Hypothermia treatment ameliorated cyclin-dependent kinase 5-mediated inflammation in ischemic stroke and improved outcomes in ischemic stroke patients

Hongwei Sun1,2, Juan Cai1,2, Shiqi Shen1,2, Xiaohui Ren1,2,*

The First Affiliated Hospital of Harbin Medical University, Harbin 150001, Heilongjiang, China.

Sun H, Cai J, Shen S, Ren X. Hypothermia treatment ameliorated cyclin-dependent kinase 5-mediated inflammation in ischemic stroke and improved outcomes in ischemic stroke patients. Clinics. 2019;74:e938

*Corresponding author. E-mail: renxiaohui2004@163.com

OBJECTIVES: The inflammatory response is a key mechanism of neuronal damage and loss during acute ischemic stroke. Hypothermia has shown promise as a treatment for ischemic stroke. In this study, we investigated the molecular signaling pathways in ischemic stroke after hypothermia treatment.

METHODS: Cyclin-dependent kinase 5 (CDK5) was overexpressed or silenced in cultured cells. Nuclear transcription factor-κB (NF-κB) activity was assessed by measurement of the luciferase reporter gene. An ischemic stroke model was established in Sprague–Dawley (SD) rats using the suture-occluded method. Animals were assigned to three groups: sham operation control, ischemic stroke, and ischemic stroke + hypothermia treatment groups. Interleukin 1β (IL-1β) levels in the culture supernatant and blood samples were assessed by ELISA. Protein expression was measured by Western blotting.

RESULTS: In HEK293 cells and primary cortical neuronal cultures exposed to hypothermia, CDK5 overexpression was associated with increased IL-1β, caspase 1, and NF-κB levels. In both a murine model of stroke and in patients, increased IL-1β levels were observed after stroke, and hypothermia treatment was associated with lower IL-1β levels. Furthermore, hypothermia-treated patients showed significant improvement in neurophysiological functional outcome.

CONCLUSIONS: Overall, hypothermia offers clinical benefit, most likely through its effects on the inflammatory response.

KEYWORDS: Overall, hypothermia offers clinical benefit, most likely through its effects on the inflammatory response.

INTRODUCTION

Ischemic stroke is one of the leading causes of mortality worldwide and the number one cause of death in the northern area of China. Inflammatory responses resulting from ischemic stroke have been recognized as a key factor in the pathophysiology of ischemic stroke. Previous studies have shown that the serum interleukin 1β (IL-1β) level is elevated in ischemic stroke patients, indicating activation of the immune system, which is associated with infiltration of immune and inflammatory cells into the central nervous system, possibly mediating neuronal damage in the brain.

Hypothermia is a promising treatment for stroke. Studies of experimental ischemic stroke models have found that the benefits of hypothermia treatment could be the result of a range of biological processes that are modulated by temperature, including reduced oxidative stress, proteolysis, and excitotoxicity (1). More importantly, hypothermia treatment has long been established to reduce the infarction size and cell death due to necrosis and apoptosis (2). Recent studies indicate that therapeutic hypothermia regulates the expression of both pro-inflammatory and anti-inflammatory cytokines, implying a close association between hypothermia and inflammatory responses in the pathogenesis of ischemic stroke (3).

Interleukin 1β (IL-1β), a pro-inflammatory cytokine and a core molecule of inflammasomes, has been found to be associated with neuronal necrosis and apoptosis. Cyclin-dependent kinase 5 (CDK5), in turn, has been reported to mediate the activation of the neuronal inflammasome, accompanied by the expression of core inflammasome molecules, such as caspase 1 (4). Furthermore, hyperactivity of CDK5, caused by the conversion of the CDK5 activator p35 to p25, has been reported to mediate neuronal death in ischemic stroke (5). Therefore, during ischemic stroke, CDK5 may induce activation of the inflammasome, which then leads to neuronal damage. The activation of nuclear transcription factor-κB (NF-κB) has been noted in infarcted cerebral areas during the early stage of ischemic stroke (6). NF-κB is involved in neuronal inflammation after cerebral stroke, but the potential association between CDK5 and NF-κB remains poorly understood.
In this study, we investigated the molecular mechanisms of the inflammatory response in ischemic stroke, particularly the correlation between the levels of CDK5 and various inflammatory molecules, including IL-1β, NF-xB, and caspase 1. Moreover, we further explored the effectiveness of hypothermia as a treatment in a cohort of ischemic stroke patients.

