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Pharmacokinetics of β-asarone in rabbit blood, hippocampus, cortex, brain stem, thalamus and cerebellum

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β-Asarone has significant pharmacological effects on the central nervous system. As a potential therapeutic agent to manage brain diseases, analysis of the pharmacokinetics of β-asarone in brain is necessary. We used cardio-perfusion method to exclude the β-asarone in the brain blood. The brain was divided into five regions: hippocampus, cortex, brain stem, thalamus and cerebellum, and pharmacokinetic differences were investigated. We found that concentration-time profile of β-asarone in blood, hippocampus, cortex, brain stem and cerebellum could be adequately described by a first-order equation, consistent with a linear two-compartmental model, but a first-order equation with a linear one-compartmental model in thalamus. The half lives of β-asarone in blood, hippocampus, cortex, brain stem, thalamus and cerebellum were 1.3801, 1.300, 1.937, 7.142, 2.832 and 8.149 h, respectively. Gender differences do not significantly influence plasma pharmacokinetics of β-asarone.

1. Introduction

β-Asarone (*cis*-2,4,5-trimethoxy-1-allyl phenyl), which has significant pharmacological effects on the central nervous system (CNS) (Fang et al. 2003; Fang et al. 2008; Zanoli et al. 1998; Cho et al. 2002), is a major component of *Acorus tatarinowii* Schott. β-Asarone could pass the blood-brain barrier (BBB) and thus enter the brain (Fang and Wei 2002). It has been reported that β-asarone could attenuate neuronal apoptosis in rat hippocampus and might be a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease (Li et al. 2010; Liu et al. 2010). Other authors found β-asarone could reduce the toxicity of excitatory amino acids in the epileptic rat brain and increase the expression of c-fos (Fang et al. 2008). Additionally, β-asarone could reduce the injuries of blood vessel endothelium and nerve cells of the cortex (Fu et al. 2008; Chen et al. 2007) and improve the cognitive function of the beta-amyloid hippocampus injection rats (Geng et al. 2010).

As a potential therapeutic agent to manage brain diseases, knowledge about the pharmacokinetics of β-asarone in brain is necessary. We have studied the pharmacokinetic of β-asarone in rat brain (Wu and Fang 2004). However, β-asarone in the brain blood did not exclude, which might lead to a false positive result. In this study, we used the cardio-perfusion method to exclude the β-asarone in the brain blood of rabbits. Meanwhile we divided the brain into five regions: hippocampus, cortex, brain stem, thalamus and cerebellum, and analyzed the pharmacokinetic differences of β-asarone among them. In addition, we analyzed the pharmacokinetic differences of β-asarone between the brain and blood to see if β-asarone has any particular affinity for brain tissues.

2. Investigations and results

For intra-day precision and accuracy, the RSD of the three concentrations (0.11100, 0.05550 and 0.00222 μg/10 μl) were 0.62%, 0.81% and 5.89%, respectively. For inter-day precision and accuracy, the RSD of the three concentrations (0.11100, 0.05550 and 0.00222 μg/10 μl) were 10.83%, 7.53% and 10.52%, respectively (Table 1).

The average recoveries of three concentrations (0.11100, 0.05550 and 0.00222 μg/10 μl) were 97.12%, 108.51% and 95.91%, respectively, and the RSD were 1.55%, 1.71% and 7.6%, respectively.

Calibration curves were constructed by plotting the peak-area (A) of β-asarone to the external standard versus β-asarone concentrations (C). A linear regression was used for quantitation. The β-asarone calibration curves of the blank blood and brain samples are shown in Table 2.

The β-asarone was very rapidly absorbed after intravenously administration (Table 3). Gender differences accounted for no significant influence on the plasma pharmacokinetics of β-asarone (Table 4). Concentration-time profile could be adequately described by a first-order equation, consistent with a linear two-compartmental model (Fig. 1). Other pharmacokinetic parameters are shown in Table 4.

