EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 3
453-458
Year: 2016

THE PHARMACOKINETICS STUDY OF TRANSCRANIAL ADMINISTRATION OF AMILORIDE HYDROCHLORIDE

Tingyu Huang[1], Zaiyou Tan*[1], Yuqing Huang[1], Chunmei Tong[1], Wenfei Chen[1], Shaokun Lu[1], Yixuan Chen[1], Xiaoxin Zhuang[1], Peishan Zheng[1], Yingqiang Lai[1], Yang Yang[1], Ruonan Wang[1], Benjamin Wong[2]

[1]Guangdong Pharmaceutical University, Guangdong Guangzhou, 510006.
[2]The University of Edinburgh, Edinburgh City,UK.

***Author for Correspondence: Zaiyou Tan**
Guangdong Pharmaceutical University, Guangdong Guangzhou, 510006.

Article Received on 08/01/2016 **Article Revised on 29/01/2016** **Article Accepted on 20/02/2016**

ABSTRACT

Objective: To develop a new route of administration,Amiloride hydrochloride has been investigated by transcranial administration in the rat. **Methods:** The concentration-time relationship of Amiloride hydrochloride was observed after Amiloride hydrochloride smeared on rats' head. The concentration of Amiloride hydrochloride was detected by validated HPLC method, fitted data by 3p97 program and calculated the pharmacokinetic parameters by AIC minimum principle. **Results:** The pharmacokinetics of Amiloride hydrochloride is in line with one-compartment model with first absorption and lag time in rat's brain(weight=1). The A of Amiloride hydrochloride was 0.266875±0.0290 μg/mL, Ke was 0.004208±0.0002min^{-1} ,Ka was 0.092585±0.0313min^{-1}, Lag time was 50.416229±1.3574 min, $T_{1/2}$(Kα) was 7.486592±1.0691min, $T_{1/2}$(Ke) was 164.7375564±11.2555 min, T_{peak} was 85.39389±2.6294 min, C_{max} was 0.219884±0.0201 μg/mL, AUC was 60.544632±4.0446 (μg/mL)*min, CL/F(s) was 1.982009±0.1319 mg/kg/min/(ug/mL), V/F(c) was 471.0563±40.2545 (mg/kg)/(ug/mL). **Conclusion:** The data indicated that Amiloride hydrochloride could penetrate into the brain for the treatment of brain diseases by transcranial administration. Amiloride hydrochloride has a potential prospect for treatment of brain diseases. The transcranial administration of drugs should be a good dosage form for diseases treatment.

KEYWORDS: Transcranial administration, pharmacokinetics, amiloride hydrochloride.

INTRODUCTION

An ambitious plan to explore the mysteries of the human brain(Brain Project) has been officially put on the agenda in 2013.The project's budget reached $4.5 billion.[1] The Brain Project is to provide a colored 3-D MRI scan of the brain's white matter pathways traces connections between cells in the cerebrum and the brain stem for researchers to better understand how we think and learn and memory. Finding new treatments for Alzheimer's disease, epilepsy and traumatic brain injury are also included in. Brain diseases such as stroke, Parkinson's disease, Alzheimer's syndrome has plagued human health. Because of the understanding of brain structure and function is restricted, there is even cerebral blood barrier, so that the efficacy of the drug treatment of these diseases are greatly affected. To improve the situation above and make a great progress in the treatment of encephalopathy, it will inevitably become an important part of Brain Project.

Mr. Theodore L. Roth[2], who is a researcher of neurological dysfunction and stroke in NIH, published an article in the journal Nature. It shows that they developed a novel route of administration and a novel murine closed-skull brain injury model that mirrors some pathological features associated with mild traumatic brain injury (TBI) in humans. They combined transcranial administration with novel murine closed-skull brain injury model to do experiments, and use this delivery route to modulate inflammation and therapeutically ameliorate brain injury through transcranial administration of glutathione. The results show that the small-molecular-weight compound can penetrate into brain through skull bones . It also provides a means to locally deliver therapeutic compounds to the site of injury. We believe that the drug can penetrate into brain through the skull bones by transcranial administration. This route of administration not only can avoid the cerebral blood barrier, but also no damage. It is conducive for patient compliance.

