. Sci Rep 2016;6:29416.