Down-regulation of Cyclin-dependent Kinase 5 Attenuates p53-dependent Apoptosis of Hippocampal CA1 Pyramidal Neurons Following Transient Cerebral Ischemia

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Introduction

Transient cerebral ischemia (TCI) leads to the "delayed neuronal death" in selectively vulnerable brain areas including the CA1 area of the hippocampus. Kitagawa et al. firstly introduced ischemic preconditioning (IPC) as a strategy for attenuating ischemia-reperfusion injury in the brain using a gerbil model of TCI2, and further studies have widely demonstrated IPC in other animal models of transient focal cerebral ischemia. This phenomenon is called cerebral "ischemic tolerance", although basic mechanisms of underlying cerebral ischemic tolerance have not been fully understood yet.

Cyclin-dependent kinase 5 (Cdk5), a proline-directed serine/threonine cyclin-dependent kinase family member, plays crucial roles in neuronal differentiation, axonal outgrowth, synaptogenesis and memory formation. The activation of Cdk5 leads to the translocation of Cdk5 from membrane to cytoplasm or nucleus. Studies using rodents have also demonstrated that the aberrant activity of Cdk5 primarily induces neuronal cell death during stroke.

p53 (a tumor suppressor) displays important roles in regulating neuronal survival in the central nervous system. However, whether this process occurs in IPC-induced neurons suffering from a subsequent TCI is not clear; variously possible explanations of neuroprotective effects of IPC against a subsequent ischemic damage are proposed.

Experimental Purpose

To the best of our knowledge, expression patterns of Cdk5, p53 and apoptosis-related proteins in IPC-induced brain following a subsequent TCI has not been studied. Thus, this study, as a part of ongoing efforts to investigate effects of both the inhibition of Cdk5 and IPC on a subsequent ischemic insult, examined changes of Cdk5, p35/p25, phospho (*p*)-p53, Bax, Bcl-2, PUMA and caspase-3 in the hippocampus with roscovitine (a potent inhibitor of Cdk5) treatment and IPC following a subsequent TCI using gerbils, which are a good animal model of TCI.

Material and Methods

Experimental animals

- Adult male Mongolian gerbils
  (Meriones unguiculatus, 6 months)
  1) Sham TCI-operated group (n=14)
  2) TCI-operated group (n=14)
  3) Roscovitine-treated and Sham group (n=14)
  4) Roscovitine-treated and TCI-operated group (n=14)
  5) IPC-treated and sham TCI-operated group (n=14)
  6) IPC+TCI group (n=14)

Surgery of IPC and TCI

Western blot analysis

- Cdk5, p35, *p*-p53, Bax, Bcl-2, PUMA and caspase-3

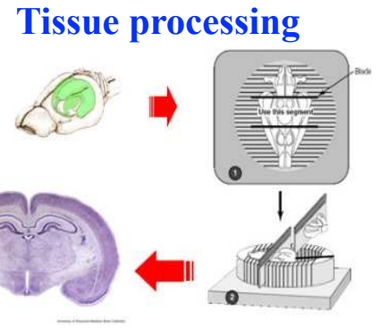
Treatment of Roscovitine

- Intravascular injection (40mg/kg)

