Ischemic Postconditioning Facilitates Brain Recovery After Stroke by Promoting mTOR Activity

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Introduction

Ischemic postconditioning represents a series of brief occlusions of blood flow before complete restoration of reperfusion after stroke, but the underlying protective mechanisms of postconditioning are not fully understood. The mTOR pathway plays a key role in cell growth and survival. We studied the hypothesis that mTOR pathway is involved in the protective effect of ischemic postconditioning.

Methods

Focal ischemia was induced by 30 min of bilateral CCA occlusion and permanent distal MCA occlusion in rats. Ischemic postconditioning was induced by 3 cycles of 30 sec reperfusion and 10 sec occlusion at the end of stroke. Rapamycin, an mTOR inhibitor, was injected into the left lateral ventricle 1 hour before stroke onset. For the behavior test, home cage and vibrissa-elicited limb use tests were performed until 21d after stroke. Peri-infarct tissues were collected for Western blotting and immunostaining. Molecular markers including Gap-43, synaptophysin, MAP-2, PSD-95, phosphorylated mTOR (p-MTOR) and 4EPB-1 in the mTOR pathway were measured.

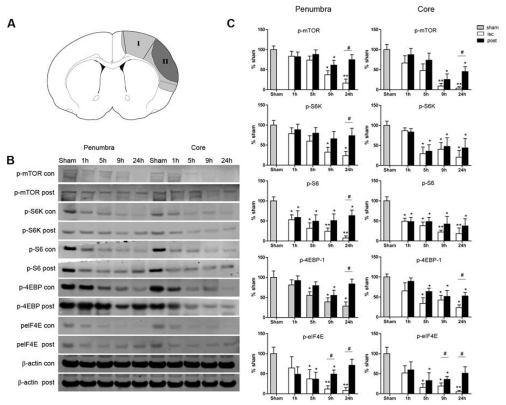
Results

Ischemic postconditioning improved neurological function when measured 2 weeks after stroke (n=6, $p<0.05$), and reduced brain injury size by 34.2% ($P<0.05$). These protective effects were abolished by rapamycin treatment. Western blotting showed that postconditioning substantially promoted the protein level of Gap-43, MAP-2 and PSD-95, but not synaptophysin. Rapamycin significantly inhibited Gap-43 levels at 1 and 3 weeks after stroke, and inhibited Map-2 level at 1 week ($P<0.05$). Postconditioning significantly increased the protein levels of p-Akt, p-mTOR, p-4EBP-1 compared with control ischemia ($p<0.05$) at 1 week after stroke injury. Rapamycin attenuated p-mTOR levels 1 and 3 weeks after stroke, and inhibited p-4EBP-1 level at 1 week ($p<0.05$), but had no effects on the expression level of p-Akt and Akt.

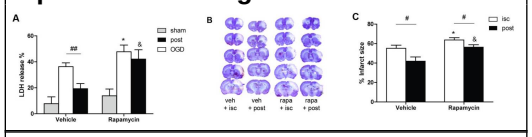
Conclusions

Ischemic postconditioning improved brain function by the enhanced mTOR activity, which is consistent with the improved expression of proteins related with synaptic function and brain plasticity.

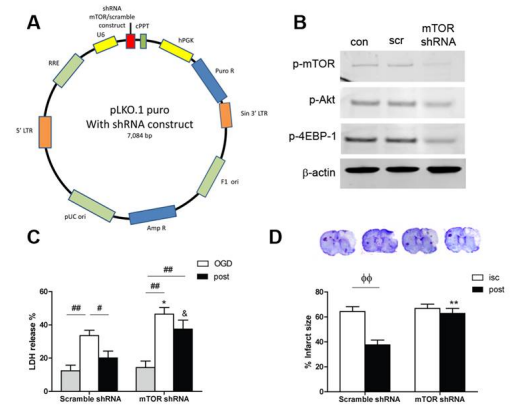
Ischemic Postconditioning promoted protein phosphorylation in the mTOR pathway



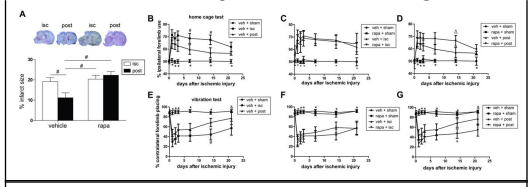
mTOR inhibition by rapamycin blocked the protective effects of Ischemic postconditioning in vitro and in vivo



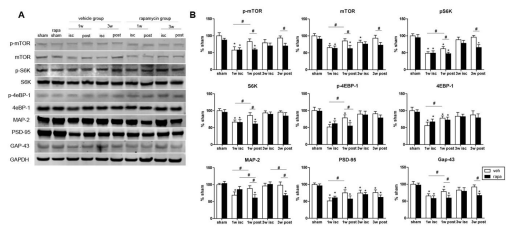
mTOR shRNA attenuated protective effects of postcon in vivo



Rapamycin administration chronically abolished the protective effects of ischemic postconditioning



Ischemic postconditioning promoted mTOR pathway in the chronic phase after stroke



mTOR-shRNA abolished the effect of posconditioning on mTOR pathway