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Pharmacodynamic action and mechanism of Du Liang soft capsule, a traditional Chinese medicine capsule, on treating nitroglycerin-induced migraine

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ABSTRACT

Ethnopharmacological relevance: Du Liang soft capsule (DL) is a traditional Chinese medicine for treating migraines; it is made from two Chinese herbs, including *Ligusticum striatum* DC., root; *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav., root.

Aim of the study: In the present study, we aimed to elucidate the pharmacodynamic action of DL and its mechanism in an animal model of migraines induced by glyceryl trinitrate (GTN).

Materials and methods: Sixty rats were randomly divided into six groups, including a normal control group, model control group, positive group (Sumatriptan 0.006 g kg^{-1}), and three DL groups (0.44, 1.31 and 3.93 g kg^{-1}). All rats were intragastrically treated with the corresponding treatment for 7 consecutive days, and they were subcutaneously injected with GTN (10 mg kg^{-1}) 30 min after the last treatment, except in the normal control group. After model establishment, the behaviors of all rats, including head scratching, cage climbing, and the development of red ears were observed continuously by digital camera every 30 min for 3 h. Four hours after GTN treatment, all rats were anaesthetized and the blood and tissue samples were collected. Plasma calcitonin gene related to peptide (CGRP) and endothelin (ET) levels were measured using the radioimmunoassay method, and serum NO was determined by the colorimetric method. Afterwards, the brainstem tissues were dissected and washed with physiological saline, and divided evenly into two parts. One part was used to test the monoamine levels, including levels of 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA), by the fluorometric method, and the other part was used to determine the nuclear factor kappaB (NF-κB) p65, nuclear c-fos, inducible nitric oxide synthase (iNOS), interleukin (IL)-1β (IL-1β), and cyclooxygenase-2 (COX-2) levels by Western blot analysis.

Results: In the pharmacodynamic action assay, DL (1.31 and 3.93 g kg^{-1}) greatly improved the abnormal behaviors of migraine rats, including head scratching and cage climbing, and the development of red ears. In the mechanism assay, compared with the control group, the plasma CGRP and serum NO levels and the brainstem 5-HT, NE and DA levels in the DL administration groups were significantly decreased; and the plasma ET levels were remarkably increased. Moreover, down-regulation of NF-κB p65, c-fos and pro-inflammatory cytokines, including iNOS, IL-1β and COX-2 in the brainstem in the DL administration groups were observed by Western blot analysis.

Conclusions: The above results suggested that DL has a therapeutic effect on migraines, and its mechanism may be related to adjusting the level of neurotransmitters and vasoactive substances, consequently relieving neurogenic inflammation.

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1. Introduction

Migraine is a complex disease characterized by a repeated attack of pulsatile, severe, unilateral or bilateral headache in with symptoms of nausea and vomiting, and it often involves paroxysmal neurovascular dysfunction. According to recent surveys, migraine has contributed to the Global Burden of Disease and seriously affects the quality of patients' life (Vos et al., 2012). The average morbidity and lifetime prevalence of migraine are 13.2% and 19%, respectively (Arroyo-Quiroz et al., 2014; Victor et al., 2010). Different pathological and genetic mechanisms can present with a variety of clinical manifestations. The complex etiology and pathogenesis of migraine are not yet completely understood. Several studies have shown that migraine is associated with neurotransmitters and vasoactive substances, such as CGRP, ET, NO, and monoaminergic transmission (Lai et al., 2011; Tassorelli et al., 2002). Moreover, with improved understanding of the molecular mechanism in migraine research, neurogenic inflammation has been found as one of the factors inducing or aggravating migraine, and it mainly affects the transcription factor NF-κB signaling pathway activation as well as the abnormal expression of inflammatory cytokines. Migraines are difficult to cure because of their complex pathogenesis, and the current clinical treatment options are not ideal.

Traditional Chinese medicine (TCM) has accumulated rich experience in migraine treatment as well as substantial attention. Beyond Asia, TCM has been popular in the United States and Europe as complementary or alternative medicine (Cheung, 2011). DL, a Chinese patent medicine (CPM) approved by the State Food and Drug Administration (SFDA) of China, is one of the TCM recipes for treating headaches due to cold-wind. DL consists of two Chinese herbs, including *Ligusticum striatum* DC., root; *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav., root. The two herbs are also known as *Rhizoma Ligustici* (Chuanxiong) and *Radix Angelicae dahuricae* (Baizhi). The combination of these two herbs has been recorded in the Chinese Pharmacopoeia for a long time (State Pharmacopeia Committee of China, 2015). Clinical studies have revealed that DL has a variety of desirable pharmacological effects on migraines (Chen et al., 2012; Wei shi et al., 2010). However, DL is short of preclinical pharmacodynamic experimental basis, and its mechanism is unknown.

