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ORIGINAL ARTICLE

Study on the Characteristics of Pharmacokinetics of Different Doses of Gastrodin in Mice

Xinyu Wang, Xin Liu, Xing Wang, Yu Dong

College of Medicine, Southwest Jiaotong University, Chengdu, Sichuan, China

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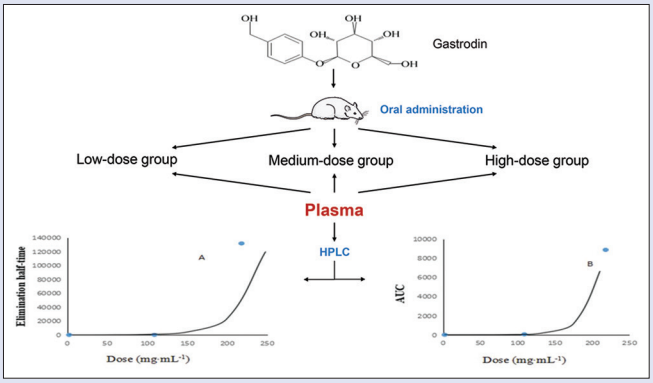
ABSTRACT

Background: *Rhizoma Gastrodiae*, the dried rhizome of *Gastrodia elata* Blume *(G. elata)*, is famous Chinese herb that belongs to the genus *Gastrodia*, family Orchidaceae. Gastrodin (GAS) is an effective monomer with one of the major active constituents in *Rhizoma Gastrodiae*. GAS has a good sedative and sleeping effect and has a mitigating effect on neurasthenia, insomnia, and headache. Moreover, in the circulatory system, it has the effect of reducing peripheral vascular resistance, blood pressure, and so on. **Objectives:** The objective is to investigate the pharmacokinetic characteristics after the administration of different doses of GAS by using pharmacokinetic method and make a preliminary judgment on whether the intake of GAS in the liver is involved in transporters. **Materials and Methods:** All healthy mice were randomly divided into three groups according to the drug concentration: low-concentration group (LC group), middle-concentration group, and high-concentration group. Then, each group received different doses of GAS by tail vein injection. The blood samples of different groups were harvested at different time points, and the blood drug concentration was evaluated by high-performance liquid chromatography method. The method was confirmed in terms of the linearity, precision, and accuracy. **Results:** The results showed that the analytical curve was linear over the concentration range of 4–40 μg/mL. In the intra- and inter-assay, the coefficient of variation was <7.23%. Moreover, the regression equation of the line was "Y = 49.43 X +0.027." The results suggested that the elimination half-life period and area under the curve were slightly decreased, then non-linearly increased accompanying with increase of the dose of GAS. **Conclusion:** The results suggested that the pharmacokinetics of different dosage GAS administration were in accordance with the two compartment model in mice.

Key words: Characteristics, dose, gastrodin, mice, pharmacokinetics

SUMMARY

- Gastrodin (GAS) is one of the major active constituents of *Rhizoma Gastrodiae* extracted from the traditional Chinese herbal agent *Gastrodia elata*. The purpose of this research is to investigate the pharmacokinetic characteristics of different doses administration of GAS using pharmacokinetic method. In the study, all healthy mice were randomly divided into three groups according to the drug concentration. Each group received different doses of GAS by tail vein injection. The blood samples of different groups were harvested at different time points, and the blood drug concentration was evaluated by high-performance liquid chromatography method. The research results suggested that the elimination half-life period and area under the curve were slightly decreased, and then non-linearly increased accompanying with increase of the dose of GAS. The results suggested that the pharmacokinetics of different dosage GAS administration were in accordance with the two-compartment model in mice. Therefore, the present results may provide a novel therapeutic strategy for the application of GAS.



Abbreviations used: TCM: Traditional Chinese medicine; GAS: Gastrodin; LC group: Low concentration group; MC group: Middle concentration group; HC group: High concentration group; HPLC: High-performance liquid chromatography; AUC: Area under the curve; RSD: Relative standard deviation; C_{max}: Peak concentration; K_{12}: Transfer rate constant from one-compartment to two-compartment; K_{21}: Transfer rate constant from two-compartment to one-compartment; Total amount of drugs in body; A: Total amount of drugs in body; V©: Apparent volume of distribution; T1/2: Half-life; CL(s): Clearance.

Correspondence:

Prof. Xing Wang,
No 111, Chengdu North 2nd Ring Road, College of Medicine, Southwest Jiaotong University, Chengdu, Sichuan, 610003, China.
E-mail: wshing@swjtu.edu.cn
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INTRODUCTION

Traditional Chinese medicine (TCM) is a system of theories and therapies through empiricism dating back to 2100 years.[1] TCM is, as main complementary and alternative medicine modalities worldwide, a viable option and plays an increasingly crucial role in international medical practice.[2] Currently, certain single Chinese herbal medicines, traditional Chinese patent medicines, bioactive ingredients, and nondrug strategies comprising acupuncture and Tai Chi Chuan are widely used for the treatment of diseases with low adverse reaction,[3] which implicate in multiple mechanisms consisting of regulation of ion channels,[4] inhibition of inflammatory factors,[5] activity of antioxidant,[6] and so on. Gastrodin (GAS) is one of the major active constituents of *Rhizoma Gastrodiae* extracted from the traditional Chinese herbal agent *Gastrodia elata* Blume (*G. elata*), and its molecular structure is shown in Figure 1. GAS has numerous

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biologic effects including anti-necrosis, anti-aging properties, and anti-apoptotic activities by effectively removing oxygen-free radicals, enhancing antioxidant activity, restraining the coupling of oxidative phosphorylation, and upregulating antioxidant enzymes.[7,8] GAS mainly has liver-calming, wind-extinguishing, and spasm-stopping effects and usually used for the treatment of headache, dizziness, epilepsy, and convulsion.[9] Meanwhile, GAS is also applied to cure complicated and chronic diseases, such as hypertension,[10] diabetes mellitus,[11] and depression.[12] In addition, GAS can effectively decrease total peripheral resistance, blood pressure, increase vascular compliance, cardiovascular blood flow, and protect myocardial cell.[13] Hence, the studies on the pharmacokinetic characters of GAS have constantly increased owing to its magical effects.

