Journal of Ethnopharmacology 222 (2018) 249–260

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Traditional Mongolian medicine Eerdun Wurile improves stroke recovery through regulation of gene expression in rat brain

Saren Gaowa[a,b,c], Narisi Bao[a,b], Man Da[c], Qiburi Qiburi[d], Tsogzolmaa Ganbold[d], Lu Chen[d], Altanzul Altangerel[d], Temuqile Temuqile[b,c,*], Huricha Baigude[d,**]

[a] School of Basic Medical Science, Beijing University of Chinese Medicine, Chaoyang District, Beijing 100029, PR China
[b] Inner Mongolia Medical University, Hohhot, Inner Mongolia 010020, PR China
[c] International Hospital of Mongolian Medicine, Hohhot, Inner Mongolia 010021, PR China
[d] Institute of Mongolian Medicinal Chemistry, School of Chemistry & Chemical Engineering, Inner Mongolia University, Hohhot, Inner Mongolia 010020, PR China

ARTICLE INFO

Chemical compounds studied in this article:
povidone-iodine (PubChem CID: 410087)
chloral hydrate (PubChem CID: 2707)
geniposide (PubChem CID: 107848)
(+)-(7 S,8 R,8′R)-lyoniresinol 9-O-β-D-(6″-O-trans-sinapoyl) glucopyranoside (PubChem CID: 10395492)
3,5-di-O-caffeoylquinic acid (PubChem CID: 6474310)
kaempferol-3-O-rutinoside (PubChem CID: 24211973)
myristicin (PubChem CID: 4276)
costunolide (PubChem CID: 6436243)
isoliquiritigenin (PubChem CID: 638278)
toosendanin (PubChem CID: 115060)
4-dihydro-2-(4' - hydroxyphenylmethyl) − 6 - [(3″,4″ -dihydroxy-5″- methoxyphenyl) methylene]-pyran-3, 5-dione
2,3- dihydro-2-(4′- hydroxy-phenylethyl)-6-[(3″,4″ -dihydroxy-5″-methoxy) phenyl]-5-pyrone

Keywords:
Ischemic stroke
Mongolian medicine
Eerdun Wurile
RNA-seq
Igf2
Microglia

ABSTRACT

Ethnopharmacological relevance: Eerdun Wurile (EW) is one of the key Mongolian medicines for treatment of neurological and cardiological disorders. EW is ranked most regularly used Mongolian medicine in clinic. Components of EW which mainly originate from natural products are well defined and are unique to Mongolian medicine.

Aim of the study: Although the recipe of EW contains known neuroactive chemicals originated from plants, its mechanism of action has never been elucidated at molecular level. The objective of the present study is to explore the mechanism of neuroregenerative activity of EW by focusing on the regulation of gene expression in the brain of rat model of stroke.

Materials and methods: Rat middle cerebral artery occlusion (MCAO) models were treated with EW for 15 days. Then, total RNAs from the cerebral cortex of rat MCAO models treated with either EW or control (saline) were extracted and analyzed by transcriptome sequencing. Differentially expressed genes were analyzed for their functions during the recovery of ischemic stroke. The expression level of significantly differentially expressed genes such as growth factors, microglia markers and secretive enzymes in the lesion was further validated by RT-qPCR and immunohistochemistry.

Results: Previously identified neuroactive compounds, such as geniposide (Yu et al., 2009), myristicin (Shin et al., 1988), costunolide (Okugawa et al., 1996), toosendanin (Shi and Chen, 1999) were detected in EW formulation. Bederson scale indicated that the treatment of rat MCAO models with EW showed significantly lowered neurological deficits ($p < 0.01$). The regional cerebral blood circulation was also remarkably higher in rat MCAO models treated with EW compared to the control group. A total of 186 genes were upregulated in the lesion of rat MCAO models treated with EW compared to control group. Among them, growth factors such as Igf1 ($p < 0.05$), Igf2 ($p < 0.01$), Grn ($p < 0.01$) were significantly upregulated in brain after treatment of rat MCAO models with EW. Meanwhile, greatly enhanced expression of microglia markers, as well as complementary components and secretive proteases were also detected.

Conclusion: Our data collectively indicated that EW enhances expression of growth factors including Igf1 and Igf2 in neurons and microglia, and may stimulate microglia polarization in the brain. The consequences of such activity include stimulation of neuron growth, hydrolysis and clearance of cell debris at the lesion, as well as the angiogenesis.

