The Role of ADAR2-AMPA Receptor GluR2 Subunit Pathway in Neuroprotection Induced by Propofol Post-Conditioning in Cerebral Ischemia-Reperfusion Injury: *in vivo and in vitro*

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Introduction: Our research team has suggested that propofol post-conditioning provided long-term neuroprotection through reducing internalization of α-amino-3-hydroxy-5-methylisox azole-4-propionic acid receptor (AMPAR) in a rat model of focal cerebral ischemia/reperfusion[1]. However the upstream of AMPAR GluR2 in propofol postconditioning have never been explored. Adenosine deaminase acting on RNA2 (ADAR2) is a nuclear enzyme essential for GluR2 pre-mRNA editing at Q/R site-607, which gates Ca^{2+} entry through AMPAR channels[2]. Here, our objective is to investigate the role of ADAR2-AMPA receptor GluR2 subunit pathway in neuroprotection induced by propofol post-conditioning in cerebral ischemia-reperfusion injury: in rats and in cultured primary hippocampus neuron.

Methods: Part1.SD rats were divided into sham group, I/R group (reperfusion after MACO 1h) and P group (20mg/kg/h propofol conditioning). Firstly we evaluated the effect of propofol on nerurological deficit scores and infarct volume for rats undergoing MCAO after 24h. Furthermore, the ratio of GluR2 membrane/total protein and ADAR2 nuclear/protein expression were analyzed by Western Bolt.

Part2.The primary hippocampus neurons cultured 7 days were divided into Control group, OGD/R group and P group. Small interfering RNAs (siRNA) were applied for studying ADAR2 gene silencing. In the following 24h, we evaluated cells viability by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As well the GluR2 and ADAR2 protein expression were analyzed by Western Bolt. Furthermore, ratio of GluR2 mRNA Q/R edited was analyzed by Nest RT-PCR and BbV1.

Results: We determined propofol statistically improved nerurological deficit scores and infarct volume in rats with MCAO. And compared with group I/R, propofol significantly increased expression of ADAR2 nuclear protein and GluR2 membrane protein while total protein of the both were of identical amounts. Interestingly, the identical phenomenon was also found in cultured primary hippocampus neuron. Moreover, propofol extremely increased cell viability of neurons with OGD/R injury and the ratio of GluR2 mRNA Q/R edited/total was higher in group P than in group OGD/R. As expected, siRNA silencing ADAR2 protein expression weakened neuroprotection of propofol.

Conclusion: Propofol post-conditioning statistically decrease ischemia-reperfusion induced acute nerve injury in rats and in cultured primary hippocampus neuron. The involved mechanism is related to promote trafficking of ADAR2 from cytoplasm to karyon and increase the ratio of GluR2 mRNA Q/R edited/unedited,

further inhibit internalization of AMPAR from cytoplasm to membrane and maintain the stability of postsynaptic membrane.

Reference:

1. Haiyun Wang, Mengqiang Luo, Guo-lin Wang, et al. Propofol post-conditioning induced long-term neuroprotection and reduced internalization of AMPAR GluR2 subunit in a rat model of focal cerebral ischemia/reperfusion. Journal of Neurochemistry. 2011.119: 210-219.
2. Horsch M, Seeburg PH, Adler T, et al. Requirement of the RNA-editing enzyme ADAR2 for normal physiology in mice. J Biol Chem. 2011. 286(21): 18614-18622.

