

Permeability of CPU-86017 through the BBB after Intravenous and Intracerebroventricular Injection in Mice

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【ABSTRACT】 **AIM:** To investigate the bi-directional penetration of CPU-86017 across the BBB (Blood Brain Barrier) following *iv* and *icv* (intracerebroventricular) administration in mice. **METHOD:** The levels of CPU-86017 (p-Chlorobenzyl-tetrahydroberberine hydrochloride) in the brain, heart, kidney and blood of mice after acute administration of 3.0 mg/kg of CPU-86017 were measured by validated HPLC assay at several time points: 5, 10, 20, 30 and 60 minutes. **RESULT:** The maximum concentrations of CPU-86017 in the brain, heart, kidney and plasma achieved at 10 minutes by both routes of administration were 0.83 ± 0.335 , 25.13 ± 4.17 , 56.0 ± 19.69 and $2.23 \pm 0.97 \mu\text{g/ml}$ in the *iv* group and 23.68 ± 4.2 , 15.9 ± 10.24 , 7.93 ± 4.68 and $3.32 \pm 2.3 \mu\text{g/ml}$ in the *icv* group, respectively. The decline in concentrations was rapid in plasma. The highest concentration of CPU-86017 was found in the kidney ($56.0 \pm 19.69 \mu\text{g/g}$) after *iv* administration and in the brain ($23.68 \pm 4.2 \mu\text{g/g}$) after *icv* injection. The difference in concentrations in the kidney and heart was not significant at 60 min after *iv* administration. Given by *icv* administration CPU-86017 reached the peripheral tissues and plasma at each of the five time points, whereas by the *iv* route at 20, 30 and 60 min the drug could not be detected in the brain. **CONCLUSION:** The permeability of CPU-86017 through the BBB was well established by two ways: blood circulation to the brain and brain to general circulation. However, a big difference exists between these ways. It is more difficult to penetrate from the blood to the brain than from brain to the peripheral.

【KEY WORDS】 CPU-86017; BBB; Pharmacokinetics; HPLC

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1 Introduction

Several investigations in China have examined p-chlorobenzyltetrahydroberberine (CPU-86017), for its antiarrhythmic activity^[1-4] in experimental animals. However, no report has appeared on its penetration through the blood brain barrier (BBB). Previously, we reported that the administration of CPU-86017 showed deeper sedation, greater loss of the right reflex and higher mortality in the *icv* route than that in the *iv* group^[3]. Since CPU-86017 was able to cross the BBB, it was possible that the brain and the cardiovascular^[5] system were the target organs of its toxic effect^[3]. However, these suggestions were related to the effect of the drug in tissues at one point of time. CPU-86017 provided protection against arrhythmia and cerebral ischemia^[1,4]. Unfortunately CPU-86017 has significant toxicity causing a lowering of blood pressure and affecting the heart (bradycardia, a delay in atrio-ventricular conduction)

when given by *iv* injection in some animal model^[5].

The present study was undertaken to explore the penetration of CPU-86017 across the blood brain barrier in two directions: brain to the blood circulation and general circulation to the brain^[7]. The study was focused on CPU-86017 levels in the brain, heart, kidney and blood at different time points after *iv* and *icv* (intracerebroventricular) medication.

2 Materials and methods

2.1 Chemicals

CPU-86017 and tetrahydroberberine (THB) were obtained from the Center of New Drug Research and Development, China Pharmaceutical University, Nanjing, PR China. All solvents were of analytical or HPLC grade. Composition of Krebs - Henseleit (K-H) solution was as follows (mmol/L): NaCl 119; NaHCO₃ 25; KCl 4.6; MgCl₂ 1.2; KH₂PO₄ 1.2; CaCl₂ 2.5; Glucose 11.

2.2 Animals

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Mice, Kwunning of both sexes (weight 18 ~22 g), were purchased from The Animal House of China Pharmaceutical University (Certificate No: SCXK 2002-0011).

They were housed in ventilated cages and maintained under standard laboratory conditions and allowed to feed ad libitum on dry mouse diet and tap water before being used.

2.3 Experimental protocol

The mice were divided randomly into 5 groups of 6 animals for each route of administration. They were assigned to receive CPU-86017 doses of 3 mg/kg. CPU-86017 was dissolved in 0.9% saline before being used and the volume of injection was 0.4 ml/20 g for *iv* and 0.05 ml/20 g for *icv*, respectively. The site for *icv* was at bregma 1mm on the left ventricle.

2.4 Measurement of CPU-86017 concentration

Measurement was performed in 5 groups of 6 mice receiving a dose of 3mg/kg either by *icv* or by *iv*. 5, 10, 20, 30 and 60 min after medication blood samples were collected in clean tubes and animals were killed. Tissue samples (brain, heart and kidney) were removed and weighed.

