Brain Research 1685 (2018) 9–18

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Neuroprotective effects of Tongxinluo on focal cerebral ischemia and reperfusion injury in rats associated with the activation of the MEK1/2/ERK1/2/p90RSK signaling pathway

Zhonghai Yu [a,b], Min Cai [a,b,1], Xianting Li [a,b,1], Jingsi Zhang [a,b], Ting Wu [a,b], Feng Yang [a,b], Wen Zhu [a,b], Yijin Xiang [a,b], Wen Zhang [a,b], Jun Xiang [a,b,*], Dingfang Cai [a,b,*]

[a] Department of Integrative Medicine, Zhongshan Hospital, Fudan University, Shanghai, China
[b] Institute of Neurology, Academy of Integrative Medicine, Fudan University, Shanghai, China

ARTICLE INFO

Article history:
Received 28 October 2017
Received in revised form 29 January 2018
Accepted 30 January 2018
Available online 7 February 2018

Keywords:
Tongxinluo
MEK12ERK12p90RSK pathway
Bad protein
Cerebral ischemia and reperfusion injury
Neuroprotection

ABSTRACT

Ischemic stroke brings a huge family and social burden. Although the reperfusion of ischemic cerebral tissue is the most important way to rescue ischemic stroke, ischemia–reperfusion (I/R) injury further results in brain damage and even lead to death. Recent studies demonstrated that Tongxinluo (TXL) helps to protect the brain against focal cerebral I/R injury in rats by reducing neuronal apoptosis, and the MEK1/2/ERK1/2/90 ribosomal S6 kinase (p90RSK) pathway may be involved in this protective effect. Therefore, our present research was designed to identify the potential mechanisms involved. Adult male Sprague-Dawley rats (n = 108) were randomly divided into 4 groups: sham, cerebral ischemia and reperfusion (I/R), I/R plus TXL (TXL), and TXL plus U0126, a highly selective inhibitor of MEK 1 and MEK 2 (TXL + U0126). Brain edema was measured by T2-weighted magnetic resonance imaging (MRI). Pathological destruction of the blood brain barrier (BBB) ultrastructure was assessed by transmission electron microscopy, and proteins involved in the MEK1/2/ERK1/2/p90RSK pathway were detected by immunofluorescence and Western blotting. Our results indicated that TXL significantly improved neurological function, reduced brain edema, ameliorated the destruction of the BBB ultrastructure and markedly reduced neuronal injury. However, these benefits were diminished when the MEK1/2/ERK1/2/p90RSK pathway was inhibited by U0126. We also found that TXL upregulated the expression levels of p-MEK1/2, p-ERK1/2, p-p90RSK and p-bad, which were all significantly reversed by U0126. Collectively, our data demonstrate that TXL provides neuroprotection against cerebral I/R injury and neuronal injury, and that these effects are mediated, in part, by activation of the MEK1/2/ERK1/2/p90RSK pathway.

1. Introduction

Ischemic stroke, a common cerebrovascular disease, is not only the main cause of death around the world, but is also the largest cause of long-term disability, thus representing a huge social burden (Ahmad et al., 2014; Pendlebury and Rothwell, 2009). Reperfusion of ischemic cerebral tissue is the most important way to rescue patients exposed to cerebral ischemia; however, restoring blood flow to the ischemic brain can also lead to ischemia-reperfusion (I/R) injury, which induces additional serious injuries in the ischemic brain. Unfortunately, the pathogenesis of this type of injury is not yet completely understood. Therefore, there is a critical need for explorative research aimed at discovering effective drugs to ameliorate or prevent cerebral I/R injury.