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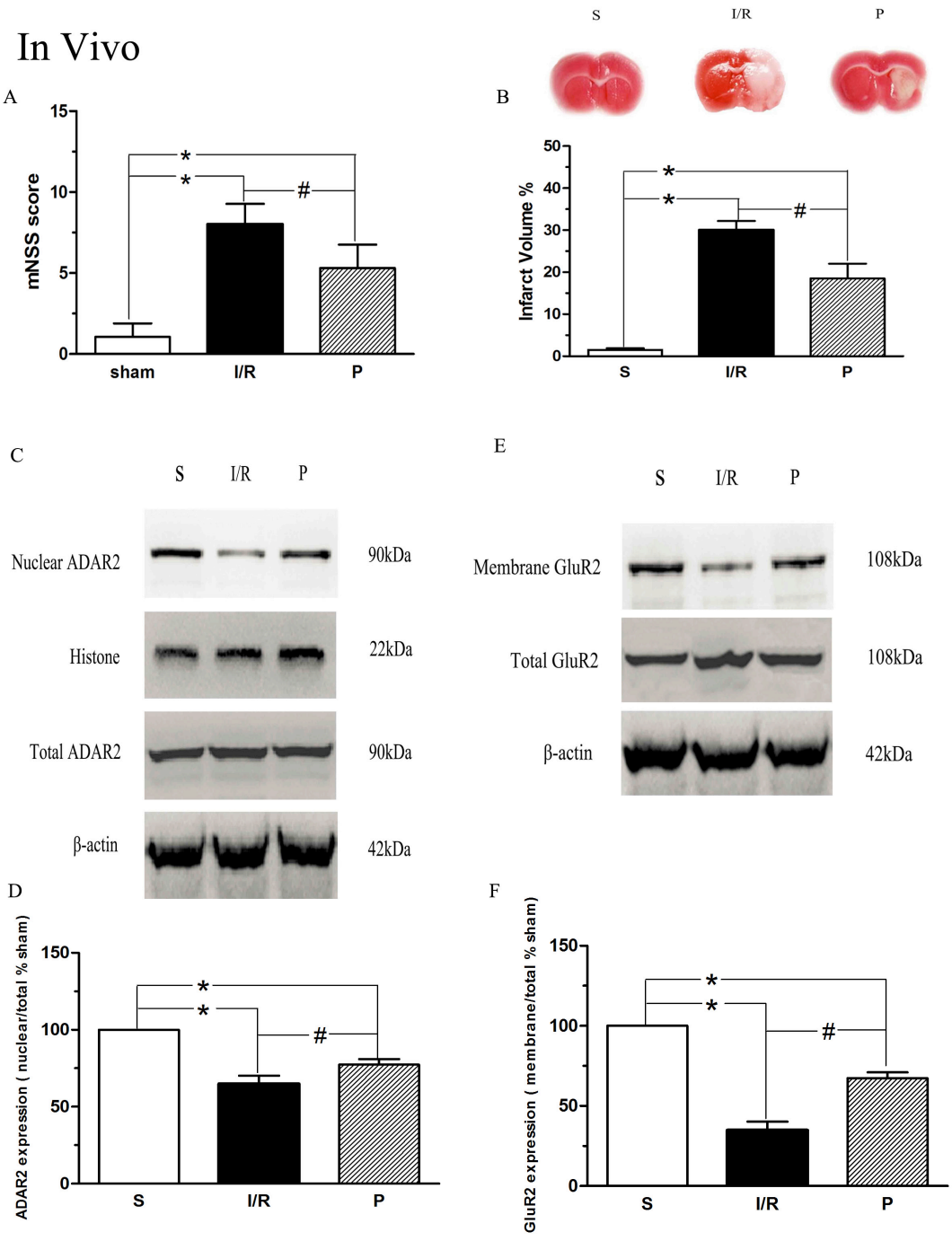

Fig.1 In Vivo

A.Effect of propofol post-conditioning on neurological function shown by mNSS score B.Effect of propofol post-conditioning on infarct volume shown TTC and Quantification of infarct volumn C and E.Effect of propofol post-conditioning on expression of ADAR2 and GluR2 shown Western Blot. D and F.Quantification of ADAR2 nuclear/total protein and GluR2 membrane/total protein expression. Bar represent mean±SE（n=10） *P<0.05;#P<0.01