2.4.1 Determination of CPU-86017 concentration in Plasma Chloroform(6 ml) was added to 1 ml plasma containing 125 μl of internal standard(THB, 0.625 μg/ml), the samples were shaking for 5 min and centrifuged at 2,500 rpm for 10 min. The organic phase was separated and evaporated at 55 °C to dryness under nitrogen. Residues were dissolved in100 μl mobile phase and an aliquot (20 μl) was analyzed by HPLC with an LC-10AT VP, SPD-10A system (Shimadzu, Japan) and a Hyper-sil (C₁₈, 5 μm particle size, 15 cm×4.6 mm ID). The mobile phase consisted of methanol - acetate buffer (53 : 47, v/v), pH 3.67 at a flow rate of 2 ml/min. The detection wavelength was 234 nm

2.4.2 Determination of CPU-86017 concentration in Brain, Heart, and Kidney Samples of tissue (1 g) were homogenized in 10 ml of K-H solution. An aliquot

(1 ml) was transferred and treated as for plasma above. For animals given drug by the *iv* route the whole hemisphere was used, but for animals given an *icv* injection into the left lateral cerebral ventricle, only the right hemisphere was taken.

The retention times of CPU-86017 and its internal standard (THB) were in blood, brain, heart and kidney; 6.848 and 3.679 min; 6.173 and 3.857 min; 6.1 and 3.673 min; 6.515and 3.557 min respectively. There was no interference of the peak of the tested compound by endogenous substances.

2.5 Assay validation

Linear regression was carried out based on area ratios of CPU-86017 to the internal standard plotted against CPU-86017 concentrations. A calibration curve was prepared by adding appropriate volumes of stock solution of CPU-86017 and THB to centrifuge tubes containing homogenized tissue samples. Final concentrations were in the range 0.04 ~30 μg/ml(*n*=5).

2.6 Data and statistics

All data were expressed as mean ±s. Student’ s test was applied to determine the statistical significance based on *P*<0.05.

3 Results

3.1 Concentration of CPU-86017 in brain, heart, kidney and plasma

The concentrations of CPU-86017 in different organs after *iv* or *icv* administrations were measured following 3 mg/kg medication at several points of time: 5, 10, 20, 30, 60 min. The data presented in Table 1 and Table 2 allow a comparison of the concentration of CPU-86017 in different organs of mice at several time points. The peak concentrations of CPU-86017 in the brain, heart, kidney and plasma were achieved at 10 min by both routes of administration. Concentrations then fell sharply at 20, 30 and 60 min after *iv* and *icv* administrations as shown in Table 1 and Table 2

Tab 1. Concentration of CPU-86017 in brain, heart, kidney (μg/g) and plasma (μg/ml) by the *iv* routes in mice ($\bar{x}\pm s$, *n*= 6 for each point time)

	5 min	10 min	20 min	30 min	60 min
Brain	0.742±0.25	0.83±0.335	0	0	0
Heart	21.48±7.12	25.13±4.17	12.67±2.69*	11.66±2.7*	11.16±2.84*
Kidney	40.08±14.0	56.0±19.69	28.54±5.61*	21.15±4.62*	10.14±2.67*
Plasma	4.71±2.5	2.23±0.97	0.335±0.03*	0.42±0.07*	0.38±0.02 ^b

* *P*< 0.05 vs 10 min

Tab 2 Concentration of CPU-86017 in brain, heart, kidney ($\mu\text{g/g}$) and plasma ($\mu\text{g/ml}$) by the *icv* routes in mice($\bar{x}\pm s$, $n=6$ for each point time)

	5 min	10 min	20 min	30 min	60 min
Brain	21.67 $\pm$ 10.34	23.68 $\pm$ 4.2	12.68 $\pm$ 8.47	10.69 $\pm$ 6.31 [*]	7.39 $\pm$ 4.42 [*]
Heart	3.8 $\pm$ 0.94 [*]	15.9 $\pm$ 10.24	8.33 $\pm$ 2.47	4.66 $\pm$ 1.55 [*]	4.5 $\pm$ 1.08 [*]
Kidney	6.45 $\pm$ 3.26	7.93 $\pm$ 4.68	6.25 $\pm$ 0.57	4.82 $\pm$ 1.59	2.93 $\pm$ 0.54 [*]
Plasma	1.54 $\pm$ 0.83	3.32 $\pm$ 2.3	1.81 $\pm$ 0.54	1.91 $\pm$ 0.85	0.565 $\pm$ 0.33 [*]

^{*} $P<0.05$ vs 10 min

3.1.1 *Concentration of CPU-86017 in brain, heart, kidney and plasma after iv route* The concentration of CPU-86017 following *iv* injection, in the kidney was approximately two times higher than that in the heart at 5, 10, 20, and 30 min. But at 60 min, the difference was not significant between kidney and heart. The cardiac concentration at 30 min was almost the same as at 60 min. The lowest concentration of CPU-86017 was found in the brain at all time points and reached an undetectable concentration after 10 min. The concentration in the heart by *iv* route was greater than that in the brain at all time points.