## MATERIALS AND METHODS

The animal experiments were approved by the Animal Ethical Committee of the local hospital.

### Reagents

Neurobasal medium, B27 supplement, high-glucose Dulbecco’s Modified Eagle Medium (DMEM), and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). pcDNA3.0, pcDNA-CDK5, and GFP-p25 plasmids were obtained from Addgene (Cambridge, MA, USA). An NF-xB luciferase reporter plasmid was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Lipofectamine 2000 transfection reagent and Opti-MEMI medium were obtained from Invitrogen (Carlsbad, CA, USA). Etoposide and roscovitine were obtained from Sigma-Aldrich (St. Louis, MO, USA). A luciferase reporter gene assay kit was purchased from Roche (Basel, Switzerland). The 96-well plate used for the luciferase reporter gene test was purchased from Greiner (Ludwigshafen, Germany).

### Cell culture and transfection

For primary cortical neuronal culture, Sprague-Dawley (SD) rats (prenatal 16–18 days old) were euthanized, and their cortex tissues were collected in D-Hanks solution. The cortex tissues were then digested with trypsin, and the cells were resuspended in neurobasal medium containing B27 supplement and glutamine. The single-cell suspension was then transferred to a 6-well culture plate precoated with poly-D-lysine at a density of 2 x 10^6 cells/well in 2 mL culture medium. The cells were incubated at 37°C for 7 days for further experiments. HEK293 cells were cultured in high-glucose DMEM that contained 10% FBS in an incubator at 37°C with 5% carbon dioxide. Transfection was conducted when cells reached 85% confluency according to the manufacturer’s instructions for Lipofectamine 2000. Cells were transfected with the NF-xB luciferase reporter plasmid. Twenty-four hours later, cells were transfected with pcDNA-CDK5 and GFP-p25 plasmids. Control cells were subjected to NF-xB luciferase reporter plasmid transfection followed by transfection with a pcDNA3.0 plasmid. Forty-eight hours after transfection, cells were lysed, and substrates were added according to the manufacturer’s instructions (Roche). The absorption was recorded on a fluorescence microplate, and NF-xB activity was analyzed.

### Murine model of ischemic stroke

Male SD rats weighing 350-380 g were used to establish the rodent ischemic stroke model. The animal experiments were approved by the Animal Ethical Committee of the local hospital. The ischemic stroke model was established using the suture-occluded method developed by Longa (7). Three hours after the surgery, rat neurophysiology was evaluated by postural reflex, and unawake or dead animals were excluded from analysis. Rats without autonomous activity or defects in neuronal function were not included in the study. The postural reflex was rated on a three-point scale, and animals that scored ≥1 point were considered ischemic stroke animals.

Animals were assigned to three groups: sham operation control (n=12), ischemic stroke (n=12), and ischemic stroke + hypothermia treatment (n=12). In the ischemic stroke + hypothermia treatment group, rats were treated with a cooling blanket at 3 h after surgery.

### Enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected from animals in the ischemic stroke (n=6) and ischemic stroke + hypothermia treatment (n=6) groups at 6 h after operation. Control animals were fed for 2 days, and blood samples were collected from animals (n=6) under anesthesia. Serum samples were prepared immediately after blood collection. The IL-1β level in serum derived from the three experimental groups was assessed by ELISA.