In the meanwhile, a variety of neurological diseases are caused by brain dysfunction at worldwide (such as Alzheimer's disease, Parkinson's disease, schizophrenia, bipolar disorder, epilepsy, attention deficit hyperactivity disorder, etc.). Transcranial administration can provide a

novel route of administration for the treatment of neurological diseases. Transcranial administration will be a breakthrough point for the treatment of neurological diseases.

We chose Amiloride hydrochloride, which have neuroprotective and treatment of stroke[3,4,5], combined with transcranial administration specially and studied transcranial absorption of Amiloride hydrochloride. The detailed work is as follows.

2 Experimental

2.1 Reagents, Chemicals and Animals: Amiloride hydrochloride was purchased from Wuhan yuanchen gongchuang Technology Co., Ltd. Methanol (high performance liquid chromatography grade) was purchased from Tianjin Kermel Chemical Reagent Co,Ltd. Phosphoric acid (analytically pure) was purchased from Guangzhou Chemical Co,Ltd. Perchloric acid (analytically pure) was purchased from Zhiyuan Tianjin chemical reagent Co, Ltd.

Sprague Dawley rats were purchased from Experimental Animal Center of Southern Medical University,license number SCxK (Guangdong) 2011-0015. Researches comply with the Principles of Laboratory Animal Care (NIH Publication No.85-23,revised 1985).

2.2 Assay procedure

2.2.1 Validation of analytical method[6]

A HPLC system with UV detection was employed for the analysis of the samples. The HPLC system consists of a Shimadzu LC-10ATvp pump,a SPD-10Avp detector and a autosampler. A Phenominex Prodigy Analytical Guard column (12.5 × 4.6mm) was used as precolumn. Phenomine Genimi C_{18} , 250×4.6mm column was used. A mobile phase consists of methanol and water (adjusted to pH 3 with phosphoric acid) (20:80). Measurement was carried out at a flow-rate of 0.5 mL/min in ambient laboratory temperature. The detection wavelength was 361nm. The injection volume was 20μL.

No interference was found in the region of Amiloride hydrochloride (see Fig.1 , Fig. 2, Fig. 3 and Fig. 4). This confirms specificity of the methods. The Amiloride hydrochloride retention time was 10.616min(Fig.2).The limit of detection(S/N=3) was 0.0002 μg/mL and limit of quantitation(S/N=10) was 0.0008 μg/mL in brain homogenates.

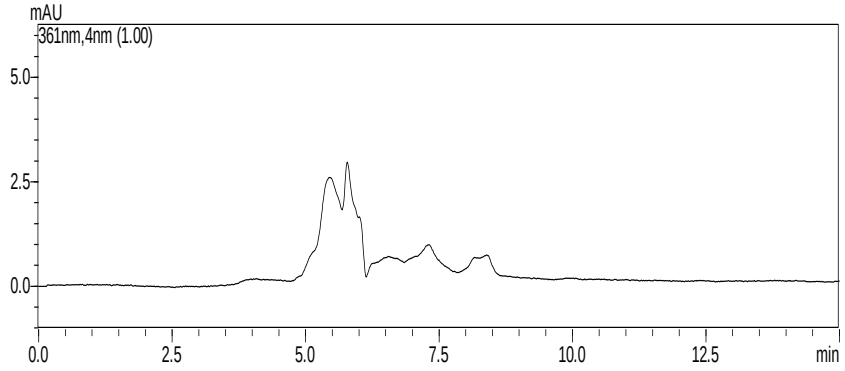

Fig. 1 The chromatogram of rat brain homogenates.