The β-asarone penetrate the BBB and be very rapidly absorbed in different brain tissues (Table 5). Concentration-time profile in hippocampus, cortex, brain stem and cerebellum could be adequately described by a first-order equation, consistent with a linear two-compartmental model, but by a first-order equation, consistent with a linear one-compartmental model in thalamus (Fig. 2). The elimination half-lifes from hippocampus, cortex, thalamus, cerebellum and brain stem were 1.300, 1.937, 2.832,

Table 1: Precision and accuracy data of the HPLC analysis (n = 5)

Concentration (ug/10ul)	RSD (%)	
	Intra-day precision and accuracy	Inter-day precision and accuracy
0.11100	0.62	10.83
0.05550	0.81	7.53
0.00222	5.89	10.52

For intra-day precision and accuracy, five replicate quality control samples at three concentrations (0.11100, 0.05550 and 0.00222 μg/10 μl) were assayed on the same day. The inter-day precision and accuracy were evaluated on three different days.

Table 2: Calibration curves of β-asarone in blank blood and brain samples

Samples	Calibration curves	Correlation coefficient	Linearity rang(mg/L)
Blood	$A = 3.33 \times 10^6 C + 1490$	0.9991	0.36–23.12
Hippocampus	$A = 2.50 \times 10^6 C + 649$	0.9996	0.34–5.52
Cortex	$A = 2.30 \times 10^6$C-216	0.9988	0.34–5.52
Thalamus	$A = 2.37 \times 10^6 C + 290$	0.9992	0.34–5.52
Cerebellum	$A = 2.42 \times 10^6 C + 124$	0.9991	0.34–5.52
Brain stem	$A = 2.40 \times 10^6 C + 1510$	0.9971	0.34–5.52

Calibration curves were constructed by plotting peak-area (A) of β-asarone to the external standard versus β-asarone concentrations (C).

Table 3: Concentration of β-asarone in plasma within 300 min

Sex	4min	7min	10min	13min	16min	20min	30min
Female (n = 5, mg/L)	12.18 ± 3.20	8.73 ± 2.51	6.82 ± 1.97	6.04 ± 1.52	4.73 ± 1.39	3.67 ± 0.92	2.46 ± 0.33
Male (n = 5, mg/L)	13.90 ± 4.03	9.14 ± 2.91	7.15 ± 2.69	5.38 ± 2.10	4.98 ± 1.70	3.85 ± 1.57	2.73 ± 0.51
Total (n = 10, mg/L)	13.04 ± 3.39	8.93 ± 2.44	6.98 ± 2.12	5.71 ± 1.68	4.86 ± 1.39	3.76 ± 1.15	2.59 ± 0.41

To be continued

Sex	40min	60min	90min	120min	180min	240min	300min
Female (n = 5, mg/L)	2.13 ± 0.75	1.19 ± 0.40	0.79 ± 0.40	0.86 ± 0.21	0.14 ± 0.09	0.13 ± 0.04	0.14 ± 0.12
Male (n = 5, mg/L)	1.47 ± 0.29	0.91 ± 0.14	0.65 ± 0.20	0.51 ± 0.11	0.17 ± 0.09	0.09 ± 0.03	0.09 ± 0.02
Total (n = 10, mg/L)	1.81 ± 0.62	1.05 ± 0.31	0.72 ± 0.29	0.63 ± 0.22	0.16 ± 0.08	0.14 ± 0.08	0.13 ± 0.09

The rabbits were administered of β-asarone via ear veins, and the plasma concentrations of β-asarone were analyzed with HPLC at 4, 7, 10, 13, 16, 20, 30, 40, 60, 90, 120, 180, 240 and 300 min, respectively. Mean% ± SD; *Significant level $p < 0.05$.

8.149 and 7.142 h, respectively. Other pharmacokinetic parameters are shown in Table 6.

