Journal of Ethnopharmacology 148 (2013) 632–639

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

The protective role of Tongxinluo on blood–brain barrier after ischemia–reperfusion brain injury ☆

Ye Liu [a], Guang Hui Tang [b], Yu Hao Sun [c], Xiao Jie Lin [b], Cong Wei [d], Guo-Yuan Yang [a,b,*], Jian Rong Liu [a,*]

[a] Department of Neurology, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai 200025, China
[b] Neuroscience and Neuroengineering Research Center, Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 200030, China
[c] Department of Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[d] Yiling Pharmaceutical Institute, HeBei, China

ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form
3 May 2013
Accepted 14 May 2013
Available online 23 May 2013

Keywords:
Brain edema
Inflammation
Ischemia
MCAO
Tongxinluo

ABSTRACT

Ethnopharmacological relevance: Tongxinluo (TXL), a renowned traditional Chinese medicine, consists of several different kinds of ingredients and has been widely used to treat myocardial infarction and ischemic stroke. However, the underlying neuroprotective mechanisms are not fully understood.

Aim of the study: We focus on the effect of TXL on blood–brain barrier (BBB) including edema formation and tight junction (TJ) protein rearrangement, and inflammatory response after transient middle cerebral artery occlusion (tMCAO). We further explore the protective mechanism of TXL on ischemia-induced BBB damage.

Materials and methods: Adult CD1 male mice ($n=168$) were randomly divided into TXL pre-treatment group, TXL pre-post treatment group, TXL post-treatment group, control group and sham group. Mice in TXL pre-treatment group were given TXL solution by 1 g/kg/day orally for 7 days before tMCAO. Mice in pre-post treatment group were continuously given TXL 7 days before and 14 days after tMCAO. Mice in TXL post-treatment group were given TXL solution immediately after tMCAO. Rotarod test and neurological severity scores were evaluated at 1–14 days following tMCAO. Brains were harvested for examining infarct volume, edema formation, and immunofluorescent staining at 1 and 3 days after tMCAO. Cytokines IL-6, IL-1β and TNF-α mRNA expression, and BBB permeability were further examined by RT-PCR and immunostaining.

Results: TXL pre-post treatment improved neurobehavioral outcomes and reduced infarct volume compared to the control ($p<0.05$). Meanwhile, hemispheric swelling, Evans blue and IgG protein extravasation reduced, while TJ protein expression up-regulated in pre-post treatment group ($p<0.05$). Further study indicated that infarct volume was smaller and BBB damage was less severe in TXL pre-post treatment group compared to TXL pre-treatment alone. It was noted that fewer myeloperoxidase (MPO) positive cells and less cytokines IL-6, IL-1β and TNF-α expression in pre-post treatment group compared to the control group ($p<0.05$).

Conclusions: TXL pre-treatment and pre-post treatment effectively protected the brain from BBB disruption via alleviating inflammatory response. Moreover, pre-post treatment has better outcomes, suggesting that continuous administration of TXL before and throughout ischemia period is necessary because of multiple functions of TXL.

1. Introduction

Two major relevant mechanisms exacerbating the processes of ischemic stroke are extensive blood–brain barrier (BBB) disruption and brain edema formation. Once ischemia occurs, discontinued focal oxygen and glucose trigger cell death cascade, consequently resulting in BBB breakdown and brain edema (Taniguchi et al., 2000). Increased BBB permeability induces vasogenic edema, causing intravascular fluid or other toxic properties influx into brain parenchyma. The detrimental edema further reduces focal blood flow, and induces cell necrosis and apoptosis (Kimelberg,

Abbreviations: BBB, blood–brain barrier; CBF, cerebral blood flow; EB, Evans blue; MCA, middle cerebral artery; MPO, myeloperoxidase; tMCAO, transient middle cerebral artery occlusion; TXL, tongxinluo; TJ, tight junction; ZO-1, Zonula occludens-1.

* Corresponding authors at: Department of Neurology, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China; 197 Rui Jin Er Road, Shanghai 200025, China. Tel.: +86 21 64370045 665201; fax: +86 21 64333548.
E-mail address: liujr300@medmail.com.cn (J.R. Liu).

0378-8741/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2013.05.018

1995; Spatz, 2010). Widespread interactions among BBB disruption, edema formation, and lower blood flow become a vicious cycle, which accelerates brain damage.

Numbers of traditional Chinese medicine, such as Nao Shuan Tong (Xiang et al., 2010), Di Huang Yin Zi (Hu et al., 2009), and Bu Yang Huan Wu decoction (Zhao et al., 2012) had beneficial responses in experimental ischemia models and could improve neurological outcomes. Tongxinluo (TXL) in powder form is a compound prescription formulated according to the meridian theory of traditional Chinese medicine. TXL is approved by the State Food and Drug Administration of China for the treatment of diabetes (Wang et al., 2012), coronary heart disease (Li et al., 2010), hyperlipidemia and atherosclerosis (Chen et al., 2009) (state medical license NO. Z20060322).