### Western blotting

Primary cultured neurons were collected and lysed by radiolabeled precipitation assay (RIPA) lysis buffer. The intracerebral ischemic penumbra was harvested from rats in the ischemic stroke and ischemic stroke + hypothermia treatment groups at 6 h after surgery or from control rats. Tissue samples were minced and lysed, and an equal amount of protein was loaded and subjected to gel electrophoresis. Protein samples were then transferred to a polyvinylidene difluoride (PVDF) membrane, which was incubated with primary antibodies against caspase 1 (1:1000 dilution), CDK5 (1:1000), p-CDK5 (1:1000), or β-actin (1:5000) at 4°C overnight. After the membrane was washed with Tris-buffered saline plus Tween-20 (TBS-T) three times, it was further incubated with a secondary antibody (1:20,000) for another 1 h at room temperature. After the membrane was washed, the bands were visualized using the BeyoECL Plus kit according to the manufacturer’s instructions (Beyotime Institute of Biotechnology). The densities of the bands of interest were analyzed by ImageJ software.

### Cohort study of hypothermia treatment

The cohort study included a total of 24 patients with acute cerebral infarction who were treated in the Department of Neurology, the First Affiliated Hospital of Harbin Medical University, Heilongjiang, China, from November 1, 2015 to December 31, 2015. Patients were randomly divided into two groups, including the standard of care group (n=12) and the standard of care + hypothermia treatment group (n=12).
In addition, a total of 12 gender- and age-matched healthy controls who underwent physical examination at the First Affiliated Hospital of Harbin Medical University, Heilongjiang, China were recruited. Informed consent forms were collected from all patients. Ethical approval for this study was granted by the local hospital.

Patients who were treated with hypothermia received cooling treatment for 24 h using a focal mild hypothermia therapy device (Harbin, China) wrapped around the head that maintained the tympanic temperature at 33-35°C, which was measured with an OMRON thermometer (Omron Dalian Co., Ltd., China). After therapy, the body temperature was gradually restored to 36.5-37.5°C within 12-20 h, increasing by 1°C every 4-6 h.

Venous blood samples (2 mL) were collected from the patients on the second day after the stroke. Morning fasting venous blood samples (2 mL) were collected from control cases. The serum IL-1β level was assessed by ELISA.

Statistical analyses
All of the data were presented as the mean ± standard error of the mean (SEM). The statistical analyses were performed with two-tailed Student’s t-tests. Analyses were carried out by GraphPad Prism5 software. p-value of less than 0.05 was considered to be statistically significant, *p < 0.05.

RESULTS

Inflammatory molecular signaling pathway in neuronal cultures
We examined the correlation between CDK5 and NF-κB, a molecule known to mediate inflammatory responses. HEK 293 cells were transfected with CDK5 together with the CDK5 activator p25 plasmids (CDK5/p25) or pcDNA3.0 plasmid, a negative control, and NF-κB activity was assessed by measurement of the luciferase reporter gene.

The NF-κB level in cells transfected with CDK5/p25 was significantly higher than that in cells transfected with pcDNA3.0 (Figure 1A). We further examined the correlation between CDK5 and NF-κB in primary cortical neuron cultures. Cultured neurons were treated with etoposide (ETOP) to stimulate CDK5 activation or ETOP and siCDK5, and untreated neurons were used as a control. The activity of NF-κB, as assessed with the luciferase reporter gene assay, was negligible in control cells (Figure 1B). In contrast, ETOP treatment induced substantial NF-κB transcription, which was significantly reduced by cotreatment with siCDK5. Thus, CDK5 may activate NF-κB.

In primary cortical neuronal cultures, inflammatory stimulation was associated with an increase in the IL-1β level. Here, neuronal cultures were treated with lipopolysaccharide (LPS) and adenosine triphosphate (ATP) or LPS and alum, and the IL-1β level in the supernatant of the culture was assessed via ELISA. Cultures treated with either LPS with ATP or LPS with alum had a significantly higher IL-1β level in the supernatant than untreated control cultures (p < 0.01; Figure 2).

Figure 1 - CDK5 activated NF-κB in HEK293 cells and primary cultured cortical neurons. (A) HEK293 cells were transfected with pcDNA3.0 or CDK5 plus p25. (B) Cultured cortical neurons were treated with ETOP to stimulate CDK5 activation or ETOP and siCDK5, and untreated neurons were used as controls. NF-κB activity was measured using a luciferase reporter gene assay. *p < 0.05; **p < 0.01.