3. Discussion

In previous studies, the plasma concentration of β-asarone was conducted by indomethacin or α-naphthol as an internal standard on account of the lack of high purity β-asarone (Tsai et al. 1992; Wei and Wu 2005). In this research, β-asarone with a purity of up to 99.55% was used as an external standard. The concentration-time profile of β-asarone in rabbit plasma can be adequately described by a first-order equation, consistent with a linear two-compartmental model. Pharmacokinetic parameters (Table 4) imply that β-asarone has a fast absorption and distribution in the rabbit blood. This result is in accordance with the results of previous studies. In addition we found that

Table 4: Pharmacokinetic parameters of β-asarone in rabbit plasma

Parameters	Values
$t_{1/2}$ (min)	82.834
AUC(0-∞) (mg/L*min)	359.357
K_{el} (1/min)	0.008
Vd (L/kg)	9.979
CL (L/min/kg)	0.083
$t_{1/2\alpha}$ (min)	1.605
$t_{1/2\beta}$ (min)	9.124
$t_{1/2\gamma}$ (min)	61.859
K_{10} (1/min)	0.071
K_{12} (1/min)	0.145
K_{21} (1/min)	0.213
K_{31} (1/min)	0.020
K_{13} (1/min)	0.070

The pharmacokinetic model and parameters were fitted and calculated by the Drug And Statistics ver2.0 software (Mathematical Pharmacology Professional Committee, China).

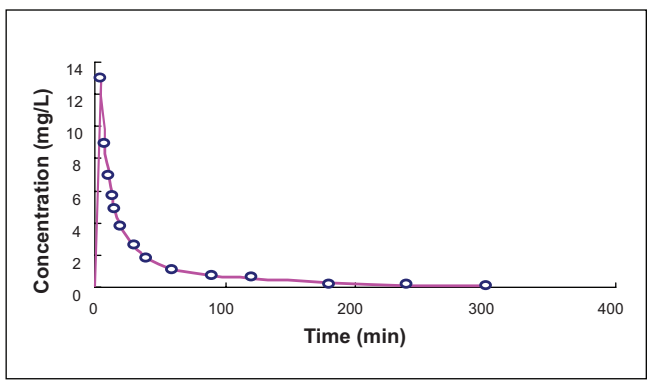

Fig. 1: The plasma elimination of β-asarone: Concentration-time profile could be adequately described by a first-order equation, consistent with a linear two-compartmental model

Table 5: Concentration of β-asarone in different brain tissues (mg/L)

Tissues	1 h	2 h	3 h	4 h	5 h
Hippocampus	2.369 ± 1.517	0.778 ± 0.240	0.404 ± 0.214	0.340 ± 0.035	0.322 ± 0.081
Cortex	1.525 ± 0.788	0.608 ± 0.206	0.650 ± 0.075	0.520 ± 0.250	0.365 ± 0.164
Thalamus	0.861 ± 0.234	0.695 ± 0.311	0.544 ± 0.193	0.426 ± 0.153	0.255 ± 0.035
Cerebellum	2.257 ± 0.426	1.223 ± 0.069	0.844 ± 0.241	0.770 ± 0.361	0.712 ± 0.221
Brain stem	1.719 ± 0.674	0.800 ± 0.249	0.745 ± 0.093	0.650 ± 0.203	0.600 ± 0.153

The rabbits were administered of β-asarone via ear veins, and the brain concentrations of β-asarone were analyzed with HPLC at 1, 2, 3, 4 and 5 h, respectively. Mean% ± SD, n = 4; *Significant level $p < 0.05$.

Table 6: Pharmacokinetic parameters of β-asarone in different brain tissues

Parameters	Hippocampus	Cortex	Thalamus	Cerebellum	Brain stem
$t_{1/2}$ (H)	1.300	1.937	2.832	8.149	7.142
$AUC_{(0-\infty)}$ (mg/L*h)	7.201	5.495	4.561	15.577	11.519
K_{el} (1/h)	0.533	0.358	0.245	0.085	0.097
Vd (L/kg)	7.814	15.261	26.879	22.649	26.842
CL (L/h/kg)	4.166	5.459	6.578	1.926	2.604
$t_{1/2\alpha}$ (H)	0.463	0.085	2.642	0.627	0.108
$t_{1/2\beta}$ (H)	69.315	4.669	2.642	69.315	7.008
K_{10} (1/h)	0.770	315.903	0.196	0.276	34.465
K_{12} (1/h)	0.678	307.776	0.066	0.690	28.084
K_{21} (1/h)	0.059	0.151	0.262	0.151	0.111

The pharmacokinetic model and parameters were fitted and calculated by the Drug And Statistics ver2.0 software (Mathematical Pharmacology Professional Committee, China).

gender differences did not significantly influence the plasma pharmacokinetics of β-asarone in rabbits.

