

Role of KCC2 in Acute and Long-Term Neuroprotection Induced by Propofol Postconditioning in a Rat Model of Focal Cerebral Ischemia/Reperfusion

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Introduction: It has been testified that propofol postconditioning plays a neuroprotective role for rats undergoing cerebral ischemia/reperfusion injury (I/R) via affecting excitatory glutamatergic nervous system^{1,2}. Regarding inhibitory central nervous system, K⁺-Cl⁻-co-transporter(KCC2) participates in gamma-aminobutyric acid (GABA) inhibitory effect in mature central neurons³. However, there is no report about the effects of propofol postconditioning on KCC2 expression. Therefore, we set out to explore the role of KCC2 in acute and long-term neuroprotection induced by propofol postconditioning in a rat model of focal cerebral I/R.

Methods: The committee of experimental animals of Tianjin Medical University approved all the procedures. 234 male Sprague-Dawley rats(250-280g), were randomly divided into 3 groups (n=78):sham operation group (group S), ischemia/reperfusion group (group I/R) and propofol postconditioning group (group P). Group I/R and P were infused saline of the same volume. At 24h postoperatively, modified neurological severity score was used to evaluate cognitive varieties, and Golgi staining to study the extent of neuron damage. Long-term cognitive function was assessed via Mirror Water Test at 9-14d and 23-28d after surgery. At the day of 1, 14 and 28 postoperatively, we used nissl stain to assess the extent of brain injury and immunofluorescence and western blot to survey expression levels of hippocampal neuronal KCC2.

Results: We found that cerebral I/R further deteriorated the extent of neural injury, reduced the number of survival neurons and downregulated KCC2 expression in I/R area in acute and long-term stage, while propofol postconditioning improved neural functions, increased the number of survival neurons and upregulated KCC2 expression.

Conclusion: These results show that cerebral I/R can further exacerbate neuron impairment via reducing neuronal KCC2 expression, whereas propofol postconditioning plays a neuroprotective role via upregulating GABA function-related KCC2 expression in ischemic area in acute and long-term stage.

References:

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Acknowledgements: The study was totally supported by grants from the Natural Science Foundation of China (81071059, 81100984, 81371245).

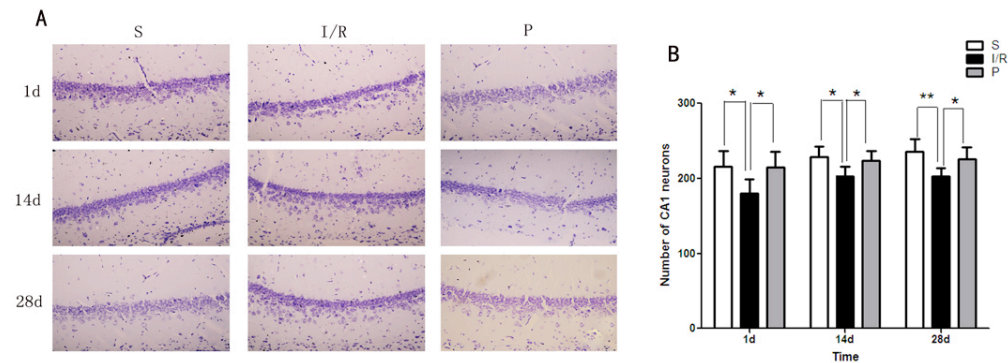


Fig.1 Effects of 3 groups on hippocampal CA1 neurons at 1d, 14d and 28d postoperatively (A) propofol postconditioning elevated the number of survival neurons (magnification 400×) (B) Quantification of survival neurons at 1d, 14d and 28d postoperatively. Nissl staining images were shown to evaluate severity of brain injury at 1d, 14d and 28d postoperatively. Bar represent mean \pm SE (n=6). **p < 0.01. *p < 0.05.