Tissue staining

- Cresyl violet (CV)
- Fluoro-Jade B (F-J B
- TUNEL staining

Tissue processing



Immunohistochemistry

Mouse anti – NeuN,

Rabbit anti – Cdk5, p53

Results

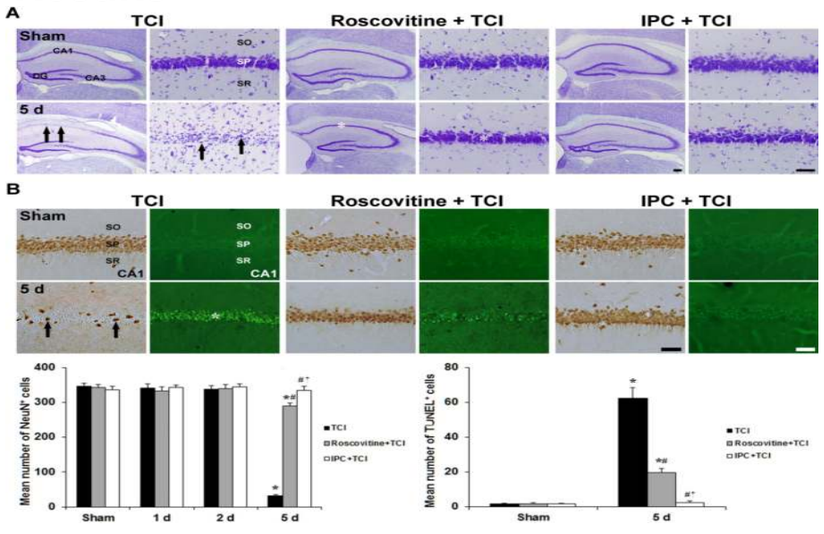


Fig. 1. Roscovitine- and IPC-mediated neuroprotection against TCI. (A) CV staining in the hippocampus of the TCI (1st, 2nd columns), roscovitine +TCI (3rd, 4th columns) and IPC+TCI (6th, 7th columns) groups. CV+ CA1 pyramidal neurons (arrows) are damaged 5 days after TCI; however, CV+ CA1 pyramidal neurons (asterisks) in the roscovitine+TCI and IPC+TCI groups are similar to those in the sham group. Scale bar = 800 μm (1st, 3rd, 5th columns), 50 μm (2nd, 4th, 6th columns). (B) NeuN immunohistochemistry (1st, 3rd columns) and F-J B histofluorescence staining (2nd, 4th columns) in the CA1 area of the TCI (1st, 2nd columns), roscovitine +TCI (3rd, 4th columns) and IPC+TCI (6th, 7th columns) groups. In the TCI group, a few NeuN+ (arrows) and many F-J B+ (asterisk) CA1 pyramidal neurons are detected 5 days after TCI. In the roscovitine +TCI and IPC+TCI groups, many NeuN+ pyramidal neurons are observed in the CA1 area; F-J B-positive cells are lower than those in the TCI group. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar = 50 μm. Quantitative graphs for numbers of NeuN+ (left) and F-J B+ (right) CA1 pyramidal neurons. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P<0.05 vs roscovitine+TCI group).

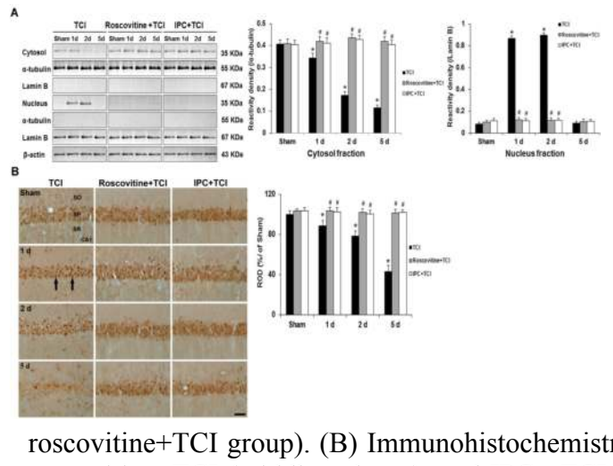


Fig. 2. Effects of roscovitine and IPC on Cdk5 expression and its translocation after TCI. (A) Western blots of Cdk5 levels in the CA1 area of the TCI, roscovitine+TCI and IPC+TCI groups at sham, 1, 2 and 5 days after TCI. α-Tubulin, lamin B and β-actin densitometric values are used to standardize for cytoplasmic and nuclear protein loading, respectively. Relative band intensity of cytoplasmic (■) and nuclear (□) Cdk5 levels is measured by densitometer. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P < 0.05 vs roscovitine+TCI group). (B) Immunohistochemistry of Cdk5 in the CA1 area of the TCI (left column), roscovitine+TCI (middle column), and IPC+ TCI (right column) groups at sham, 1, 2 and 5 days after TCI. Cdk5 immunoreactivity is translocated into nuclei (arrows) in CA1 pyramidal neurons of the TCI group 1 and 2 days after TCI and hardly detected 5 days after TCI. In the roscovitine+sham, IPC+sham, roscovitine+TCI and IPC+TCI groups, Cdk5 immunoreactivity in CA1 pyramidal neurons is similar to that in the sham group. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar = 50 μm. Quantitative graph of Cdk5 immunoreactivity in CA1 pyramidal neurons. A ratio of the ROD was calibrated as %, with the sham group designated as 100%. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P < 0.05 vs roscovitine+TCI group).

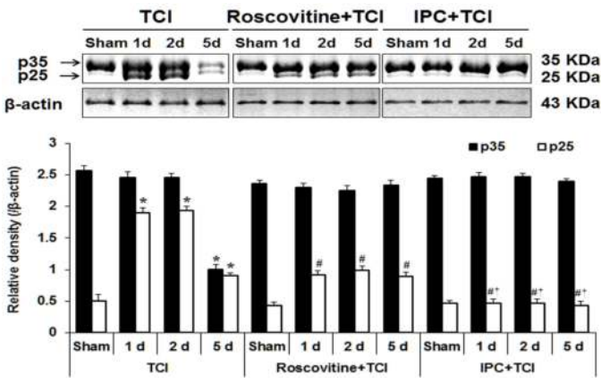


Fig. 3. Effects of roscovitine and IPC on levels of p35 and p25 proteins in the TCI, roscovitine+TCI and IPC+TCI groups at sham, 1, 2 and 5 days after TCI. β-actin is used as a protein loading control. Relative band intensity of p35 and p25 levels was measured by densitometer. Levels of p25 protein in the roscovitine+TCI and IPC+TCI groups are significantly low compared with those in the TCI group. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P < 0.05 vs roscovitine+TCI group).