Abbreviations: tPA, tissue plasminogen; MCAO/R, middle cerebral artery occlusion/reperfusion; EW, Eerdun Wurile; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; DEGs, differentially expressed genes; Igf2, insulin-like growth factor 2; IGFBP2, insulin-like growth factor binding protein 2; Tgf-b1, transforming growth factor beta 1; Vim, vimentin; Grn, granulin; ApoD, apolipoprotein D; Aif1, allograft inflammatory factor 1; Csf1r, colony stimulating factor 1 receptor; CX3CR1, C-X3-C motif chemokine receptor 1; C3, complementary component 3; C1qa, complementary component 1, q subcomponent, A chain

* Corresponding author at: International Mongolian Hospital, Hohhot 010021, PR China.
** Corresponding author.
E-mail address: hbaigude@imu.edu.cn (H. Baigude).

https://doi.org/10.1016/j.jep.2018.05.011
Received 29 November 2017; Received in revised form 28 April 2018; Accepted 10 May 2018

1. Introduction

Stroke happens when blood supply to brain is insufficient, or due to bleeding in the brain, leading to the damage of brain tissue and subsequent dysfunction in central nervous system. Ischemic stroke, which is a predominant type of stroke, may happen due to blockage of blood vessel caused by thrombosis, embolism, or systemic hypoperfusion (Donnan et al., 2008; Shuaib and Hachinski, 1991). Cryptogenic stroke, which occurs without obvious reasons listed above, constitutes about 35% of all ischemic stroke (Guercini et al., 2008). Ranked after cardiovascular disease and before cancer, stroke is the second major cause of death worldwide (Donnan et al., 2008). It is a tremendous threat to the health of world population. Although seriousness of stroke hit varies depending on the size and location of the damaged brain area, considerably high disability rate has been reported that can lead to numbness, incontinence, speech and vision loss. The main principle for stroke therapy is quick restoration of blood supply by removing the blockage (Saver, 2006). Early thrombolysis using tissue plasminogen (tPA) can remarkable decrease the rate of disability (Emberson et al., 2014). Stroke rehabilitation is crucial to the reduction of brain injury as well as promotion of recovery. Constraint-induced movement therapy (Siebers et al., 2010), brain repair by electrical stimulation (Brown, 2006), and Bobath (Brock et al., 2011) are among the popular stroke rehabilitations.

Natural products such as isoliquiritigenin (root of *Glycyrrhiza glabra*) has protective effects in rat middle cerebral artery occlusion (MCAO)-induced ischemic stroke model (Zhan and Yang, 2006). Diphenylheptanes (fruits of *Amomum tsaoko*) protects H_2O_2-induced nerve injury (Zhang et al., 2016). Some traditional Chinese folk medicines have significant therapeutic efficacy for post-stroke recovery (Young et al., 2010).

Eerdun Wurile (EW) is a well established Mongolian medicine with a long history of clinical application for treatment of CNS diseases. Effective compounds groups are molecular base for the various biological activities of Mongolian medicine such as neuroprotective effect (Liu et al., 2011). The main components originated from plants in EW are *Terminalia chebula Retz* (fruits), *Carthamus tinctorius* (flowers), *Gardenia jasminoides Ellis* (fruits), *Amomum tsaoko* (fruits), *Glycyrrhiza uralensis Fis* (roots and rhizomes), *Myristica fragrans* (seeds), *Abutilon theophrasti* (seeds), *Melia toosendan Sieb* (fruits), *Cassia obtusifolia* (seeds), *Saussurea costus* (roots), and *Cinnamomum cassia* (bark) (Table S1). The key components in EW contain a group of neuroactive natural products. For example, *Gardenia jasminoides Ellis* contains geniposide, (+)-(7S,8R,8′R)-lyoniresinol 9-O-β-D-(6″-O-trans-sinapoyl) glucopyranoside and 3,5-di-O-caffeoylquinic acid; *Amomum tsaoko* contains 4-dihydro-2-(4' - hydroxyphenylmethyl) -6-[(3″,4″ -dihydroxy-5″- methoxyphenyl) methylene]-pyran-3, 5-dione and 2,3- dihydro-2-(4′- hydroxy-phenylethyl)-6-[(3″,4″ -dihydroxy-5″-methoxy) phenyl]-5-pyrone. The therapeutic effect of EW in the treatment of limb numbness and other nerve related diseases has been clinically proved. It is one of the key remedies used in post-stroke recovery in Mongolian medicine (Hua et al., 2014; Tian, 2011). Recent study on MCAO/R injury rat model has shown that EW may improve nerve growth by up-regulating expression of neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Hua et al., 2016). However, full spectrum of gene regulation by EW is not understood, and more detailed neuroprotective and possible neuroregenerative mechanism of EW is not clear. In this report, we first confirmed the therapeutic efficiency of EW on MCAO rat model by Bederson scaling, brain microcirculation measurement, TTC staining and H&E staining of infarction area. Then, we analyzed full transcription patterns in the cerebral infarction of MCAO model animals treated with EW using RNA-seq technology. Significant differential expression was observed in genes functioning in cell recovery and growth, as well as immune activity, which may provide the neurorecovery mechanism of EW.

