Journal of Chromatography B, 1029–1030 (2016) 95–101

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Pharmacokinetic studies of phellodendrine in rat plasma and tissues after intravenous administration using ultra-high performance liquid chromatography–tandem mass spectrometry

Yi Li, Xin-Guang Liu, Hui-Ying Wang, Xin Dong, Wen Gao, Xiao-Jun Xu, Ping Li*, Hua Yang*

State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form 28 June 2016
Accepted 3 July 2016
Available online 5 July 2016

Keywords:
Phellodendrine
Pharmacokinetic
Tissue distribution
UHPLC–MS/MS
Cortex phellodendri

ABSTRACT

Phellodendrine, a quaternary ammonium alkaloid extracted from the dried bark of *Phellodendrom chinensis* Schneid and *Phellodendrom amurense* Rupr, has the effect of suppressing cellular immune response, reducing blood pressure and antinephritis. However, few investigations have been conducted for the pharmacokinetic study of phellodendrine. Thus, a rapid, simple and reliable ultra-high performance liquid chromatography–tandem quadrupole mass spectrometry (UHPLC–QQQ MS/MS) method has been established for quantification of phellodendrine in rat plasma and tissues by using magnoflorine as internal standard. The chromatographic separation was achieved on an Agilent ZORBAX SB-C18 column (4.6 mm × 50 mm, 1.8 μm) by gradient elution using 0.1% aqueous formic acid (A) and methanol (B). Triple quadrupole mass detection with multiple reaction monitoring mode was used to monitor the ion transitions, at *m/z* 342.20 → 192.20 for phellodendrine and *m/z* 342.20 → 58.20 for internal standard, respectively. The developed method was fully validated and successfully applied to the pharmacokinetics and tissue distribution study of phellodendrine after intravenous administration. The lower limits of quantification were 0.5 ng/mL for plasma samples, 2.5 ng/g for brain and 1 ng/g for other tested tissues. Precisions and accuracy values were within the Food and Drug Administration acceptance criteria, the recovery and matrix effects were between 87.8–113.5%. The area under the curve (AUC_{0-t}) ranged from 15.58 to 57.41 mg/L min and Cmax were between 1.63–4.93 mg/L. The results showed that phellodendrine was eliminated in 120 min in plasma and most of tissues and the highest concentrations of phellodendrine were found in the kidney. This study may provide a basis for the further study of phellodendrine.

1. Introduction

Cortex Phellodendri, commonly known as "Chuanhuangbai" or "Guanhuangbai", is the dry bark of *Phellodendrom chinensis* Schneid or *Phellodendrom amurense* Rupr. It is a traditional Chinese medicine used for clearing away heat, eliminating dampness, purging pathogenic fire, relieving consumptive fever, counteracting toxicity, curing furuncles and also can cure dysentery, diarrhea and other syndromes [1,2]. Modern pharmacological researches have demonstrated that Cortex Phellodendri has the effect of anti-inflammatory, anti-tumor, anti-microbial, anti-fungal, anti-oxidant, antibiosis, anti-diarrheal, anti-arrhythmic, anti-pyretic, anti-ultraviolet radiation, anti-angiogenic potential, neuropreotective, immunoenhancement, hypotensive, inhibit lipid peroxidation [3–13].

Cortex Phellodendri contains various alkaloids such as berberine, jatrorrhizine and phellodendrine [14,15]. Previous studies mainly focus on berberine [16,17], while little attention was paid to phellodendrine. Chinese Pharmacopeia (Edition 2015) take phellodendrine ((7S,13aS)-3,10-dimethoxy-7-methyl-6,8,13,13a-tetrahydro-5H-isoquinolino[2,1-b]isoquinolin-7-ium-2,11-diol), a quaternary ammonium alkaloid, as one of the evaluating indexes of Phellodendri chinensis cortex. Pharmacological studies reported that phellodendrine possess antinephritic effect [18], has a weak hypotensive effect through an autonomic ganglion blocking action, and also suppresses the cellular immune response [19,20].

Pharmacokinetics and features of tissue distribution are key to the comprehension of the *in vivo* behavior and action mechanism [21]. Thus, it's necessary to investigate the systemic tissue exposure

* Corresponding author.
E-mail addresses: liping2004@126.com (P. Li), 104yang104@163.com (H. Yang).