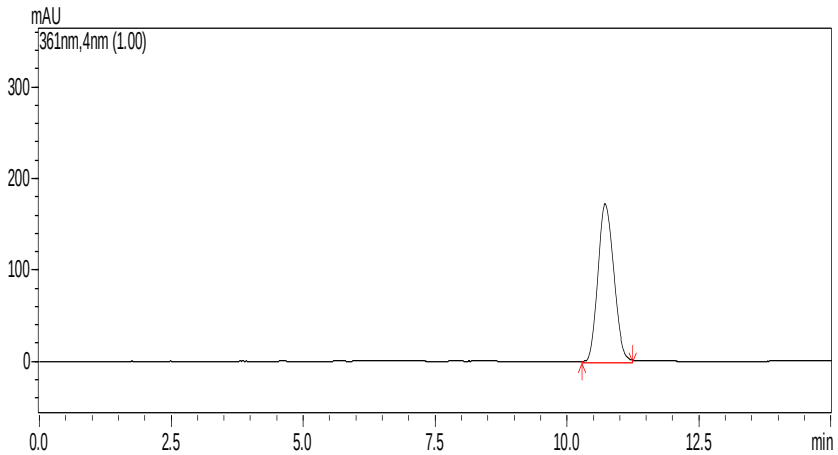

Fig. 2 The chromatogram of Amiloride hydrochloride solution

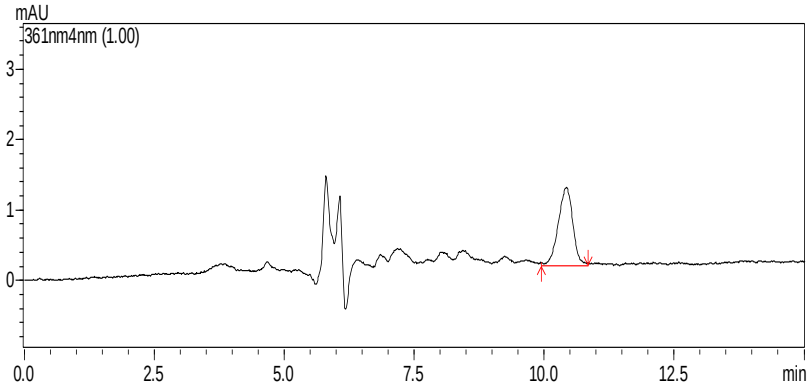

Fig. 3 The chromatogram of Amiloride hydrochloride added in rat brain homogenates.

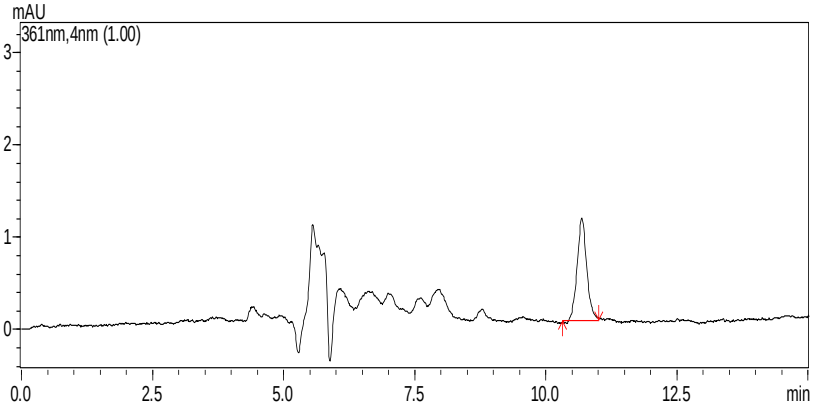

Fig. 4 The chromatogram of Amiloride hydrochloride in rat brain homogenates.