We studied the pharmacokinetics of β-asarone in rat brain (Wu and Fang 2004). The result showed that the concentration-time profile of β-asarone in brain could be adequately described by a first-order equation, consistent with a linear one-compartmental model. In this study, the cardio-perfusion method was used to exclude the influence of β-asarone in the brain blood of rabbits (Liu and Fang 2011). Concentration-time profile of β-asarone in hippocampus, cortex, brain stem and cerebellum can be adequately described by a first-order equation, consistent with a linear two-compartmental model, but by a first-order equation, consistent with a linear one-compartmental model in thalamus. The results given in Table 6 show that β-asarone can penetrate the BBB rapidly and be widely distributed in cortex, cerebellum, brain stem and hippocampus. The half-lives of β-asarone in five brain tissues are longer than that in plasma, and half-lives of brain stem and cerebellum are longer than other parts of brain. The concentrations of β-asarone in five brain tissues are higher than that in plasma at the same time points, which implies that β-asarone has a particular affinity for brain tissues.

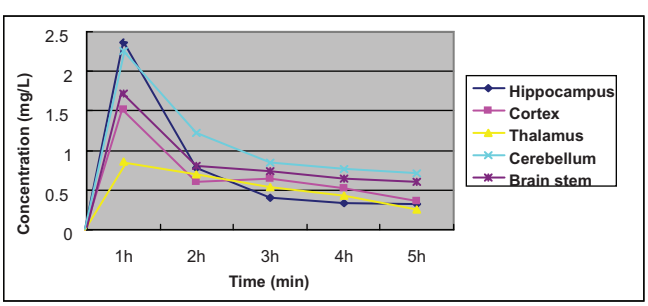

Fig. 2: Elimination of β-asarone in different brain tissues: the treatment groups were intravenously administered of 30 mg/kg β-asarone-propylene glycol-water (6:60:34, v/v/v) via ear veins, and anesthetized by 1 g/kg ethylurethan via intraperitoneal injection at 1, 2, 3, 4 and 5 h, respectively. The concentration-time profile could be adequately described by a first-order equation, consistent with a linear two-compartmental model

β-Asarone is a strong fat-soluble substance with a small molecular weight, which can go through the BBB rapidly and affect the brain directly. The results suggest β-asarone may be a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with brain diseases such as Alzheimer's disease.

4. Experimental

4.1. Materials

β-Asarone was obtained as reported by Liu and Fang (2011). Purity up to 99.55% was confirmed by gas chromatography–mass spectrometry (GC-MS), infrared spectrum (IR) and nuclear magnetic resonance (NMR) detection.

Ethylurethan was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Methanol and propylene glycol were purchased from Guangzhou Chemicals (Guangzhou, China). All solvents used were of analytical grade but methanol of chromatographic pure.

4.2. Animals and treatment

The study and its experimental protocol were approved by the Ethics Committee of Guangzhou University of Chinese Medicine. Local institutional approval for research was obtained before initiation of the study. Seventeen male and 17 female Dutch belted rabbits (1.7–2.3 kg) were placed in metabolism cages and acclimatized to the laboratory conditions for two weeks before commencement of the experiment. The room temperature was kept at 19–27 °C, and relative humidity was kept at 35–55%. The rabbits received diet (K1, lactamin) and water *ad libitum*.

Five male and 5 female rabbits received 1 g/kg ethylurethan via i.p. administration, and the blank blood samples (1.5 ml) were collected *via* a jugular cannula. Then the rabbits were intravenously administered of 30 mg/kg β-asarone-propylene glycol-water (6:60:34, v/v/v), and blood samples(1.5 ml) were collected at 4, 7, 10, 13, 16, 20, 30, 40, 60, 90, 120, 180, 240 and 300 min, respectively.