In this study, using the classic migraine model induced by GTN, we aimed to explore the pharmacodynamic action and mechanism of DL as well as provide further pharmacological basis and interpretation of mechanisms for clinical medication.

2. Materials and methods

2.1. Animal preparation

The adult male SPF-grade Sprague-Dawley rats (200 ± 20 g) used in the study were provided by the Experimental Animal Center at Chongqing Medical University (Chongqing, China) and were maintained in plastic cages at 22 ± 2 ℃ on a 12 h light/dark cycle with free access to food and water in the Experimental Animal Center at the College of Pharmaceutical Sciences of Southwest University (Chongqing, China). The animal approval number of the Experimental Animal Center was SYXK 2009-0002. All studies were strictly performed in accordance with the international ethical guidelines and related ethical regulations of the Research Institute of Surgery at the College of Pharmaceutical Sciences of Southwest University.

2.2. Preparation of experimental reagents

DL was provided by Chongqing Pharscin Pharmaceutical Group Co., Ltd (Chongqing, China), and dissolved in the solvent containing 2% Tween-80 and 2% sodium carboxymethyl cellulose saline. CGRP and ET were detected by the Beijing North Institute of Biological Technology (Beijing, China). An NO kit was purchased from Nanjing jiancheng Bioengineering Institute (Nanjing, China). 5-HT, NE, and DA standards were from Sigma (USA). Antibodies against iNOS and IL-1β were from Santa Cruz (CA, USA). Antibodies against NF-κB and histone H3 were from Boster Biological Engineering Co., Ltd. (Wuhan, China). Antibodies against COX-2, c-fos, β-actin, and horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG were from ZSGB-BIO (Beijing, China). Polyvinylidene difluoride (PVDF) membranes and an enhanced chemiluminescence (ECL) kit were purchased from Millipore (Bedford, MA, USA).

2.3. HPLC analysis of the main DL components

DL consists of *Rhizoma Ligustici* (Chuanxiong) and *Radix Angelicae dahuricae* (Baizhi) at a ratio of 1:4 (w/w). To ensure the reliability and repeatability of the present study, the main components, including imperatorin, isoimperatorin, and ferulic acid, in DL were detected by HPLC. The components were extracted by ethanol or a mixture of methanol and ethylic acid. An ODS-C18 column (4.6 mm×250 mm, 5 μm) was used as the solid phase for separation. The mobile phase was a mixture of methanol and acetonitrile (52:48) for detecting imperatorin and isoimperatorin (Hai wei et al., 2011) as well as a mixture of methanol and 0.1% acetic acid (35:65) for detecting ferulic acid (State Pharmacopeia Committee of China, 2010). The sample was delivered at a flow rate of 1.0 mL min^{-1} at a column temperature of 30 °C. The detection wavelengths were set at 248 nm and 321 nm, and 10 μL was loaded.

The HPLC results were shown in Fig. 1. T contents of three components, including imperatorin, isoimperatorin, and ferulic acid, were 1.54 mg g^{-1}, 0.49 mg g^{-1}, and 0.051 mg g^{-1}, respectively. HPLC assays had good repeatability and adequate stability, and the RSDs of precision were all less than 2.0%. The average recoveries were 95–105%.

2.4. Experimental methods

Sixty rats were randomly divided into six groups, including a normal control group, model control group, positive group (Sumatriptan 0.006 g kg^{-1}), and DL groups (0.44, 1.31, and 3.93 g kg^{-1}). The selection of the dosages of DL was based on the dose-related effects of DL in rats with nitroglycerin-induced migraines in our previous study (The data is shown in Supplementary Fig. 1 and Supplementary Fig. 2). The normal and model control groups were orally administered with physiological saline containing 2% Tween-80 (TW-80) and 2% carboxymethylcellulose sodium (CMC-Na) for 7 consecutive days. Based on the previous methods (Tassorelli and Joseph, 1995), 30 min after the last treatment, all rats, except those in the normal control group, were subcutaneously injected with GTN (10 mg kg^{-1}). The rats in the normal control group were injected with an equivalent volume of vehicle.

2.4.1. Behavioral test (Xian-Jun et al., 2005)

After model establishment, the behaviors of model rats, including the numbers of head scratching and cage climbing were observed continuously by digital camera (SONY DSC-WX9, Japan) every 30 min for 3 h. In the meantime, the duration of the climbing cage response was recorded if the behavior appeared five times in a time interval, and red ear symptoms were immediately recorded.

2.4.2. Assay of plasma CGRP, plasma ET, serum NO and brainstem monoamine levels

Four hours after model establishment, all rats were anaesthetized with 1.5% isoflurane. Blood samples were collected from the orbital venous plexus to determine plasma ET and CGRP levels with the radioimmunoassay method and serum NO with the colorimetric