Calibration curves equation of Amiloride hydrochloride is y = 70724x + 1983.7. Lnearity of the methods was confirmed(R^2=0.9991),over a range of 0.02~0.32 μg/mL. The relative standard deviation (RSD) of intra-day precision were 3.33%,1.73% and 7.98% respectively in low(0.08 μg/mL), medium(0.16 μg/mL) and high(0.24 μg/mL) concentrations and RSD of inter-day precision were 0.47%, 0.72%, 1.04% respectively (Table 2 and Table 3).All RSD of intra-day and inter-day precision were small than 10%, they are corresponding to quality control samples of the method.[7] RSD of stability at ambient laboratory temperature were 0.61%, 0.06%, 1.59% within 10 hours(Table 4). All of them were small than RSD<10%, they are in line with the methodology requirements[7] The extraction recoveries were 88.2±2.08%,87.9±1.97% and 90.4±2.36% respectively in low, medium and high concentrations(Table 5). All of extraction recoveries were more than or equal to 70%, they are in line with the methodology requirements.[7] The relative recoveries were 105.9±1.12%, 101.2±1.42% and 112.0±2.78% in low, medium and high concentrations(see Table 6). All of relative recoveries were between 85% and 115% , they are in line with the methodology requirements.[7]

Table 1. Calibration curves of Amiloride hydrochloride in rat brain homogenates (n=5)

C(mg/mL)	0.02	0.08	0.12	0.16	0.20	0.24	0.32
A	3626±12	7348±20	10618±50	13332±56	15852±65	18965±68	24770±58

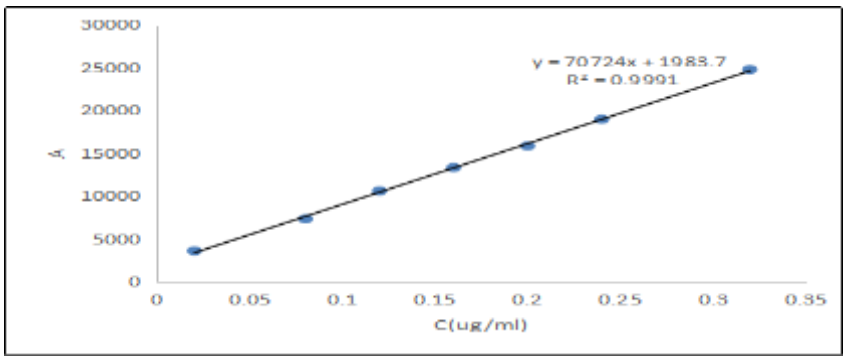

Fig.5 Calibration curves of Amiloride hydrochloride in rat brain homogenates

Table 2. Intra-day Precision of Amiloride hydrochloride in rat brain homogenates (n=5)

C(μg/mL)	1	2	3	4	5	mean±SD(%)	RSD(%)
0.08	0.0714	0.0726	0.0773	0.0743	0.0764	0.0744±0.0025	3.33
0.16	0.1620	0.1620	0.1651	0.1575	0.1601	0.1613±0.0028	1.73
0.24	0.2855	0.2887	0.3009	0.2878	0.3004	0.2926±0.0234	7.98

Table 3. Inter-day Precision of Amiloride hydrochloride in rat brain homogenates (n=5)

C （μg/mL）	0.08	0.16	0.24
Day 1	0.0734	0.1605	0.2917
Day 2	0.0728	0.1603	0.2863
Day 3	0.0734	0.1624	0.2913
mean±SD(%)	0.0732±0.0003	0.1611±0.0012	0.2898±0.0030
RSD(%)	0.47	0.72	1.04

Table 4. Stability of Amiloride hydrochloride in rat brain homogenates (n=5)

C(μg/m)	0.08	0.16	0.24
0 hour	0.7924	0.1605	0.2605
10 hour	0.7856	0.1603	0.2547
mean±SD(%)	0.7890±0.0048	0.1604±0.0001	0.2576±0.0041
RSD%	0.61	0.06	1.59

Table 5. Extraction Recovery of Amiloride hydrochloride in rat brain homogenates(n=5)

C(μg/mL)	1	2	3	4	5	Mean±SD(%)	RSD(%)
0.08	86.2	88.8	90.6	85.7	89.8	88.2±2.08	2.36
0.16	87.1	87.9	89.1	84.9	90.0	87.9±1.97	2.24
0.24	91.9	92.3	92.1	88.6	87.2	90.4±2.36	2.61