As an important approach for human health, traditional Chinese medicine (TCM) has a long history and is widely used across China. A large number of traditional Chinese medicines, such as Xiao-Xu-Ming Decoction (Lan et al., 2013a,b; Lan et al., 2014), Bu Yang Huan Wu decoction (Zhao et al., 2012), Cerebralcare Granule (Huang et al., 2012) and NaoShuan Tong (Xiang et al., 2010), have shown beneficial responses in experimental animal models and could ameliorate I/R injury. Furthermore, Tongxinluo (TXL) has been widely prescribed across China to remedy cardio-cerebrovascular diseases, including atherosclerosis, angina pectoris and ischemic stroke (Chen et al., 2009; Cui et al., 2014; You et al., 2005; Zhang et al., 2009).

* Corresponding authors at: 180 Fenglin Road, Shanghai, China.
E-mail addresses: xiang.jun@mail.zs-hospital.sh.cn (J. Xiang), dingfangcai@126.com (D. Cai).
[1] Co-first author.

https://doi.org/10.1016/j.brainres.2018.01.036
0006-8993/

Our previous studies confirmed that TXL helps to protect the brain against focal cerebral ischemia and reperfusion injury in rats by activating the PI3k/Akt signaling pathway, reducing neuronal apoptosis (Yu et al., 2016), alleviating brain edema and inhibiting post-ischemic inflammation (Cai et al., 2016). However, when we investigated the importance of the PI3K/Akt signaling pathway in exerting the neuroprotective effect of TXL, we found that the MEK1/2/ERK1/2/p90RSK pathway might also be involved. Therefore, the present study aimed to investigate whether TXL modulated the activation of the MEK1/2/ERK1/2/p90RSK pathway and its downstream targets, 90 kDa ribosomal S6 kinase (p90RSK) and bad against focal cerebral I/R injury in rats.

2. Results

2.1. TXL improved neurological function

It was possible to evaluate the effects of TXL on the neurological function of our experimental rats 24 h after reperfusion by analyzing neurological deficit scores. Rats in the I/R group and the TXL + U0126 group showed prominent neurological deficits which were markedly diminished in response to TXL treatment, while rats in the sham group performed normally after operation. These group differences were statistically significant ($^{*}P < 0.05$; $^{\#}P < 0.05$; $^{\star}P < 0.05$) (Fig. 1).

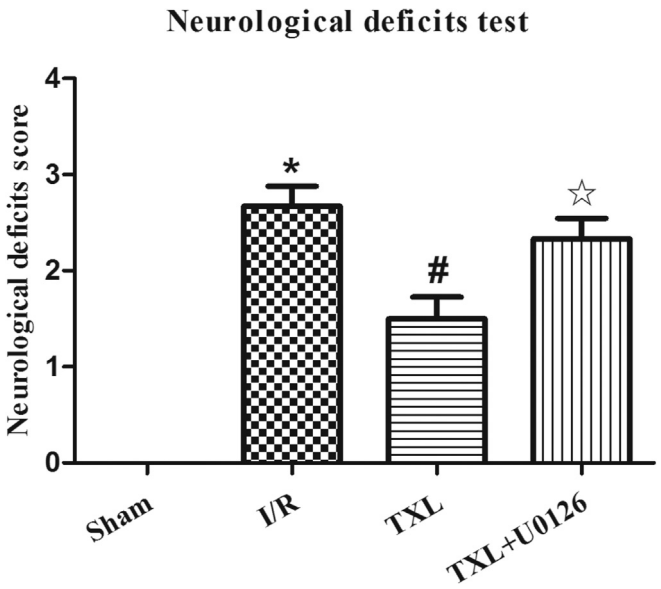

Fig. 1. Effects of TXL on neurological deficit scores. TXL treatment markedly improved the neurological deficits score, while U0126 reversed the benefits of TXL treatment. Data are reported as means ± standard error of the mean (SEM). $n = 6$; $^{*}P < 0.05$ *versus* Sham group; $^{\#}P < 0.05$ *versus* I/R group. $^{\star}P < 0.05$ *versus* TXL group.