3.1.2 *Concentration of CPU-86017 in brain, heart, kidney and plasma after icv route* The highest concentrations of CPU-86017 following *icv* injection, were found in brain (the opposite hemisphere to the *icv* injection) followed by the heart. At 5 min the concentration of CPU-86017 in the kidney was higher than that in the heart but at 30 min the difference between the heart and kidney was not significant.

The concentration of CPU-86017 in the brain was significantly higher following *icv* administration than following *iv* administration. Conversely, following *icv* administration the cardiac concentration of CPU-86017 was significantly lower than that following *iv* administration.

The concentration of CPU-86017 in plasma was much lower than that in the kidney and heart at all time points after both routes of administration. However, the concentration was higher in the *icv* group than in the *iv* group at 10, 20, 30 and 60 min as shown in Table 1 and Table 2.

4 Discussion

The results of this investigation provide a better description of the permeability of CPU-86017 through the BBB after *iv* and *icv* in mice than it was available previously^[3]. The permeability of CPU-86017 was confirmed by HPLC assay^[8,9] of brain levels following two routes of

injection. The appearance of CPU-86017 in the brain was substantial and higher by the *icv* route than by the *iv* route and was accompanied by higher plasma levels of CPU-86017 at all time points save at the 5 minute time point. This report indicates that by the *icv* route, CPU-86017 exits easily through the blood brain barrier and is continuously released from the brain to the blood circulation. In addition, this finding reveals that the drug not only passes easily through the BBB into the brain, but also diffuses faster from the cerebral tissue to the blood circulation than it distributes into tissues. Thus, the biological responses after *icv* injection are considered to be due to an acute toxic effect on cerebral tissue and peripheral target organs after diffusion into the circulation^[3].

Intravenously injected CPU-86017 appeared in cardiac tissue, reaching a significantly higher concentration than in plasma and the brain. This finding indicates that CPU-86017 does not easily cross the blood brain barrier from the general circulation to the brain and supports our previous report that the heart is an important target organ of CPU-86017's pharmacological effects^[1,6]. In addition, the low concentration of CPU-86017 found in the brain at 5 and 10 min reached an undetectable value at 20, 30, 60 min. Since CPU-86017 could reach the cerebral tissue by *iv* route, the brain might be also the target organ of CPU-86017. This confirms that CPU-86017 does not only exert a central effect together with peripheral effect on the circulation but also gets into the brain from the general circulation after *iv* injection. The deep sedation observed after administration of CPU-86017 by both routes in mice presumably reflects this central effect.

CPU-86017 produces a hypotensive effect in some animal models following *iv* administration^[3]; the locus of this effect remains to be determined although the high concentrations in the kidney, imply that the kidney could be considered as one of the main target organs of

this compound.

The results of this research demonstrate that CPU-86017 crosses the blood brain barrier by two directions: from the blood circulation to the brain and from the brain to the general circulation. However, a significant difference exists between the two directions. CPU-86017 exits from the brain to the general circulation more easily than it enters the brain from the blood circulation. The BBB is a two-way transport phenomenon for CPU-86017, although its transport mechanism^[1] through the BBB remains to be investigated.

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氯苄律定 CPU-86017 在小鼠静脉给药和脑室给药后通过血脑屏障的渗透性

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【摘 要】 目的: 研究小鼠静脉给药和脑室给药后 CPU-86017 通过血脑屏障的双向渗透性。方法: 小鼠给予 3.0 mg/kg CPU-86017(盐酸对氯苄四氢小檗碱)后, 在 5, 10, 20, 30, 60 min 时用 HPLC 测脑、心、肾和血中 CPU-86017 的水平。结果: 两种途径给药的 CPU-86017 在小鼠脑、心、肾和血浆中的最大浓度基本上在 10 min 时都达到最大值, 在静脉给药组分别为 0.83±0.335, 25.13±4.17, 56.0±19.69, 2.23±0.97 μg/ml, 在脑室给药组分别为 23.68±4.2, 15.9±10.24, 7.93±4.68, 3.32±2.3 μg/ml。小鼠血浆浓度很快减少。静脉给药后 CPU-86017 在肾中的最大浓度是 56.0±19.69 μg/g, 脑室给药后, 在脑中的最大浓度是 23.68±4.2 μg/g。静脉给药 60 min 后, CPU-86017 在肾中和脑中的浓度没有显著性差异。脑室给药后, CPU-86017 到达外周组织和血浆中的浓度每 5 分钟都会有变化, 而静脉给药后, 在 20, 30 和 60 min 时, 脑中的药物浓度仍不能被检测出。结论: CPU-86017 通过血脑屏障的渗透可以通过两条途径: 从血液循环到脑和从脑到全身循环。然而, 这两条途径存在很大的不同。从血渗透到脑比从脑渗透到外周更困难。

【关键词】 CPU-86017; 血脑屏障; 药动学; HPLC