Figure 2 - IL-1β was produced in cortical neurons in response to inflammatory stimuli. Cultured primary cortical neurons were treated with LPS and ATP or LPS and alum, and the IL-1β level in the supernatant of the culture was assessed with ELISA. *p < 0.05; **p < 0.01.
Next, we examined the correlation between CDK5 and IL-1β. Cultured primary cortical neurons were treated with ETOP, ETOP plus roscovitine (ROS), a CDK5 inhibitor, and ETOP plus siCDK5. Untreated cells were used as the control group. ELISA revealed that the IL-1β level in the supernatant of the cell culture was significantly higher in the ETOP-treated cells than in the control group (p < 0.01; Figure 3A). Blocking CDK5 activity with either ROS or siCDK5 significantly reduced the IL-1β level (p < 0.05). There was no significant difference in the protein expression of pro-caspase 1 (p45) among the three groups as assessed via Western blotting (Figure 3B). The level of activated caspase 1 (p20) in these cells was consistent with the IL-1β level; the p20 subunit level was significantly higher in ETOP-treated cells than in the control group (p < 0.01), and treatment with either ROS or siCDK5 significantly reduced the IL-1β level (p < 0.05).

**IL-1β and CDK5 levels in a murine model of ischemic stroke**

We next investigated the correlation between hypothermia treatment and IL-1 and CDK5 levels in a rat model of ischemic stroke. Ischemic stroke was surgically induced in rats, and rats in the hypothermia treatment group were cooled immediately after the surgery. Both the serum IL-1β content and IL-1β level in the brain tissue assessed by ELISA were significantly higher in ischemic stroke rats than in rats in the sham surgery group (p < 0.01; Figure 4A and Figure 4B). Hypothermia treatment significantly reduced the increase in IL-1β (p < 0.05 compared to ischemic stroke).

In addition, the protein expression levels of CDK5 and p-CDK5 in the intracerebral ischemic penumbra area and normal brain tissues were analyzed. As shown in Figures 4C and 4D, there was no obvious change in the overall expression of CDK5 among the three experimental groups. However, the level of p-CDK5 was significantly upregulated in the intracerebral ischemic penumbra derived from animals with ischemic stroke compared with that in the penumbra from animals who underwent sham surgery (p < 0.05). Note that treatment with hypothermia greatly reversed the elevation in p-CDK5 in the intracerebral ischemic penumbra in ischemic rats (p < 0.05).

**Serum IL-1β level in a cohort of ischemic patients treated with hypothermia**

This cohort study included 24 patients who had experienced acute ischemic stroke and were randomized into groups treated with hypothermia with standard of care or standard of care alone and 24 healthy volunteers. There was no significant difference in the age between the two cohorts (Table 1; p > 0.05). Neurophysiological function was assessed using the National Institutes of Health Stroke Scale (NIHSS). At the start of treatment, hypothermia-treated patients scored 13.8 ± 7.8, and the standard of care patients scored 14.2 ± 7.2 (p > 0.05). After therapy, the decrease in the NIHSS score was 2.6 ± 1.3 in the standard of care group and 3.9 ± 1.6 in the hypothermia-treated patients (p < 0.05).

The serum IL-1β level was assessed in all patients on the second day after the stroke. The IL-1β level was significantly higher in patients with stroke than in healthy volunteers (p < 0.01; Figure 5). Furthermore, hypothermia-treated patients showed a significantly lower serum IL-1β than patients in the standard of care only group (p < 0.05).

**DISCUSSION**

Hypothermia as a treatment option for ischemic stroke has shown promise. This study examined the molecular mechanism of the inflammatory response and the possible effects of hypothermia on the signaling pathway. We found that in cultured neurons, activation of CDK5 was associated with increased levels of key inflammatory molecules, including IL-1, NF-κB, and caspase 1. These findings are consistent with available studies of inflammatory responses.

