Journal of Ethnopharmacology 209 (2017) 50–61

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Xuefu Zhuyu decoction ameliorates obesity, hepatic steatosis, neuroinflammation, amyloid deposition and cognition impairment in metabolically stressed APPswe/PS1dE9 mice

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ARTICLE INFO

Keywords:
Metabolic stresses
Alzheimer's disease
Xuefu Zhuyu decoction
Amyloid plaque
Glia
Cognitive dysfunction

ABSTRACT

Ethnopharmacological relevance: Metabolic syndrome and vascular dysfunction was suggested to be the risk factors for Alzheimer's disease (AD). Xuefu Zhuyu decoction (XZD) is a traditional Chinese medicine used to treat metabolic syndrome and cardiac-cerebral vascular disease. The effects of XZD on ameliorating metabolic syndrome, amyloid-related pathologies and cognitive impairment in an animal model of AD with metabolic stress was investigated.

Materials and method: The animal model of AD with metabolic stress was created by administrating high-fat diet and a low-dose injection of streptozotocin prior to the appearance of senile plaques in APP/PS1 transgenic mice. The diabesity-associated metabolic changes and AD-related pathological alterations were examined.

Results: We found that XZD reduced body weight, insulin and leptin level, HOMA-IR, hepatic triglyceride, serum Aβ42 in the metabolic stressed AD animal. XZD also ameliorated oral glucose tolerant, Aβ deposition, astrocyte and microglia activation in the vicinity of plaques, and nesting behavior in the metabolic stressed AD animal.

Conclusion: The results of this study suggest that XZD is able to reduce the peripheral metabolic stress-mediated vascular hypoperfusion, neuroinflammation and AD-related pathology in APP/PS1 mice.

1. Introduction

Obesity, hyperglycemia, and insulin resistance are the core characteristics of metabolic syndrome, in which diverse pathological mechanisms including cerebral hypoperfusion, glucose hypometabolism, endothelial dysfunction, neuroinflammation and oxidative-nitrosative stress are synergistically promoted. Thereby, amyloid beta (Aβ) deposition and neurofibrillary tangle formation are enhanced (Kaur,

Abbreviations: Aβ, amyloid-β;; AD, Alzheimer's disease; ADL, activity of daily living; ANOVA, analysis of variant; APP, amyloid precursor protein; AICD, APP intracellular domain; APP/PS1, APPswe/PS1ΔE9; AUC, area under the curve; BBB, blood-brain barrier; BSB, 1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy) styrylbenzene; CNS, central nervous system; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; HE, hematoxylin and eosin; HFD, high-fat diet; HFSTZ, HFD and low dose injection of STZ; HOMA-IR, homeostasis model assessment of insulin resistance; Iba-1, ionized calcium-binding adaptor molecule-1; NCD, normal chow diet; OGTT, oral glucose tolerance test; STZ, strptozotocin; TCM, traditional Chinese medicine; TG, triglyceride; XZD, Xuefu Zhuyu decoction

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http://dx.doi.org/10.1016/j.jep.2017.07.036
Received 1 June 2017; Received in revised form 18 July 2017; Accepted 21 July 2017
Available online 22 July 2017

2014; Daulatzai, 2017). Eventually, these factors lead to neurodegeneration and Alzheimer's disease (AD) (Heneka et al., 2015). The development of AD-related pathology has been suggested to be positively correlated with impaired metabolic function (De Felice and Ferreira, 2014; McCrimmon et al., 2012; Zuloaga et al., 2016). Alternatively, Aβ angiopathy restrains the function of endothelium cells (Stukas et al., 2014), and the drainage of parenchyma Aβ may be blocked by a defective vascular system (Prasad et al., 2014). Therefore, during the progression of AD pathogenesis, there is a vicious spiral among vascular impairment and accumulated Aβ-mediated neurotoxicity.

Transgenic mice has been used to revealed that dietary manipulations (e.g., a high-fat diet, HFD) and nitrosamine exposure (i.e., administration of streptozotocin, STZ) may accelerate AD pathogenesis (Maesako et al., 2012; Wang et al., 2010). In our previous studies, the combination of HFD and administration of STZ (HFSTZ) were employed to investigate the effect of metabolic stress in APP/PS1 mice (Yeh et al., 2015; Shie et al., 2015). We found that there is an interplay between genetic background of AD and HFSTZ-induced metabolic stress. Moreover, HFSTZ-aggravated vascular inflammation, amyloid deposition and astrocyte activation, and impairment of cerebral glucose metabolism and daily living skill in APP/PS1 mice were observed.

Xuefu Zhuyu Decoction (XZD), a traditional Chinese classical herbal formula, is used for promoting blood circulation and removing blood stasis. XZD is first described in the medical classic Yi Lin Gai Cuo by Wang Qingren (Shoja et al., 2010). XZD has been found to be used for the treatment of metabolic syndrome (Jang et al., 2016), cardiac-cerebral vascular disease (Teng et al., 2014; Wang et al., 2011; Lee et al., 2011), traumatic brain injury and post-craniocerebral traumatic mental disorders (Xing et al., 2016; Sun et al., 2008), through removing blood stasis (Wang et al., 2005). The multiple cardiovascular protective actions of XZD with no adverse effects have been documented recently (Huang et al., 2007; Yi et al., 2014). It is effective in lowering blood pressure and alleviating blood pressure related symptoms [Ding et al., 2001]. XZD consists of 11 crude herbs: *Prunus persica (L.) Batsch (Tao Ren), Angelicae sinensis (Oliv.) Diels (Dang Gui), Ligusticumi chuanxiong Hort. (Chuan Xiong), Carthamus tinctorius L. (Hong Hua), Paeonia lactiflora Pall. (Chi Shao), Rehmannia glutinosa Libosch. (Di Huang), Citrus aurantium* L. *(Zhi Qiao), Bupleurum chinense DC. (Chai Hu), Platycodon grandiflorum (Jacq.) A. DC. (Jie Geng), Achyranthes bidentata Bl. (Niu Xi)* and *Glycyrrhiza uralensis Fisch. (Gan Cao)*.

