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Cerebral nuclei distribution study of dehydrodiisoeugenol as an anxiogenic agent determined by RP-HPLC

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ABSTRACT

A sensitive RP-HPLC–DAD method was established to quantify dehydrodiisoeugenol (DDIE) in rat cerebral nuclei. The assay procedure involved one-step extraction of DDIE and daidzein, as an internal standard, from rat plasma and various cerebral nuclei with ethyl acetate. Chromatographic separation was performed on a Diamonsil™ ODS C_{18} column with methanol–water (81:19, v/v) as a mobile phase. The UV absorbance of the samples was measured at the wavelength of 270 nm. The analysis method was proved to be precise and accurate at linearity ranges in plasma and each cerebral nucleus with correlation coefficients of ≥0.9971. The results indicated that the method established was successfully applied to cerebral nuclei distribution study of DDIE after intravenous administration at a single dose of 40 mg/kg to rat. DDIE showed high concentration in all of cerebral nuclei at 8 min, which indicated that DDIE could cross the blood–brain barrier rapidly and might be one of the main bioactive substances of nutmeg. The results provide fundamental data for evaluating the effects of DDIE on the central nervous system and to be developed into an effective anxiogenic agent.

1. Introduction

Nutmeg, the seeds of *Myristica fragrans* Houtt. (family Myristicaceae), is popular in most parts of West Africa, Asia and Europe as a spice. It has been used in medicine since at least the seventh century and is generally applied for strengthening the stomach and intestine as a frequently used traditional medicine in Korea, Japan and China [1]. Researches have revealed that the extract of nutmeg possesses learning and memory-improving [2], stimulant, hallucinogenic [3], anxiogenic [4], and antidepressant-like [5] effects, which may be attributed to its nervous system stimulating effect. Dehydrodiisoeugenol (DDIE; chemical structure shown in Fig. 1) and myrislignan are the main neolignanoid components in the nutmeg [6,7] and the aril [8] of *M. fragrans*, and the DDIE content in the nutmeg is 0.14%–0.23% w/w [6,9]. As one of the main components, DDIE was determined as the index component to control the quality of the nutmeg by Pharmacopoeia of the People's Republic of China [1]. Previous studies have shown DDIE to have bioactivities of anti-inflammation, anti-lipid peroxidation and antibiosis *in vitro* and *in vivo* [8,10–12] as well as inhibitory activity of hepatic drug-metabolizing enzyme [13]. The results for tissue distribution studies have indicated that both DDIE [14] and myrislignan [15] could permeate the blood–brain barrier (BBB) to distribute into the brain of rats. In order to further confirm if the nervous system stimulating effect of nutmeg is related with DDIE, a cerebral nuclei distribution study was performed. In the present paper, a simple and rapid HPLC method utilizing a Diamonsil™ ODS C_{18} column with diode array detector (DAD) and daidzein as an internal standard (IS) for the quantification of DDIE in rat cerebral nuclei was established for the first time. The method developed was successfully applied to cerebral nuclei kinetic distribution study of DDIE in rats after intravenous (i.v.) administration at a single dose of 40 mg/kg.

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2. Experimental

2.1. Chemicals and reagents

DDIE (purity > 99.5%) was isolated and purified in our laboratory from the nutmeg as described in a previous paper [7]. Daidzein (chemical structure shown in Fig. 1) was of HPLC grade (purity > 99%) and purchased from National Institutes for Food and Drug Control (batch no.111502-200101, Beijing, China). HPLC-grade methanol (MeOH) was obtained from Fisher Scientific Co. (Somerville, NJ, USA). The water (H_2O) used in the experiment was collected from a Milli-Q Ultra-pure water system (Millipore, Billerica, MA, USA). Other chemicals (analytical grade) were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd. (Beijing, China).

2.2. Animals

Male Sprague–Dawley (SD) rats with an initial body weight of 200 ± 10 g were obtained from the Laboratory Animal Center of Peking University Health Science Center (Beijing, China). The rats were maintained in a 12 h light/12 h dark cycle and a temperature-controlled environment at 22 ± 1 °C with a humidity of 60 ± 5% for one week before the start of the experiment, and fed standard laboratory chow with water *ad libitum*. The experimental procedures were in compliance with the European Community guidelines for the use of experimental animals and approved by the Peking University Committee on Animal Care and Use (SYXK [Jing] 2006-0025).

