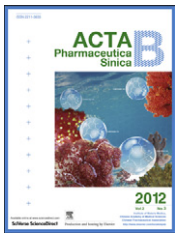
Acta Pharmaceutica Sinica B 2012;2(3):274–277

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Studies on tissue distribution of scutellarin and methyl polyethylene glycol (mPEG)-scutellarin prodrug in mice

Juan Lu[a], Xia Zhan[b], Qingfei Liu[c], Xi Chen[a,*], Xinge Zhao[d], Yiming Wang[c], Guoan Luo[c,*]

[a]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100094, China*
[b]*Beijing Technology and Business University, Beijing 100048, China*
[c]*School of Medicine, Tsinghua University, Beijing 100084, China*
[d]*Jiangsu Simcere Pharmaceutical Research Institute, Nanjing 210042, China*

Received 13 January 2012; revised 15 March 2012; accepted 22 March 2012

KEY WORDS

PEGylation;
Scutellarin;
Tissue distribution;
Cerebral I/R injury;
Brain-targeting

Abstract This study was to determine tissue distribution and pharmacokinetics of mPEG-scutellarin prodrug (**7e**), a chemical entity previously shown to have a beneficial effect in cerebral ischemia/reperfusion (I/R) injury. After injecting scutellarin or prodrug **7e**, the concentrations of scutellarin and **7e** in tissues were determined and the pharmacokinetic parameters were calculated. The results showed that the distribution of scutellarin in tissues was enhanced by PEGylation. The distribution of **7e** in brain was approximately 2.1-fold higher than that of scutellarin, indicating that PEGylation increased the brain penetration of scutellarin. We conclude that **7e** could exert more effective protection on cerebral I/R injury in mice. This study also provided a simple and convenient strategy to identify novel drugs with potential protective function for I/R injury in mice.

*Corresponding authors.
E-mail addresses: chenxi@implad.ac.cn (Xi Chen), luoga@mail.tsinghua.edu.cn (Guoan Luo).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
http://dx.doi.org/10.1016/j.apsb.2012.03.007

Production and hosting by Elsevier

1. Introduction

Scutellarin (4′,5,6-trihydroxyflavone-7-glucuronide) (Fig. 1) is the major active component of traditional Chinese herb, *Erigeron breviscapus* (Vant.) Hand-Mazz., which has been used to treat acute cerebral infarction and paralysis induced by cerebrovascular diseases such as hypertension, cerebral thrombosis, and cerebral hemorrhage in China since 1984[1–3].

Although scutellarin has been clinically used for a long time, its low solubility, poor bioavailability and short half-life *in vivo* limit its clinical application[4,5]. Numerous efforts[6–10] have been made to improve the clinical usefulness of scutellarin. PEGylation has been recognized as a promising strategy. PEGylation may extend the retention time in body, enhance distribution into tissues, decrease adverse effects, and increase resistance to degradation[11–14]. These advantages generally improve patient compliance[15].

In a previous study[1], fifteen PEGylated scutellarin prodrugs were designed and synthesized. Four prodrugs were selected for exploration of their activities according to water solubility and protective effect on cerebral I/R injury. The protective effect of the prodrugs on cerebral I/R injury was explored through a rat middle cerebral artery occlusion (MCAO) model. The prodrug **7e** (Fig. 1) exhibited a 7,000-fold increase in water solubility over that of scutellarin, reduced infarct area from 27.2 to 12.2% (33.3% for the control) and decreased the neurological deficit score from 2.77 to 1.32 (2.85 for the control). The data indicated that the prodrug **7e** had a protective effect on ischemic injury induced by cerebral I/R in rats when compared with scutellarin. Moreover, the pharmacokinetics of **7e** and scutellarin revealed a slower elimination of **7e** in blood.

However, no comprehensive analysis of the protective effect of **7e** on cerebral I/R injury was reported. The investigation of its distribution in tissues, especially in brain is highly desirable to determine if slow elimination of **7e** in blood can promote better accessibility to its targets to validate its ability to protect the brain from cerebral I/R injury.