The aim of the present study was to examine the effect of XZD on metabolic stress-aggravated pathological progression of AD. We hypothesized that XZD may ameliorate diabesity-associated metabolic changes on cerebral and hepatic hypoperfusion that in turn reduce amyloid-related pathologies. We examined blood glucose, insulin, HOMA-IR, Leptin, body weight, epididymal fat, hepatic steatosis, Aβ burden, glial activation, and nesting behavior in APP/PS1 mice.

2. Materials and methods

2.1. XZD preparation

The extracts of XZD (*Prunus persica (L.) Batsch, Angelicae sinensis (Oliv.) Diels, Carthamus tinctorius L., Rehmannia glutinosa Libosch., Achyranthes bidentata Bl., Paeonia lactiflora Pall., Citrus aurantium* L., *Ligusticumi chuanxiong Hort., Platycodon grandiflorum (Jacq.) A. DC., Bupleurum chinense DC.,* and *Glycyrrhiza uralensis Fisch.* at a ratio of 8:6:6:6:6:4:4:3:3:2:2) were prepared from crude plant medicines. ALL materials were purchased from a local supplier and identified by Dr. Lee, I-Jung, Ph. D., the leader of the herbarium of the National Research Institute of Chinese Medicine (NRICM). The ZXD was prepared by boiling in distilled water at 100 °C for 30 min, twice. The drug suspension was lyophilized, and performed HPLC fingerprinting (Fig. 1a). The drug powder was dissolved in normal saline to a final concentration of 2.0 g/mL (equivalent to the dry weight of the raw materias) for animal administration. The voucher specimens were deposited at the herbarium of the NRICM.

2.2. Characterization of XZD

The powder of XZD (50 g) was extracted with 50% ethanol (250 mL) at 40 °C for 20 min and followed by filtration to yield diluted ethanol extract (16 g). The extract was then partitioned with H_2O/*n*-butanol (each 200 mL) system three times. Combined the *n*-butanol layer and removed the solvent to yield *n*-butanol extract (4 g). The *n*-butanol extract was subjected to Si gel column (4 × 46 cm) eluting with $CHCl_3$/EtOAc/ MeOH/H_2O (15:40:22:10) solvent system to give subfractions Bu-1–Bu-5. A precipitate was filtered from Fr. Bu-3 and recrystalized in MeOH to give **5**. The filtrate of Fr. Bu-3 was purified by semipreparative HPLC (20% ACN/H_2O, Purospher® STAR RP-18e column, 5 μm, 10 × 250 mm, flow rate = 4.7 mL/min, UV = 254 nm) to yield **2**, **4**, and **5**. Fr. Bu-5 was purified by semipreparative HPLC (45% ACN/H_2O, Purospher® STAR RP-18e column, 5 μm, 10 × 250 mm, flow rate = 4.7 mL/min, UV = 254 nm) to yield **6**. The structures of compounds **2, 4–6** were deduced by spectral means and comparison with the published data, as liquiritin apioside, naringin, hesperidin, and glycyrrhizin, respectively. Compounds paeoniflorin (**1**) and ferulic acid (**3**) were purchased from Qualiflex Co., Ltd. Then, the identified compounds **1–6** served as standards in the following analytical experiment.

2.3. HPLC analysis

Ten mg of *n*-butanol extract of XZD or 1 mg of standards were dissolved in 1 mL methanol individually and vortexed for 30 s. These solutions were filtrated by syringe filter (0.4 μm), then the filtrates were transferred to HPLC analysis.

HPLC analysis was performed using a Hitachi system (Tokyo, Japan) consisting of an L-7100 HPLC pump, an L-7200 autosampler equipped with a 100-μl sample loop, an L-7450A photodiode array detector and a D-7000 HPLC Multi-System Manager chromatographic data system. Samples were performed on a reverse-phase C18 column (Purospher STAR, 4 × 250 mm, 5 μm, Merck, Darmstadt, Germany). Mobile phases consisted of solvents A (water with 0.1% formic acid) and B (acetonitrile). An eluting program was performed as following: from 5% to 35% of B over 30 min, then increasing the percentage to 50% of B in the next 5 min, and maintaining 50% B for further 5 min, up to 90% B for the next 1 min, and finally maintaining 90% B to the end of 50 min, at a flow rate of 1 mL/min. An aliquot (10 μl) of sample was injected for analysis and the profile was recorded at UV 254 nm.

2.4. Animal management and administration

The Institutional Animal Care and Use Committee at the National Research Institution of Chinese Medicine approved the animal protocol (IACUC No: 103–417-1, 104–417-1 and 105–417-1). All experimental procedures involving animal and their care were carried out in accordance with Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH). Male APP/PS1 transgenic mice (No. 005864) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) for breeding with female wildtype C57BL/6J mice. To be Brief, the transgenic mouse line expresses human APP with Swedish mutation and mutant human Presinilin 1 (PS1 delta E9) both under the control of the mouse prion protein promoter resulting in abundant amyloid plaques in cortex and hippocampus (Jankowsky et al., 2004). Experiments were conducted using wild type siblings and AD transgenic female C57BL/6J mice. Animals were housed under controlled room temperature (24 ± 1 °C) and humidity (55–65%) with a 12:12-h (07:00–19:00) light-dark cycle. Experiments were conducted using male APP/PS1 transgenic mice and