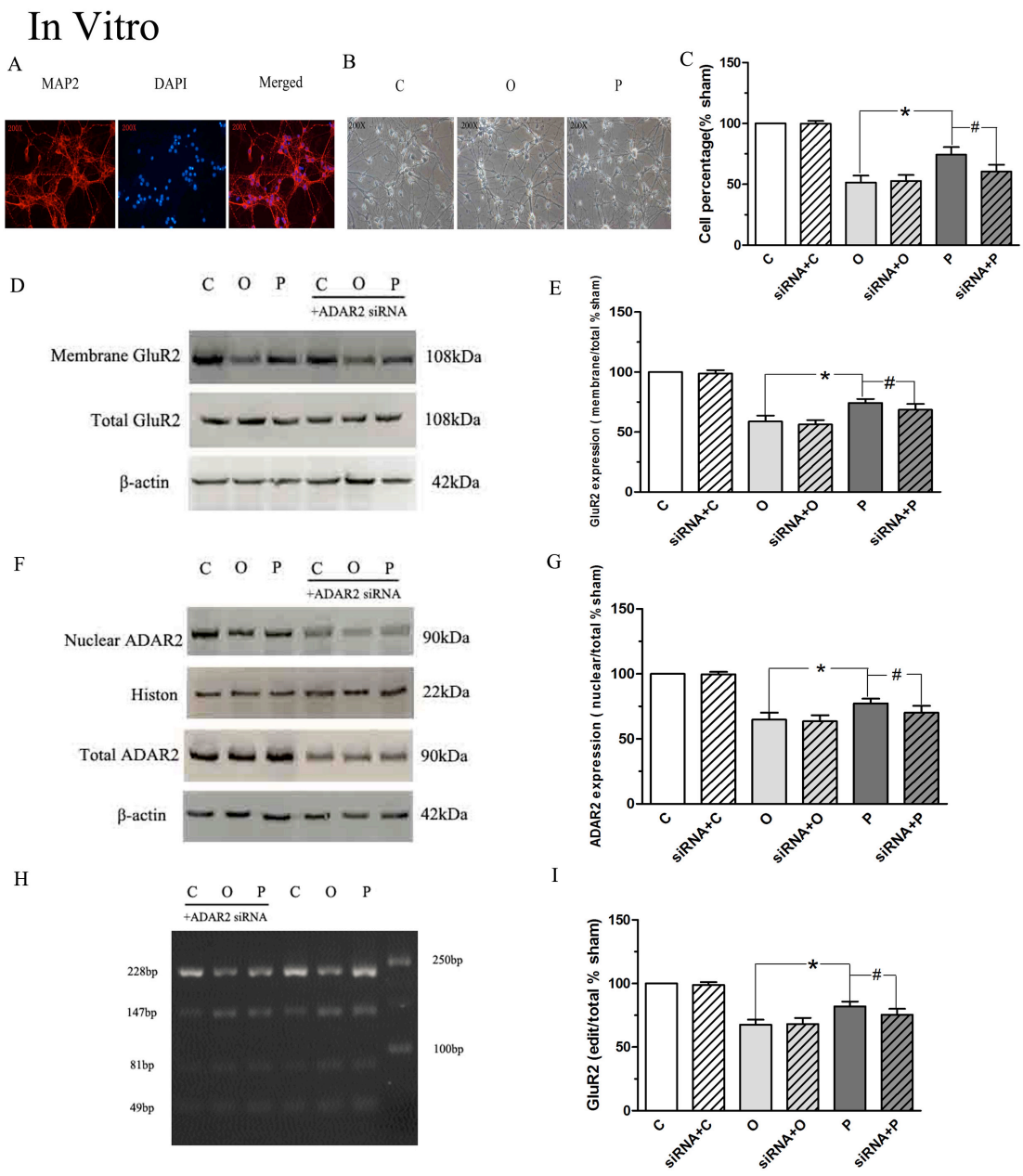

Fig.2 In Vitro

A.Neuron purity be up to above 90% B.Effect of propofol post-conditioning on cellular morphology change C.Effect of propofol post-conditioning on neuron viability shown by MTT D.Effect of propofol post-conditioning expression of GluR2 protein shown by Western Blot E.Quantification of membrane/total GluR2 expression F.Effect of propofol post-conditioning expression of ADAR2 protein shown by Western Blot G.Quantification of nuclear/total GluR2 expression H.Effect of propofol post-conditioning on GluR2 mRNA edited shown by Nested RT-PCR I.Quantification of GluR2 mRNA edited/total expression Bar represent mean±SE（n=10） *P<0.05;#P<0.01