2. Materials and methods

2.1. Chemicals and instruments

Eerdun Wurile (internal medicine number M14010080, batch number 20150301) was provided by National Mongolian Pharmaceutical Preparation Center, International Mongolian Hospital, Inner Mongolia, China. Voucher specimens were deposited in the Virtual Herbarium of Inner Mongolia Medical University (Hohhot, China). Chloral hydrate was purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China). Monofilament nylon suture was purchased from Beijing Cinontech Co. Ltd. (Beijing, China). Waters Acquity UPLC/qTOF (Waters, USA) includes quaternary solvent manager, online degasser, sample manager, column manager, Acquity PDA detector, ESI source, Lockspray source, Xevo G2-XS QTof four-pole flight time tandem mass spectrometer and Masslynx V4.1 Workstation. Acetonitrile is purchased from Fisher Scientific, USA. Formic acid solution is purchased from Sigma-Aldrich, China. Leucine enkephalin is purchased from Waters, USA. Ethanol absolute, petroleum, ethyl acetate and n-butanol were purchased from Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd (Tianjin, China).

2.2. Sample preparation for UPLC-qTOF mass measurement

First, EW powder was weighed into 5 round bottom flasks (0.2 g per flask), and added 20 mL of distilled water (I), anhydrous ethanol (II), n-butanol (III), ethyl acetate (IV) and petroleum ether (V), respectively. After immersing for 16 h, the flasks were heated in oil bath to reflux for 8 h at 35 °C while stirring at 700 r/min, followed by filtration through a sand funnel with diatomite. Then, the solvents were removed under nitrogen flow and the samples were stored at 4 °C.

2.3. Analysis of UPLC-QTof-MS

For analysis, the following chromatographic conditions were applied: the column (Waters ACQUIY UPLC® BEH Shield RP18, 2.1 × 100 mm Column, 1.7 μm) was connected to a Vanguard HSS T3 guard column. Column temperature was 40 °C; the mobile phase was as following: A: water (containing 0.1% formic acid), B: acetonitrile (containing 0.1% formic acid); the mobile phase gradient elution was: 50% → 90% B, 10 → 15 min: 100% B, 15 → 20 min: 50% B; the flow rate was 0.4 mL/min; the injection volume was: 2 μL. The mass spectrometry conditions were as following: Electrospray Ionization (ESI) positive ion mode was used for detection. Mass detection range was 100–1200 Da; capillary voltage was 3 kV; sample cone was 40 V; extraction cone was 4 V; source temperature was 100 °C; desolvation temperature was 400 °C; desolvation gas was 800 L/h, lockmass was 556.2771 (positive ion mode). The accuracy error threshold was fixed at 5 mDa. Data acquisition is controlled by MassLynx 4.1 software.

According to the report of the chemical composition of the EW medicinal materials which were associated with nerves system, the information on the chemical constituents were collected and that chemical compositions structure were drawn through the "Chemdraw" software, building the molecular formula and theoretical relative molecular mass database.

2.4. Animals

A total of 55 eight-week-old male Wistar rats weighing 200–240 g (purchased from Experimental Animal Center of Inner Mongolia University, Hohhot, Inner Mongolia, China) were used for this experiment. The rats were acclimated for 7 days before the start of any experiments. They were housed in a controlled environment (4 animals per cages, 55 ± 5% relative humidity, 22 °C, 12 h:12 h light/dark cycle) and provided with free access to food and water. All experimental procedures involving animals were approved by the Animal