2.3. Preparation of standard and quality control (QC) samples

Stock solution of DDIE at a concentration of 1 mg/mL in MeOH was prepared. Working solutions of DDIE were prepared by dilution of the stock solution with MeOH. For constructing the calibration curves, a series of DDIE standard solutions for plasma or cerebral nuclei was prepared by spiking blank rat plasma or cerebral nuclei with appropriate DDIE and 20 μL of IS working solutions. The IS working solution was obtained from further diluting the IS stock solution (1 mg/mL) to result in a final concentration of 50 μg/mL. Standard solutions of DDIE and IS were spiked into blank plasma or cerebral nuclei homogenates and were extracted to give low, medium and high levels of quality control (QC) samples.

2.4. Sample preparation

Before analysis, each collected blood sample was centrifuged at 4000 *g* for 10 min at room temperature. The plasma sample (200 μL) spiked with 20 μL of IS working solution was added with 800 μL of ethyl acetate (EtOAc) and vortexed for 1 min. After centrifugation at 16,000 *g* for 10 min, the supernatant was transferred into a clean glass tube. The remnant plasma layer was added with 800 μL of EtOAc and the procedure above was repeated. Two organic layers were combined and evaporated under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 100 μL of MeOH. An aliquot of 20 μL of solution was injected into the HPLC system for analysis.

For the cerebral nuclei, the cortex, hippocampus, striatum, hypothalamus, cerebellum and brainstem were thawed and homogenized in 1 mL of precooled normal saline solution. After spiking 20 μL of IS working solution, the homogenized solution was added with 4 mL of EtOAc and vortexed for 1 min, then the sample was centrifuged at 16,000 *g* for 10 min. The supernatant was removed and dried under a gentle stream of nitrogen at 40 °C. The remnant homogenate layer was added with the same volume of EtOAc and extracted as described above. Two sections of the supernatant were mixed and evaporated. The residue was reconstituted in 100 μL of MeOH. An aliquot of 20 μL of solution was injected into the HPLC system for analysis.

2.5. Instrumentation and analytical conditions

The analytical HPLC system consisted of a G1311C quaternary gradient pump, a G1329B autosampler, a G1316A column oven, and a G1315D DAD (Agilent Co., USA). A Diamonsil™ ODS C_{18} column (250 × 4.6 mm i.d., 5 μm; Dikma Technologies, Beijing, China) coupled with a C_{18} guard column (8 × 4 mm i.d., 5 μm) was used. The ratio of the mobile phase was MeOH–H_2O (81:19, v/v) and its flow-rate was 1 mL/min. Detection was performed at a wavelength of 270 nm and 25 °C. The injection volume was 20 μL.

2.6. Method validation

2.6.1. Specificity

Specificity of the method was assessed by comparing chromatograms of the blank plasma and cerebral nuclei with the corresponding analytical samples to exclude the interference of the analyte and IS.

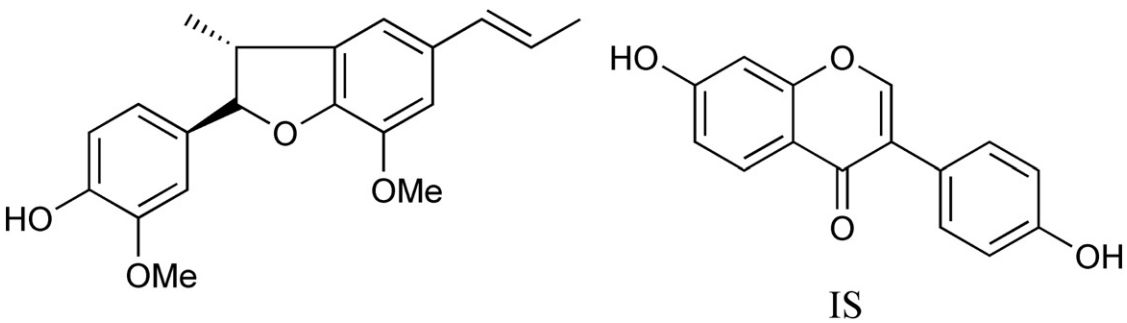

Fig. 1. Chemical structures of DDIE and IS (daidzein).