Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2015, **13**(7): 0535–0539
Chinese Journal of Natural Medicines

Pharmacokinetics and tissue distributions of veratric acid after intravenous administration in rats

PENG Yu-Shuai, LIU Li-Jia, ZHAO Can, YANG Xin, LIU Chen, WANG Ru-Feng*

School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China

Available online 20 July 2015

[ABSTRACT] The present study was designed to investigate the pharmacokinetics and tissue distributions of veratric acid following intravenous administration in rats. The concentrations of veratric acid in rat plasma at various times after administrated at doses of 2.5, 5, and 10 mg·kg^{-1} were quantified by HPLC. The tissue distributions of veratric acid at various times after a single intravenous dose of 2.5 mg·kg^{-1} were also analyzed. The plasma pharmacokinetic parameters at the three doses were as follows: $t_{1/2}$, (86.23 ± 6.83), (72.66 ± 4.10) and (71.20 ± 2.90) min; C_0, (11.10 ± 1.47), (23.67 ± 1.24) and (39.17 ± 3.90) μg·mL^{-1}; and $AUC_{0\to\infty}$, (1 240.90 ± 129.14), (2 273.84 ± 132.47) and (3 516.4 ± 403.37) min·μg·mL^{-1}, respectively. The compound was distributed into tissues rapidly and extensively after intravenous administration and was mainly distributed into the liver, heart and kidneys.

[KEY WORDS] Veratric acid; Pharmacokinetics; Tissue distribution

[CLC Number] R969.1 **[Document code]** A **[Article ID]** 2095-6975(2015)07-0535-05

Introduction

Veratric acid (Fig. 1) is one of the phenolic acids abundant in the flowers of *Trollius chinensis* [1-4]. It is considered as one of the major effective components in these flowers, having anti-inflammatory, anti-oxidant, and hypotensive effects [5-9]. It is well known that the pharmacokinetics of a compound is crucial to evaluate its efficacy and toxicity *in vivo* and predict its potential in drug research and development. Our previous study has proven the good absorbability of veratric acid after oral administration to rats [10]; however, little information on its tissue distribution is available. Thus, we carried out the present investigation on the pharmacokinetics and tissue distribution of veratric acid after intravenous administration to rats for a better understanding of its disposition *in vivo* and potential as a novel therapeutic agent.

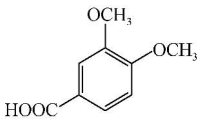

Fig. 1 Structure of veratric acid

Materials and methods

Instrument and reagents

The reference compound of veratric acid was extracted and purified from the flowers of *T. chinensis* in our laboratory. Its purity was over 99% as determined by HPLC analysis. Acetonitrile of HPLC grade was supplied by Fisher Scientific (Pittsburgh, PA, USA). Acetic acid and 1, 2-propanediol of analytical grade were purchased from Beijing Chemical Factory (Beijing, China). The water used was purified by a Milli-Q system (Millipore Corporation, Billerica, MA, USA).

The major instruments used in the present study included a Waters 1500 series high performance liquid chromatography system (Waters, Milford, MA, USA), equipped with a 1525 binary pump, an online degasser, a manual injector and a 2489 UV/Visible detector, as well as a T10 Basic tissue homogenizer (IKA Company, Staufen, Germany).

HPLC conditions

Chromatographic analysis was carried out on a C_{18} column (250 mm × 4.6 mm, 4 μm; Phenomenex, Torrance, CA, USA)

[Received on] 10-Oct.-2014
[Research funding] This work was supported by NSFC (Nos. 81274044 and 81073018).
[*Corresponding author] E-mail: wangrufeng@tsinghua.org.cn
These authors have no conflict of interest to declare.

using acetonitrile as mobile phase A, and 0.1% acetic acid in purified water as mobile phase B. The gradient elution was performed at a flow rate of 1.0 mL·min^{-1} with the gradient program including 5% A–30% A (0–2 min), 30% A–45% A (2–8 min), and 45% A–5% A (8–10 min). The column temperature was kept at 35 ºC and the detector wavelength was set at 254 nm.

Animals

Sprague-Dawley rats weighing 200 ± 20 g were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and were housed in plastic cages at a temperature of 20–26 ºC under 12 h light/dark cycle and 70% relative humidity. They were allowed free access to standard rodent diet and tap water. The diet was removed from the cages 24 h before the experiment, but water was supplied *ad libitum*.

Drug preparation

Veratric acid was dissolved in 1, 2-propanediol-redistilled water (1 : 5, *V*/*V*) to the concentration of 2 mg·mL^{-1} and filtered through a 0.22-μm filter before administration.