2.2. TXL reduced brain edema

Nowadays, MRI is one of the most non-invasive and promising approaches for examining brain edema formation, and T2-weighted MRI is always used to evaluate the edema induced by cerebral ischemia and reperfusion. Consequently, this technique was used to investigate cerebral edema under each experimental condition. As expected, no edema was detected in the Sham group. However, the extent of brain edema area was diminished in the TXL group in comparison with the I/R group. Furthermore, a larger area of brain edema was observed in the TXL + U0126 group compared with the TXL group. These differences were statistically significant (Edema volume Ratio: $^{*}P < 0.05$; $^{\#}P < 0.05$; $^{\star}P < 0.05$) (Hemisphere intensity: $^{*}P < 0.05$; $^{\#}P < 0.05$; $^{\star}P < 0.05$) (Fig. 2).

2.3. TXL ameliorated destruction in the BBB ultrastructure after ischemia-reperfusion

We evaluated ultrastructural destruction of the tight junction (TJ) to investigate whether TXL treatment ameliorated damage caused to the BBB. The cerebral cortical microvessels of the sham group and TXL group were normal, with a continuous basal lamina, regular microvascular endothelial cells, and a dense and firm TJ. However, a loose connection between the two endothelial cells layers with an impaired TJ were observed in the I/R group and the TXL + U0126 group, and we also found that the basal laminas of rats in these two groups were swollen and deformed in the penumbra region (Fig. 3).

2.4. TXL blocked the neuronal injury induced by ischemia and reperfusion

Neuronal injury in the ischemic penumbra of the cortex was evaluated by the double staining of DAPI and immunofluorescence labeling with NeuN at 24 h after reperfusion. As depicted in Fig. 4 (A) and (B), the number of surviving cells in the I/R group was lower than that of the Sham group, and TXL significantly improved the number of surviving cells. In addition, double staining showed that neuronal injury was strikingly induced in the I/R group, whereas the number of cells double-stained with DAPI and NeuN were significantly increased in the TXL group. However, a marked increase in the number of surviving neurons was observed in the TXL + U0126 group. These differences between them were statistically significant ($^{*}P < 0.05$; $^{\#}P < 0.05$; $^{\star}P < 0.05$) (Fig. 4).

2.5. Effects of TXL on the expression of p-ERK1/2 (Thr202/Tyr204) and p-p90RSK (Ser380)

To identify whether p-ERK1/2 (Thr202/Tyr204) and p-p90RSK (Ser380) signals participate in modulating the neuroprotective effects of TXL on focal cerebral ischemia and reperfusion injury in rats, we respectively evaluated p-ERK1/2 (Thr202/Tyr204) and p-p90RSK (Ser380) positive cells in the ischemic cortical penumbra using fluorescence microscopy. As depicted in Fig. 5, data indicated that the expression levels of p-ERK1/2 (Thr202/Tyr204) and p-p90RSK (Ser380) positive cells in the I/R group were significantly reduced at 24 h after reperfusion ($^{*}P < 0.05$). However, TXL treatment was able to markedly increase the levels of these phosphorylated proteins in the TXL group ($^{\#}P < 0.05$), which were all significantly blocked by U0126 in the TXL + U0126 group ($^{\star}P < 0.05$).

2.6. TXL improved the expression of proteins in the MEK1/2/ERK1/2/p90RSK pathway

To elucidate whether the MEK1/2/ERK1/2/p90RSK pathway participated in the neuroprotective effect of TXL, the expression level of proteins related to the pathway in the ischemic cortical penumbra were carefully detected by Western blots. As depicted in Fig. 6, data indicated that the expression levels of p-MEK1/2(Ser221), p-ERK1/2(Thr202/Tyr204), p-p90RSK (Ser380) and bad (Ser112) in the I/R group were significantly reduced at 24 h after reperfusion ($^{*}P < 0.05$). However, TXL treatment was able to markedly increase the levels of these phosphorylated proteins in the TXL group ($^{\#}P < 0.05$), which were all prominently suppressed by pretreatment with U0126 in the TXL + U0126 group ($^{\star}P < 0.05$). In addition, there were no changes in the expression levels of total proteins, such as MEK1/2, ERK1/2, p90RSK and Bad.