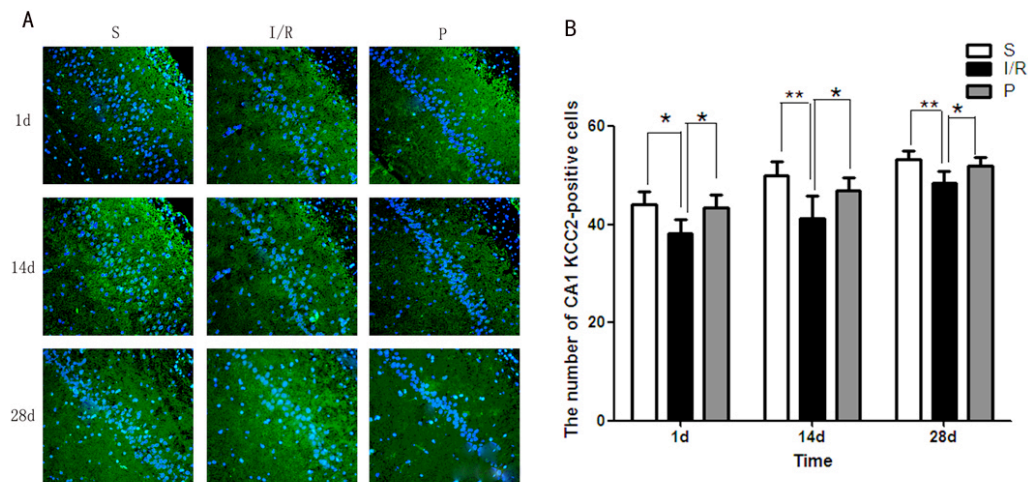


Fig.2 Effects of propofol postconditioning on KCC2-positive cells of hippocampal CA1 after transient MCAO in 3 groups at 1d, 14d and 28d postoperatively. (A) KCC2-positive cells were shown through immunofluorescence imaging (magnification 400×). (B) Quantification of CA1 survival KCC2-positive cells at 1d, 14d and 28d postoperatively. Bar represents mean \pm SE (n=6). **p < 0.01. *p < 0.05.

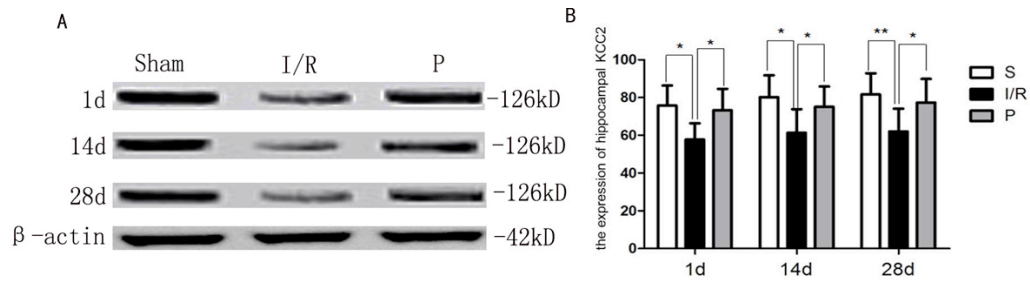


Fig.3 Propofol postconditioning increased the expression of KCC2 during cerebral isc injury. (A) Western blot analysis showed the expression of hippocampal KCC2 at 1d,14d and 28d after reperfusion. (B) Quantification of the expression of hippocampal KCC2 at 1d,14d and 28d after reperfusion. Bars represent mean \pm SE(n=6). **p<0.01. *p<0.05

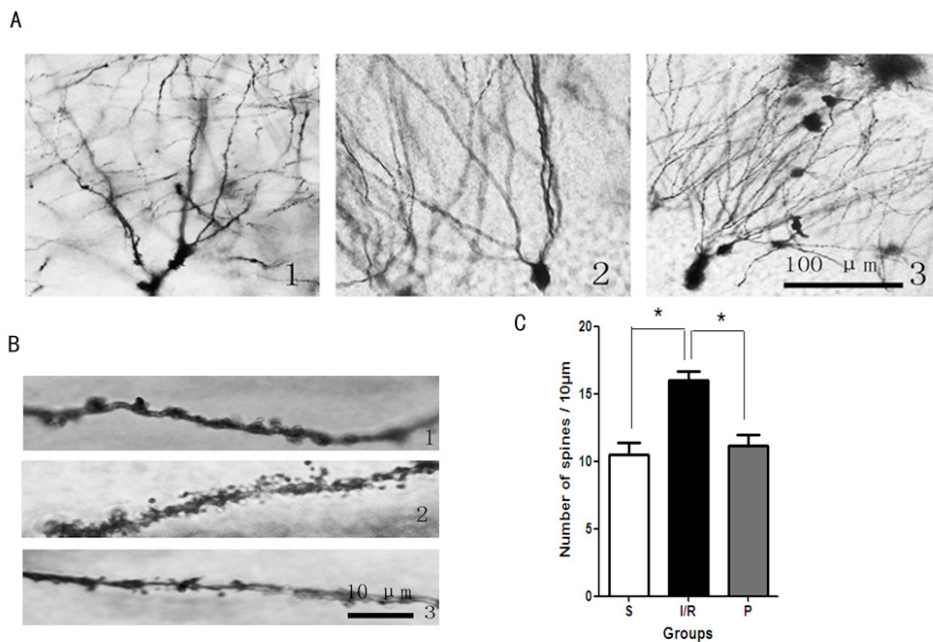


Fig.4 (A) Alterations of the total number of spines on basal dendrites were shown in ischemic area at 24h postoperatively. Scale bar=100 μ m. (B) Propofol postconditioning decreased density of spines in ischemic area at 24h postoperatively. Scale bar=10 μ m (C) Quantification of the density of spines. Bars represent mean \pm SE(n=6). *p<0.05.