A high body temperature is associated with a larger infarct size and poor functional outcome in patients with acute...
ischemic stroke (8). The effectiveness of therapeutic hypothermia for the management of ischemic stroke has been well documented in several animal studies as well as preclinical trials (9,10). In clinical trials, the use of hypothermia after conventional therapy (e.g., recanalization) has been proven to prevent cerebral edema and improve therapeutic outcomes in patients with acute ischemic stroke (11,12). In rodent studies, therapeutic hypothermia efficiently reduced the infarct size in an ischemic stroke animal model when compared to normothermia (13,14). Although the underlying mechanism by which therapeutic hypothermia protects against cerebral ischemia has not yet been fully clarified, hypothermia is
were treated with hypothermia. The serum IL-1β level was assessed in all patients (healthy control, n=12; stroke, n=12; stroke + hypothermia treatment, n=12) on the second day after stroke. *p<0.05; **p<0.01.

Figure 5 - Serum IL-1β levels in a cohort of ischemic patients who were treated with hypothermia. The serum IL-1β level was assessed in all patients (healthy control, n=12; stroke, n=12; stroke + hypothermia treatment, n=12) on the second day after stroke. *p<0.05; **p<0.01.

suggested to regulate the inflammatory responses by reducing the expression of pro-inflammatory factors in mice with acute ischemic stroke (15).

The pro-inflammatory cytokine IL-1β plays a crucial role in the pathogenesis of ischemic stroke (16,17). Increased generation of IL-1β in patients with ischemic stroke may induce cell apoptosis, resulting in brain tissue injury in the intracerebral ischemic penumbra area and ultimately enlarging the area of cerebral infarction. In this study, hypothermia treatment efficiently decreased the IL-1β production induced by inflammation in ischemic rodents as well as in patients. Our findings are consistent with a previous study revealing reduced IL-1β expression in stroke mice that received hypothermia therapy (15).

CDK5 is a key molecule that mediates multiple signaling pathways that lead to neuronal loss, including the autophagy process (18,19), apoptosis (20,21), and neuronal death processes (22,23). In particular, CDK5 activity during oxidative stress has been found to contribute to the pathophysiology of various central nervous system disorders (24-26). Our study revealed an increase in CDK5 in animals after ischemic stroke. In addition, CDK5 signaling is closely associated with inflammasome activation; as in primary cultured cortical neurons, activation of the CDK5 pathway in our study promoted the maturation and secretion of IL-1β through activation of caspase 1. In addition, CDK5 activation resulted in increased NF-kB activity in cultured neurons as well as HEK293 cells. Compared to stroke animals, rats receiving hypothermia therapy had a lower p-CDK5 level, indicating that hypothermia suppressed the activation of the CDK5 signaling pathway, consequently decreasing IL-1β generation.

Consistent with previous clinical studies, our study of a small cohort of patients showed clinical benefits of hypothermia treatment. Hypothermia-treated patients showed a better outcome than patients who received only standard of care. This clinical benefit could result from the inhibitory effects of hypothermia on the inflammatory response. Both the brain and serum levels of IL-1β decreased with hypothermia treatment, suggesting that hypothermia at least partially blocks the inflammatory response signaling pathway. However, a recent study found that the combination of mild hypothermia and inhibition of CDK5 showed a trend toward better outcomes (13), indicating that the effects of hypothermia are not limited to suppression of inflammatory responses.

In summary, our present study demonstrates that therapeutic hypothermia exerts neuroprotection in ischemic stroke via a novel mechanism. Therapeutic hypothermia suppresses the abnormal activation of CDK5 in neurons following ischemic stroke, which results in a reduction in NF-kB activity and inhibition of caspase 1, ultimately decreasing the caspase 1-dependent production of IL-1β. In rats with ischemic stroke, therapeutic hypothermia decreases the secretion of the pro-inflammatory cytokine IL-1β, inhibits inflammatory responses, and prevents neuronal apoptosis, thereby providing favorable therapeutic outcomes. Our findings may provide clinical benefits to patients with acute ischemic stroke. Nevertheless, future studies will continue to explore the underlying mechanism of neuroprotection induced by therapeutic hypothermia.

**ACKNOWLEDGMENTS**

This study was supported by the Special Foundation for Innovative Talents of Harbin Science and Technology (Grant No. 2013RAQXJ097).