Twelve male and 12 female rabbits were randomly divided into six groups: blank group and five treatment groups according to the time points (1, 2, 3, 4 and 5 h). The rabbits in the blank group were intravenously administered of 30 mg/kg propylene glycol-water (60:40, v/v) via ear veins, and anesthetized with 1 g/kg ethylurethan via i.p. administration. The blank brain tissues (cortex, cerebellum, thalamus, brain stem and hippocampus) were harvested on a glacial table and weighted. The rabbits in the treatment groups were intravenously administered of 30 mg/kg β-asarone-propylene glycol-water (6:60:34, v/v/v) via ear veins, and anesthetized with 1 g/kg ethylurethan via

i.p. at 1, 2, 3, 4 and 5 h, respectively. The cardio-perfusion method was used to exclude the influence of β-asarone in the brain blood. Normal saline was perfused into the left ventricle and evacuated from the right atrial appendage. The perfusion could be stopped when the rabbit eyes and claws turned pale. After the perfusion, the treatment brain tissues were harvested as the same as the blank group.

4.3. Analytical techniques

4.3.1. Chromatographic conditions

Analysis was performed on a Waters 2695 HPLC system (Waters Corp., USA). The analytes were separated on 5-μm Symmetry C18 column (150 mm × 4.6 mm) with column temperature 40 °C. The mobile phase consisted of methanol-water (60:40, v/v). The flow rate was 1.2 ml/min and retention times were monitored by UV detection at 253 nm.

4.3.2. Preparation of standard solutions, calibration standards

Two primary stock solutions of β-asarone (0.680 g/L, 0.864 g/L) were prepared by dissolving appropriate amounts of β-asarone in methanol, respectively. One (0.680 g/L) was for the study of blood pharmacokinetic and the other (0.864 g/L) for brain pharmacokinetic. The stock solutions of β-asarone were diluted with methanol to produce appropriate working solutions before using. All the standard solutions were stored at 4 °C.
The blank blood samples were spiked with working solutions to achieve the following calibration standard concentrations: 0.361, 0.722, 1.445, 2.890, 5.780, 11.560 and 23.120 mg/L. And the blank brain tissues were prepared in the same way to achieve the following calibration standard concentrations: 0.340, 0.690, 1.380, 2.760 and 5.520 mg/L.

4.4. Sample preparation

The calibration standards of blank blood samples were homogenized at a speed of 3000 rpm for 15 min. 300 μL plasma for every samples were prepared by making an plasma-methanol (1:3, v/v) homogenate (10000 rpm for 10 min at -4 °C), and then the supernatants were filtered through a 0.45-μm microporous membrane. The blood samples of treatment groups were prepared in the same way. 10 μl of the upper layer was drawn off carefully and injected for analysis.
The calibration standards of blank brain samples were prepared by making a tissue-methanol (1:20, w/v) homogenate (10000 rpm for 10 min at -4 °C), and the supernatants were filtered through a 0.45-μm microporous membrane. The brain samples of treatment groups were prepared in the same way. 10 μl of the upper layer was drawn off carefully and injected for analysis.

4.5. Precision and accuracy

The precision and accuracy of the method were examined by adding known amounts of β-asarone to the drug-free plasma of blank groups (quality control samples). For intra-day precision and accuracy, five replicate quality control samples at three concentrations (0.11100, 0.05550 and 0.00222 μg/10 μl) were assayed on the same day. The inter-day precision and accuracy were evaluated on three different days.

4.6. Recovery

The analytical recovery for plasma at three different concentrations of β-asarone (0.11100, 0.05550 and 0.00222 μg/10 μl) was determined. Known amounts of β-asarone were added to drug-free plasma and assayed (five samples for each concentration level).

4.7. Statistical analyses

The pharmacokinetic model and parameters were fitted and calculated by the Drug And Statistics ver2.0 software (Mathematical Pharmacology Professional Committee, China). Measurement data were expressed as mean ± standard deviation ($x \pm s$) and compared by analysis of variance and $p < 0.05$ was considered significantly different. All statistical analyses were performed with version SPSS 13.0 statistical software.

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