Table 6. Relative recovery of Amiloride hydrochloride in rat brain homogenates (n=5)

C(μg/mL)	1	2	3	4	5	mean±SD(%)	RSD (%)
0.08	104.9	104.8	106.0	107.5	106.4	105.9±1.12	1.06
0.16	101.1	103.0	101.1	99.1	101.8	101.2±1.42	1.40
0.24	113.7	113.3	113.5	107.1	112.2	112.0±2.78	2.48

2.2.2 Sample Preparation

1 g brain tissues samples was mixed with 1.5 mL double-stilled water,and homogenized for 4 min by FJ-200 tissuemizer (Jingke Huarui Instrument Company,Peking). Then 200 μL 6% perchloric acid was added to 0.5 mL centrifuge tube with 200 μL brain tissue homogenate organization for protein precipitation. After vortexing 2 min,the mixture was centrifuged at 12000 rpm for 5 min. The supernatant was filtered through 0.22 μm organic membrane filter. 20 μL of this reconstituted solution was injected into the HPLC system for analysis after filtration.

2.2.3 Pharmacokinetics study of transcranial administration[8]: 35 SD rats (250 ± 10 g) were divided into 7 groups randomly . All of SD rats have been fasting for 8 hours before the experiment. A single dose of Amiloride hydrochloride(120mg/kg) was smeared on each rat's hairless head by swab. After transcranial administration,brain tissues were collected at 60,90,120,150,180,240,300 min respectively. To remove blood, brain tissues were cleaned with NS immediately and then bolted dry with filter paper.

3. RESULTS AND DISCUSSION

The experimental data were processed by 3p97.[9] Concentration of Amiloride hydrochloride at each time point are shown in Fig. 6,the pharmacokinetic parameters are shown in Table 7.

Table 7. The pharmacokinetic parameters of transcranial administration of Amiloride hydrochloride

Parameters	Unit	Value
A	$\mu g*mL^{-1}$	0.266875±0.0290
Ke	min^{-1}	0.004208±0.0002
Ka	min^{-1}	0.092585±0.0313
Lag time	min	50.416229±1.3574
$T_{1/2}(K\alpha)$	min	7.486592±1.0691
$T_{1/2}(Ke)$	min	164.7375564±11.2555
T_{peak}	min	85.39389±2.6294

C_{max}	μg*mL^{-1}	0.219884±0.0201
AUC	(μg*mL^{-1})*min	60.544632±4.0446
CL/F(s)	mg/kg/min/(ug*mL^{-1})	1.982009±0.1319
V/F(c)	(mg*kg-1)/(ug*mL^{-1})	471.0563±40.2545

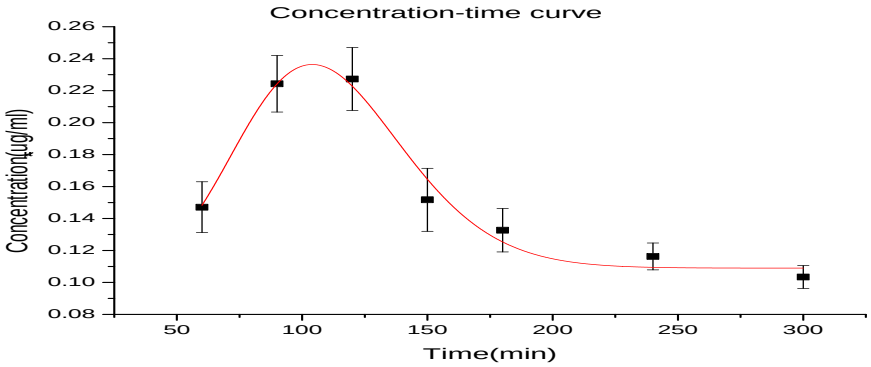

Fig.6 Concentration of Amiloride hydrochloride in different time after transcranial administration