**AUTHOR CONTRIBUTIONS**

Sun H designed the study. Ren X analyzed the data. Shen S and Cai J established the rat animal model and carried out immunohistochemistry and electrophoresis studies.

**REFERENCES**

1. Linares G, Mayer SA. Hypothermia for the treatment of ischemic and hemorrhagic stroke. Crit Care Med. 2009;37(7 Suppl):S243-9. https://doi.org/10.1097/CCM.0b013e3181aa3d61
2. Maier CM, Ahern K, Cheng ML, Lee JE, Yenari MA, Steinberg GK. Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. Stroke. 1998;29(10):2171-80. https://doi.org/10.1161/01.STR.29.10.2171
3. Lee JH, Zhang J, Yu SP. Neuroprotective mechanisms and translational potential of therapeutic hypothermia in the treatment of ischemic stroke.
Hypothermia treatment for ischemic stroke

Sun H et al.

Neural Regen Res. 2017;12(2):341-50. https://doi.org/10.4103/1673-5374.202915

4. Zhang P, Shao XY, Qi GL, Chen Q, Bu LL, Chen LJ, et al. Cdk5-Dependent Activation of Neuronal Inflammamasomes in Parkinson’s Disease. Mov Disord. 2016;31(5):366-76. https://doi.org/10.1002/mds.26488

5. Meyer DA, Torres-Alturo MI, Tan Z, Tozzi A, Di Filippo M, DiNapoli V, et al. Ischemic stroke injury is mediated by aberrant Cdk5. J Neurosci. 2014;34(24):8259-67. https://doi.org/10.1523/JNEUROSCI.4368-13.2014

6. Zhang S, Zou O, Ly PT, Wu Y, Zhang S, Zhang M, et al. Down-regulation of MIF by NFκB under hypoxia accelerated neuronal loss during stroke. FASEB J. 2014;28(10):4394-407. https://doi.org/10.1096/fj.14-253625

7. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without cranectomy in rats. Stroke. 1989;20(1):84-91. https://doi.org/10.1161/01.STR.20.1.84

8. Geurts M, Scheijmans FE, van Seeters T, Biessels GJ, Kappelle LJ, Velthuis BK, et al. Temporal profile of body temperature in acute ischemic stroke: relation to infarct size and outcome. BMC Neurol. 2016;16(1):233. https://doi.org/10.1186/s12883-016-0760-7

9. van der Worp HB, Sena ES, Donnan GA, Howells DW, Macleod MR. Hypothermia in animal models of acute ischemic stroke: a systematic review and meta-analysis. Brain. 2007;130(Pt 12):3063-74. https://doi.org/10.1093/brain/awm083

10. Dumitrascu OM, Lamb J, Lyden PD. Still cooling after all these years: Meta-analysis of pre-clinical trials of therapeutic hypothermia for acute ischemic stroke. J Cereb Blood Flow Metab. 2016;36(7):1157-64. https://doi.org/10.1038/sj.emboj.7600441

11. Hong JM, Lee JS, Song HJ, Jeong HS, Choi HA, Lee K. Therapeutic hypothermia after recanalization in patients with acute ischemic stroke: outcome in rat. Iran J Basic Med Sci. 2014;17(7):476-82.

12. Hwang YH, Jeon JS, Kim YW, Kang DH, Kim YS, Liebeskind DS. Impact of immediate post-reperfusion cooling on outcome in patients with acute stroke and substantial ischemic changes. J Neurointerv Surg. 2017;9(1):21-5. https://doi.org/10.1136/neurintsurg-2015-012233

13. Ji YB, Zhuang PP, Ji Z, Huang KB, Gu Y, Wu YM, et al. TFP5 is comparable to mild hypothermia in improving neurological outcomes in early-stage ischemic stroke of adult rats. Neuroscience. 2017;343:337-45. https://doi.org/10.1016/j.neuroscience.2016.12.009

14. Allahavakoli M, Kahrnouei MH, Rezaazadeh H, Rohobakhsh A, Mahmoody MF, Moghadam-Ahmad A, et al. Delayed combination therapy of local brain hypothermia and decompressive craniectomy on acute stroke outcome in rat. Iran J Basic Med Sci. 2014;17(7):476-82.