Pharmacokinetic study

For the plasma pharmacokinetic study, the veratric acid solution was injected to rats (n = 5) via a tail vein injection at the doses of 2.5, 5, and 10 mg·kg^{-1}, respectively. Blood samples were obtained from the fossa orbitalis vein and collected in heparinized tube at 0, 5, 10, 25, 40, 70, 100, 160, 220, 340, and 490 min after drug injection. The blood samples were centrifuged at 8 000 r·min^{-1} for 10 min, and the plasma was collected.

Tissue distribution study

For the tissue distribution study, 24 rats were assigned randomly to six groups (4/group) and were given the veratric acid solution via tail vein injection at 2.5 mg·kg^{-1}. Tissue samples from the liver, heart, spleen, lungs, kidneys, stomach, small intestine, trachea, and brain were obtained at 5, 25, 40, 70, 100 and 160 min after administration, respectively. The tissue samples were put into normal saline solution to remove the blood or content, blotted with filter paper, and then weighed and homogenized in saline solution (1 : 2, g·mL^{-1}). The tissue homogenates were extracted with ultrasound at 50 ºC for 30 min and then centrifuged at 8 000 r·min^{-1} for 10 min. Finally, the supernatant was collected for further treatment.

Sampling

The plasma or tissue homogenate samples (100 μL each) were mixed well with 10 μL of 100 μg·mL^{-1} vitexin (as the internal standard), and 250 μL of acetonitrile was then added to remove protein [11-12]. The resultant solution was thoroughly vortex-mixed for 1 min. After centrifugation at 12 000 rpm for 15 min, the supernatant was collected and filtered through a 0.45-μm filter, and 20 μL of the filtrate was injected onto the HPLC.

Preparation of standard and quality control samples

To prepare the standard calibration samples and quality control samples (QC), several veratric acid standard solutions at the concentration range of 0.01–40.00 μg·mL^{-1} in plasma or 0.01–5.00 μg·mL^{-1} in tissue homogenates were prepared by adding veratric acid stock solutions to 100 μL of plasma or supernatant of tissue homogenates from untreated rats, and then the samples were extracted as above. The QC samples (0.1, 10, and 40 μg·mL^{-1}) used in the validation were prepared in the same way as the standard calibration samples.

Results

Method validation

Specificity

With the HPLC conditions described above, no significant interfering peak was observed. The retention time of veratric acid was 8.627 min, and that of vitexin was 6.489 min (Fig. 2). For veratric acid, the theoretical plate number was about 6 400, and the tailing factor was 1.15.

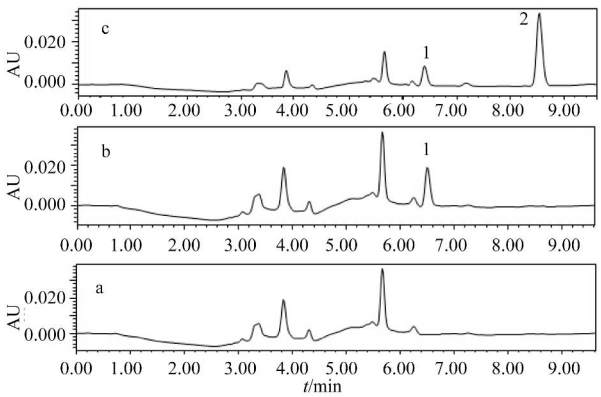

Fig. 2 Chromatograms of the blank plasma and plasma samples of veratric acid treated rats
a. Blank plasma; b. Blank plasma with internal standard; c. Plasma sample of veratric acid; 1. Vitexin; 2. Veratric acid

Linearity and calibration curve

The standard calibration curves of the peak area ratio of veratric acid to vitexin (*Y*) versus the concentrations of veratric acid (*X*) are listed in Table 1.

Table 1 The standard calibration curves of plasma and tissue homogenates

	Calibration curves	Correlation coefficients (r)	Linear ranges (μg·mL^{-1})
Plasma	Y = 0.119 5 X − 0.024 0	0.999 5	0.01–40
Liver	Y = 0.249 5 X + 0.138 6	0.999 4	0.01–5
Heart	Y = 0.307 7 X + 0.095 0	0.999 8	0.01–5
Spleen	Y = 0.208 9 X + 0.067 5	0.997 7	0.01–3
Lungs	Y = 0.242 3 X + 0.057 6	0.997 3	0.01–3
Kidneys	Y = 0.416 1 X + 0.057 3	0.999 4	0.01–5
Stomach	Y = 0.220 2 X + 0.086 7	0.996 2	0.01–3
Small intestine	Y = 0.238 6 X + 0.066 1	0.997 6	0.01–3
Trachea	Y = 0.262 9 X + 0.072 8	0.999 7	0.01–3
Brain	Y = 0.203 7 X - 0.013 5	0.996 7	0.01–3