This thesis was for the study of brain tissue pharmacokinetics of Amiloride hydrochloride in the rat after transcranial administration. Amiloride hydrochloride was in line with one-compartment model with first absorption and lag time in rat's brain(weight=1). The T_{peak} of Amiloride hydrochloride was about 85.39389±2.6294 min and C_{max} was about 0.219884±0.0201μg/mL, which indicated that Amiloride hydrochloride could penetrate into the brain. The data also shows that Amiloride hydrochloride had effect after the time of transcranial administration about 50.416229±1.3574 min. And Ke is smaller than Ka ,which indicated that absorption is faster than elimination in rat' brain. The AUC value of Amiloride hydrochloride was 60.544632±4.0446 (μg/mL)*min in brain, which indicated that a amounts of Amiloride hydrochloride could be found in brain.

4. CONCLUSION

In this study, the pharmacokinetics of Amiloride hydrochloride is in line with one-compartment model with first absorption and lag time in rat's brain(weight=1). The A of Amiloride hydrochloride was 0.266875±0.0290 μg/mL, Ke was 0.004208±0.0002min^{-1} ,Ka was 0.092585±0.0313 min^{-1}, Lag time was 50.416229±1.3574 min, $T_{1/2}$(Kα) was 7.486592±1.0691min, $T_{1/2}$(Ke) was 164.7375564±11.2555 min,T_{peak} was 85.39389±2.6294 min, C_{max} was 0.219884±0.0201 μg/mL, AUC was 60.544632±4.0446 (μg/mL)*min, CL/F(s) was 1.982009±0.1319 mg/kg/min/(ug/mL), V/F(c) was 471.0563±40.2545 (mg/kg)/(ug/mL). The pharmacokinetic data shows that drugs can enter the brain tissue through the skull and can achieve therapeutic plasma concentrations of brain diseases. Transcranial administration has potential therapeutic route of administration of various brain diseases. Furthermore, Transcranial administration, which has no damage and can be overcome brain blood barrier, is more conducive for patient compliance during the treatment of brain diseases. As a probe in this experiment, amiloride hydrochloride exhibit valuable penetration. Amiloride hydrochloride has a nutritional effect of nerve cell, it can develop new formulations for the treatment of encephalopathy by transcranial administration.

REFERENCE

1. Sara Reardon,Ambious plans for Brain project unveiled[J].Nature,2014,6:dio:10.1038/nature. 2014; 15375.
2. Theodore L. Roth,Transcranial amelioration of inflammation and cell death after brain injury[J].Nature, 2014; 1(9): 223-228.
3. Phillis JW , Estevez AY, Guyot LL, O'Regan MH.5-(N-Ethyl-N-isopropyl) -amiloride,an Na(+)-H(+) exchange inhibitor, protects gerbil hippocampal neurons from ischemic injury[J] . Brain Res, 1999 Aug 21; 839(1): 199-202.
4. Suzuki Y, Matsumoto Y ,Ikeda Y ,et al .SM -20220,a Na+/H+ exchanger inhibitor: effects on ischemic brain damage through edema and neutrophil accumulation in rat middle cerebral artery occlusion model. Brain Res, 2002; 945: 242.
5. Pilitsis JG,Diaz FG,O'Regan MH,et al. Inhibition of Na(+)/H(+) exchange by SM-20220 attenuates free fatty acid efflux in rat cerebral cortex during ischemia- reperfusion injury. Brain Res, 2001 Sep 21; 913(2): 156-8.
6. David G.Watson. Pharmaceutical Analysis,A Textbook for Pharmacy Students and Pharmaceutical Chemists[M]. Churchill Livingstone (London). 2009; 1-11: 238-274.
7. Li Haozhi. Biopharmaceutical Analysis[M].Beijing:People's Medical Publishing House, 2008: 118-138.
8. Sunil S Jambhekar and Philip J Breen. Basic Pharmacokinetics [M]. Pharmaceutical Press. [London.Chicago]. 2009; 1-1.

9. Wei Shuli, Zhang Qiang. Biopharmaceutics and Pharmacokinetics[M]. 2nd Edition. Beijing: Peking University Medical Press, 2004; 452-463.