15. Lee JH, Wei ZZ, Cao W, Won S, Gu X, Winter M, et al. Regulation of therapeutic hypothermia on inflammatory cytokines, microglia polarization, migration and functional recovery after ischemic stroke in mice. Neurobiol Dis. 2016;96:248-60. https://doi.org/10.1016/j.nbd.2016.09.013

16. Dinarello CA. A clinical perspective of IL-1β as the gatekeeper of inflammation. Eur J Immunol. 2011;41(5):1203-17. https://doi.org/10.1002/eji.201141550

17. Fann DY, Lee SY, Manzano S, Chunduri P, Sobey CG, Amrugham TV. Pathogenesis of acute stroke and the role of inflammamasomes. Ageing Res Rev. 2013;12(4):941-66. https://doi.org/10.1016/j.ager.2013.09.004

18. Ren Z, Shu Y, Gao C, Wang X, Qi G, Zhang P, et al. Cdk5-mediated phosphorylation and autophagy of RKIP regulate neuronal death in Parkinson’s disease. Neurobiol Aging. 2014;35(2):2870-80. https://doi.org/10.1016/j.neurobiolaging.2014.05.034

19. Dong Wei Sheng Zhi Ye Bing Za Zhi. 2012;30(2):85-8.

20. Zheng YL, Kesavapany S, Gravell M, Hamilton RS, Schubert M, Amin N, et al. Cdk5 inhibitory peptide reduces tau hyperphosphorylation and apoptosis in neurons. EMBO J. 2005;24(1):209-20. https://doi.org/10.1038/sj.emboj.7600441

21. Hisanaga S, Asada A. Cdk5-induced neuronal cell death: the activation of the conventional RB-E2F G1 pathway in post-mitotic neurons. Cell Cycle. 2012;11(11):2049. https://doi.org/10.4161/cc.20536

22. Fu AK, Hung KW, Choi HA, Lee K. Deregulated Cdk5 promotes oxidative stress and mitochondrial dysfunction. J Neurochem. 2008;107(1):265-78. https://doi.org/10.1111/j.1471-4159.2008.05616.x

23. Lee JH, Jeong MW, Kim W, Choi YH, Kim KT. Cooperative roles of c-Abl and Cdk5 in regulation of p53 in response to oxidative stress. J Biol Chem. 2008;283(28):19826-35. https://doi.org/10.1074/jbc.M702602200

24. Zambrano CA, Egana JT, Nunez MT, Maccioni RB, Gonzalez-Billault C. Ischemic stroke injury is mediated by aberrant Cdk5. J Neurosci. 2014;34(24):8259-67. https://doi.org/10.1523/JNEUROSCI.4368-13.2014

25. Lee JH, Wei ZZ, Cao W, Won S, Gu X, Winter M, et al. Regulation of therapeutic hypothermia on inflammatory cytokines, microglia polarization, migration and functional recovery after ischemic stroke in mice. Neurobiol Dis. 2016;96:248-60. https://doi.org/10.1016/j.nbd.2016.09.013

26. Sun KH, de Pablo Y, Vincent F, Shah K. Deregulated Cdk5 promotes oxidative stress and mitochondrial dysfunction. J Neurochem. 2008;107(1):265-78. https://doi.org/10.1111/j.1471-4159.2008.05616.x

27. Lee JH, Jeong MW, Kim W, Choi YH, Kim KT. Cooperative roles of c-Abl and Cdk5 in regulation of p53 in response to oxidative stress. J Biol Chem. 2008;283(28):19826-35. https://doi.org/10.1074/jbc.M702602200

28. Zambrano CA, Egana JT, Nunez MT, Maccioni RB, Gonzalez-Billault C. Oxidative stress promotes tau dephosphorylation in neuronal cells: the roles of cdck5 and PPI. Free Radic Biol Med. 2004;36(11):1393-402. https://doi.org/10.1016/j.freeradbiomed.2004.03.007