These studies confirmed multiple functions such as platelet aggregation inhibition and vascular protective effects when TXL was administrated. Hence, in present study, using a mouse model of ischemia–reperfusion injury, we test whether TXL attenuates the ischemia-induced BBB disruption and improves neurological behavioral outcomes. Furthermore, we explore the mechanism underlying the function of TXL during ischemia injury.

2. Materials and methods

2.1. Drug and preparation

TXL powder was provided by Shijiazhuang Yiling Pharmaceutical Incorporated Company (Shijiazhuang, China). It was obtained in the form of a dried superfine powder (≤10 μm) from a mixture of 12 components (Table 1) by a micronizer, and the powder was prepared as capsule, which was authenticated and standardized on the basis of marker compounds in the Chinese Pharmacopoeia (Committee, 2005). The ingredients of TXL capsule were carefully analyzed and quality controlled as previously described (Chen et al., 2009). The powder was finally dissolved in 0.9% NaCl (0.08 g/ml) and stored at 4 °C until being used.

2.2. Suture transient middle cerebral artery occlusion (tMCAO) in mice

Focal cerebral ischemia was induced by tMCAO (Yang et al., 1994). Briefly, mice were anesthetized with ketamine/xylazine (100 mg/10 mg/kg, Sigma, San Louis, MO). Body temperature was maintained at 37 ± 0.3 °C using a heating pad (RWD Life Science, Shenzhen, China). After isolation of left common carotid artery (CCA), external and internal carotid artery (ECA, ICA), a silicone-coated 6-0 suture (Coviden, Mansfield, MA) was gently inserted from the ECA stump to the ICA, and stopped at the opening of the middle cerebral artery (MCA). The distance from the bifurcation of ICA/ECA to MCA was 10 ± 0.5 mm. To confirm successful obstruction and reperfusion of the artery, relative cerebral blood flow (CBF) in the left MCA core territory was monitored through an optic fiber glued to the skull (3.5 mm lateral to the sagittal suture and 1 mm posterior to the coronal suture) and connected to a laser Doppler flowmeter (Moor Instruments, Devon, UK).

2.3. Animals and drug administration

Animal experiment protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Shanghai Jiao Tong University, Shanghai, China. Adult male CD1 mice (n=168) weighing 25–30 g were randomly divided into five groups: TXL pre-treatment group: TXL administration for 7 days before tMCAO (1 g/kg/day). TXL pre-post treatment group: TXL administration 7 days before and continuing to 14 days after tMCAO (1 g/kg/day). TXL post-treatment group: TXL administration immediately after tMCAO (1 g/kg). Control group: normal saline (NS) administration before and after tMCAO. Sham group: subjected to the same surgery without tMCAO or solution administration. Animals except those in sham group were fed with TXL or NS daily through a gastric needle. The dose of TXL was chosen by referring to previous studies (Chen et al., 2009). The whole experiment design was illustrated in Fig. 1.

2.4. Behavioral assessment

All animals underwent behavioral test after surgery and were scored by experimenters who were blind to both neurological and treatment conditions. Rotarod test was used to evaluate fore and hind limb motor coordination and balance by measuring the duration of animals remaining on the accelerating rotating rod. The velocity was slowly increased from 4 to 40 g within 4 min. Modified neurological severity score (Li et al., 2000) was calculated as the sum of scores on motor, sensory, balance, and reflexes, including raising the mouse by the tail (0–3), walking on the floor (0–3), beam balance tests (0–6), and relaxes absence (0–2). The neurological function was graded on a scale of 0 to14 (normal score 0, maximal deficit score 14), and the higher score implied greater neurological injury. The tests were performed at 1, 3, 7 and 14 days post-injury.

Table 1
12 Ingredients of Tongxinluo.

Ingredients (Latin name)	Family	Voucher specimen number	Part used	Processing	Amount used (%)
Plants					
Panax ginseng C.A.Mey.	*Araliaceae*	11,001	Root and rhizome	Extraction	1.677
Ziziphus jujuba Mill. Var. spinosa (Bunge) Hu H.F.Chou	*Rhamnaceae*	11,002	Seed	Extraction	1.173
Paeonia lactiflora Pall.	*Ranunculaceae*	11,003	Root	Extraction	1.558
Santalum album L.	*Santalaceae*	11,004	Heartwood of stem	Extraction	0.354
Dalbergia odorifera T.Chen	*Leguminosae*	11,005	Heartwood of stem and root	Extraction	4.000
Boswellia carteri Birdw	*Burseraceae*	11,006	Resin	Farina	5.927
Borneolum syntheticum	*Dipterocarpaceae*	11,007	Resin	Artificial	3.626
Insects					
Scolopendra subspinipes mutilans L. *Koch*	*Psittacidae*	12,001	Dried body	Farina	3.623
Buthus martensii Karsch	*Buthidae*	12,002	Dried body	Farina	18.111
Steleophage plancyi (Boleny)	*Corydiidae*	12,003	Female dried body	Micro-oryzae farina	18.111
Hirudo nipponica Whitman	*Hirudinidae*	12,004	Dried body	Farina	27.330
Cryptotympana pustulata Fabricius	*Cicadidae*	12,005	Skin	Farina	18.111