2. Materials and methods

2.1. Reagents and chemicals

7e (purity > 98%, determined by HPLC-UV-ELSD) was separated and purified and its structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy. Scutellarin and rutin (IS) standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile was LC-grade and purchased from Merck. Deionized water was prepared with a Milli-Q system (Millipore, France). Other reagents were analytical grade.

Kunming mice (SPF Grade) weighing 18–22 g were obtained from the Experimental Center of Chinese Academy of Medical Sciences and housed at a constant temperature of 22–25 °C under a 12 h light–dark cycle with free access to food and drinking water. The protocol was approved by the Institutional Animal Care and Use Committee of Tsinghua University (Beijing, China).

2.2. Analytical conditions and sample pretreatment procedure

Analytical LC was performed with a Hitachi (Japan) system comprising of pump (L-2130), UV detector (L-2400), autosampler (L-2200), and column oven (L-2300) connected to a computer installed with appropriate software. Reversed-phase chromatographic separation was performed on a 250 mm × 4.60 mm, 5 μm particle, Phenomenex RP-18C column with a mobile phase gradient prepared from pure acetonitrile (component A) and 0.3% aqueous formic acid (component B). The LC gradient program was: 0.01–10 min, 78% (*v*/*v*) B; 10–12 min, 78–60% B; 12–14 min, 60–50% B; 14–15 min, 50–40% B; 15–20 min, 40% B; 20–25 min, 40–78% B; 25–30 min, 78% B. The flow rate was 0.8 mL/min, the injection volume was 20 μL, the detection wavelength was 335 nm, and the column temperature was 40 °C.

To each tissue sample, 20 μL of IS solution was added to make a concentration of 0.1 μg/mL, followed by 50 μL of 1:20 phosphate solution and 5 mL of methanol, then vigorously vortexed for 3 min and centrifuged at 10,000 rpm for 15 min. The supernatant was transferred to a new centrifuge tube and evaporated to dryness under a stream of nitrogen gas at 40 °C. The residue was reconstituted in 100 μL mobile phase, and then mixed for 3 min through vortexing followed by the centrifugation at 10,000 rpm for 15 min. The supernatant was transferred to an inner glass sample tube and 20 μL of supernatant was injected for analysis.

2.3. Tissue distribution examination

Kunming mice were divided into eight groups with 10 mice in each group, and fasted for 12 h prior to intravenous administration of scutellarin at a dose of 144 mg/kg (or **7e** at a dose of 799 mg/kg). Tissues were collected at 5, 15, 30, 45, 60, 90, 120 and 240 min following drug administration. Various tissues (brain, heart, liver and spleen) were immediately harvested and kept in normal saline to remove the blood. After blotted on filter paper, the filter paper with sample blot was weighted. In addition, after homogenization using a 3-fold volume of normal saline in 10 mL centrifuge tube, tissue samples were stored at −80 °C until assay[16].

2.4. Statistical analysis

The AUC_{0-t}, C_{max}, MRT and $T_{1/2}$ were calculated by Data and MAX Statistics (DAS 2.0, Shanghai, China). Statistical

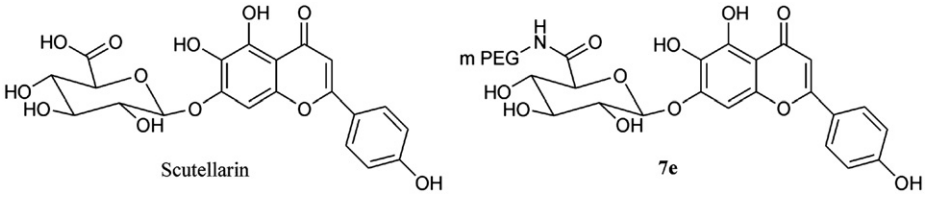


Figure 1 Chemical structures of scutellarin and its prodrug **7e**.