http://dx.doi.org/10.1016/j.jchromb.2016.07.006
1570-0232/

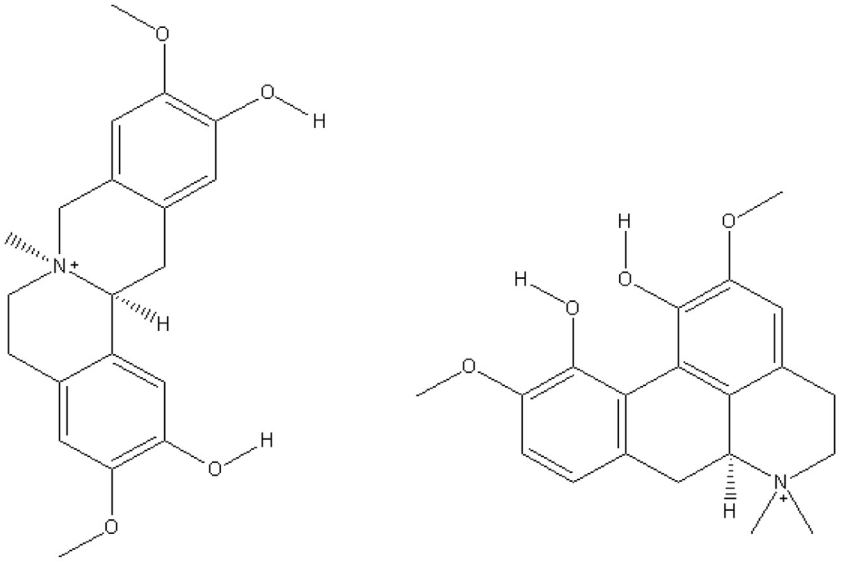

Fig. 1. Chemical structure of phellodendrine and magnoflorine (IS).

Table 1
Calibration curves of phellodendrine in biological samples.

Samples	Calibration curves	Correlation coefficients (r)	Liner ranges (μg/ml)	LLOQs (μg/ml)
Plasma	Y = 7.198X + 0.036	0.992	0.0005–25	0.0005
Liver	Y = 11.459X + 0.005	0.997	0.001–20	0.001
Spleen	Y = 10.736X + 0.001	0.992	0.001–20	0.001
Kidney	Y = 10.033X + 0.005	0.996	0.001–20	0.001
Brain	Y = 11.214X + 0.001	0.997	0.001–20	0.0025

Table 2
Precision and accuracy of phellodendrine in plasma and tissue homogenates of rats (n = 6).

Samples	Spiked concentration (ug/ml)	Inter-day			Intra-day		
		Measured concentration (μg/ml)	Accuracy (RE, %)	Precision (RSD, %)	Measured concentration (μg/ml)	Accuracy (RE, %)	Precision (RSD, %)
Plasma	0.001	0.0011 ± 0.013	6.35	12.67	0.0010 ± 0.0078	4.48	7.43
	0.1	0.095 ± 0.44	−5.45	4.70	0.095 ± 0.30	−5.45	3.18
	20	18.41 ± 118.38	−7.95	6.43	18.77 ± 83.10	−6.14	4.43
Liver	0.005	0.0054 ± 0.056	8.40	10.39	0.0052 ± 0.053	3.60	10.22
	0.1	0.11 ± 0.68	7.91	6.37	0.11 ± 0.50	9.63	4.60
	1	1.1 ± 7.0	8.75	6.42	1.09 ± 4.71	8.78	4.75
Spleen	0.005	0.0053 ± 0.045	5.50	8.53	0.0048 ± 0.016	−3.90	3.37
	0.1	0.10 ± 0.59	3.11	5.72	0.099 ± 0.44	−0.60	4.43
	1	0.97 ± 5.8	−2.81	5.96	0.99 ± 4.6	−0.51	4.66
Kidney	0.005	0.0050 ± 0.043	0.38	8.64	0.0055 ± 0.013	10.70	2.42
	0.1	0.11 ± 0.62	12.16	5.57	0.11 ± 0.50	11.90	4.47
	1	1.087 ± 6.8	8.71	6.27	1.05 ± 7.28	4.86	6.95
Brain	0.005	0.0057 ± 0.047	14.42	8.27	0.0052 ± 0.027	4.95	5.67
	0.1	0.11 ± 0.64	9.43	5.85	0.11 ± 0.25	6.70	2.59
	1	1.12 ± 6.1	11.78	5.42	1.082 ± 5.77	8.19	5.84

and plasma pharmacokinetic of phellodendrine. However, there are few reports about phellodendrine in rat plasma and tissues.

In this study, a rapid, simple and reliable ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) method has been developed to determinate phellodendrine in rat plasma and tissues. The validated UHPLC–MS/MS method was successfully applied to quantify the phellodendrine in the plasma and tissues, enabling reliable and fast evaluation of *in vivo* behavior.

2. Experimental

2.1. Chemical and reagents

Phellodendrine (purity ≥ 96%) was purchased from Pufei De Biotec Co. Ltd. (Chengdu, China). The internal standard (IS), magnoflorine (purity ≥ 98%), was purchased from Phystandard Technology Co. Ltd. (Shenzhen, China). Fig. 1 illustrates both their chemical structures.