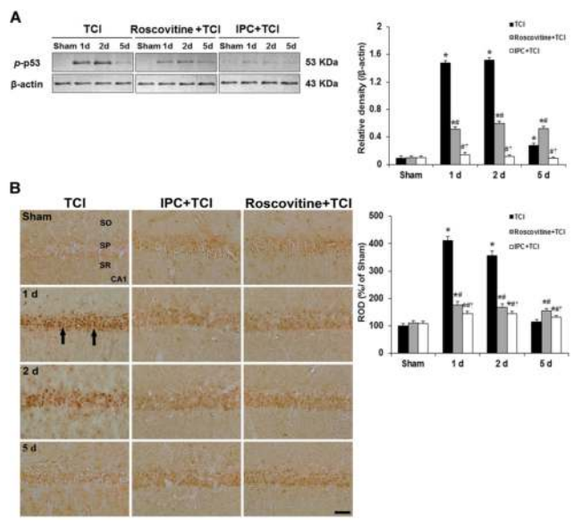


Fig. 4. Effects of roscovitine and IPC on p-p53 expression and its translocation after TCI. (A) Western blots of p-p53 in the CA1 area of the TCI, roscovitine+TCI and IPC+TCI groups at sham, 1, 2 and 5 days after TCI. β-actin is used as a protein loading control. Relative band intensity of p-p53 level is measured by densitometer. p-p53 levels are significantly low in the roscovitine+TCI and IPC+TCI groups compared with the TCI group. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P < 0.05 vs roscovitine+TCI group). (B) p-p53 immunoreactivity in the CA1 area of the TCI (left column), roscovitine+TCI (middle column) and IPC+ TCI (right column) groups at sham, 1, 2 and 5 days after TCI. p-p53 immunoreactivity in the TCI group is very strong in nuclei (arrows) of CA1 pyramidal neurons 1 and 2 days after TCI. In the roscovitine+TCI and IPC+TCI groups, p-p53 immunoreactivity in CA1 pyramidal neurons is moderated 1 and 2 days after TCI , and the immunoreactivity is shown in both nuclei and cytoplasm. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bar = 50 μm. Quantitative graph of p-p53 immunoreactivity in CA1 pyramidal neurons. A ratio of the ROD was calibrated as %, with the sham group designated as 100%. The bars are reported as means ± SEM from three independent experiments (n = 6, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P< 0.05 vs roscovitine+TCI group).

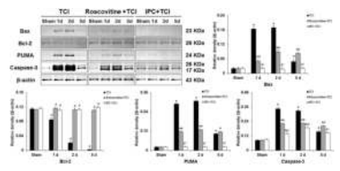


Fig. 5. Effects of roscovitine and IPC on Bax, Bcl-2, PUMA and active caspase-3 levels in the TCI, roscovitine+TCI and IPC+TCI groups at sham, 1, 2 and 5 days after TCI. β-actin is used as a protein loading control. Relative band intensity of Bax, Bcl-2, PUMA and active caspase-3 levels is measured by densitometer. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P < 0.05 vs roscovitine+TCI group).

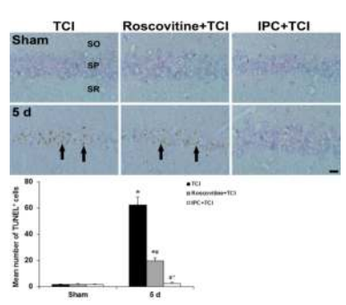


Fig. 6. Effect of roscovitine and IPC on apoptosis of CA1 pyramidal neuroins using TUNEL staining in the TCI (left column), roscovitine+TCI (middle column), and IPC+ TCI (right column) groups at sham and 5 days after TCI. Many TUNEL+ CA1 pyramidal neurons (arrows) are found in the stratum pyramidale (SP) of the TCI group 5 days after TCI. However, TUNEL+ cells are significantly low in the roscovitine+TCI and IPC+TCI groups compared with the TCI group, respectively. SO, stratum oriens; SR, stratum radiatum. Scale bar = 50 μm. The quantitative graph is shown the percentage of TUNEL+ cells in the SP. The bars are reported as means ± SEM from three independent experiments (n = 7, *P < 0.05 vs. sham group; #P<0.05 vs TCI group; †P< 0.05 vs roscovitine+TCI group).

Conclusion

In summary, our present findings showed that roscovitine treatment and IPC clearly protected CA1 pyramidal neurons from a subsequent severer TCI and that roscovitine- and IPC-mediated neuroprotection were closely related with the down-regulation of Cdk5 and p25. In addition, the down-regulation of Cdk5 by roscovitine treatment and IPC might be a key factor in attenuating p53-dependent apoptosis after TCI. Thus, we strongly suggest that the down-regulation of Cdk5 is critical in neuroprotection as well as IPC-mediated tolerance against various ischemic insults.

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