Currently, the mainly study method, including a single dose and intragastric administration were utilized to investigate the pharmacokinetic characters of GAS.[14] The administration of Parishin B and *G. elata* extract in clinic result in a longer duration time of action than that of the administration of free GAS[15,16] while multi-dose administration was rarely used. The concentration-time profile in rats after *Gastrodia* administration of GAS was fitted to a one-compartment open model.[17] However, in human plasma, it was fitted to a two-compartment open model.[18]

At the same time, the liver is the most important organ of the body's material metabolism, which mediates the transport and metabolism of various substances. Some studies found that there were a number of drug transporters on the vascular side membrane and the bile duct side membrane in the liver cells, which took drugs from the blood vessels into the liver cells.[12] Moreover, some drugs were secreted from the liver into the bile or systemic circulation in the original form or its metabolites.[16]

Using the pharmacokinetic method of blood concentration, the pharmacokinetic characteristics of different doses of GAS *in vivo* were studied, and the characteristics of the liver transport were preliminarily judged to determine whether the GAS intake in the liver was involved in transporters and the relationship between the dosage of GAS and drug transporters or drug-metabolizing enzyme.[18]

MATERIALS AND METHODS

Animals and grouping

Healthy male Kunming mice (weighing 18–22 g) were purchased from the Center of Experimental Animals, Sichuan Province People's Hospital. Guidelines for laboratory animal care and safety from NIH have also been followed. Animal care and all experimental protocols were approved by the guidelines of the Institutional Medical Experimental Animal Care Committee of Southwest Jiaotong University. All animals were housed in individual cages in a temperature (21°C–25°C) and humidity (45%–50%)-controlled room (laboratory animal room of specific pathogen-free [SPF] on a 12 h light/dark cycle controlled artificially with free access to water and pellet feed). All animals were randomly divided into three groups: Low-dose group (LD group), medium-dose group (MD group), high-dose group [Table 1], 30 rats in each group, 6 rats at each time point.

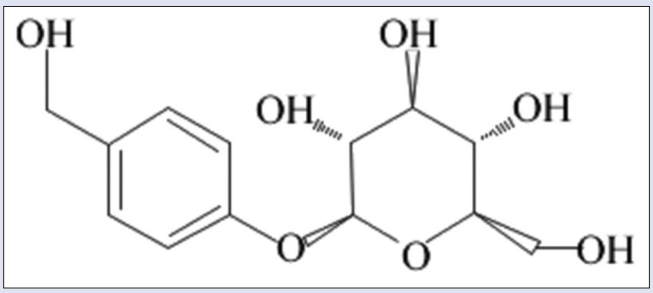

Figure 1: Chemical structure of gastrodin

Instrumentation

Waters 2695 Alliance high-performance liquid chromatography (HPLC) system (Milford, MA, USA) was equipped with column and sample compartment with temperature control and photodiode array wavelength detector (PDA) (Waters 2998), quaternary pump, autosampler, and on-line degasser. Data acquisition, analysis, and reporting were employed using Empower chromatography software (Milford, MA, USA). Experimental conditions were precolumn: Wondasil (4.0 × l0Mm, $C_{18,}$ 5 μm); mobile phase: Acetonitrile 0.2% phosphoric (2.7:97.3), velocity of flow: 1 mL/min; detection wavelength: 220 nm; column temperature: 30°C; and sample volume: 10 μL.

Preparation of chemicals and reagents

GAS (98%, Xi'an Baichuan biological technology co., Ltd, batch lot GA140715) was accurately weighed and confected into different concentration solutions comprising 2.18 mg/mL (low dose), 109 mg/mL (medium dose), and 218 mg/mL (high dose). Moreover, 4.0 mg GAS reference substance (The National Institutes for food and drug Control, 110807-201507) and methyl alcohol (Avantor Chemical Products trading Shanghai Co. Ltd., batch lot 000080789) were compounded as the reference solution (400 μg/mL). 8.0 mg phloroglucinol (Sinopharm Group Co. Ltd, batch lot 20020827) was diluted with methanol (Avantor Chemical Products trading Shanghai Co. Ltd., batch lot 000080789) to prepare internal standard solutions (800 μg/mL). All reagents were stored at 4°C for further usage.

Drug administration and sample harvest

The animals were acclimated to SPF laboratory conditions for 1 week prior to the drug treatments. Before the experiment, the mice were fasted for 12 h. According to the maximum solubility of GAS in physiological saline and preliminary experiment, the high-dose GAS concentration was determined to be 1635 mg/kg.[19] The low-medium-high-dose ratio of 1:50:100 in this experiment was used in this experiment to reach saturation in mice with high-dose mice and compare the pharmacodynamic parameters of three different dose groups to better observe the pharmacodynamic characteristics of GAS in mice. Under the relevant research, there were no acute toxic reactions in the mice after intravenous injection and intragastric administration at a dose of 2500–5000 mg/kg and 1250–5000 mg/kg.[20] Then, different concentrations drug (including 2.18 mg/mL: Low dose, 109 mg/mL: Medium dose, 218 mg/mL: High dose; 0.15 mL per 20 g body weight) were injected into the caudal vein of the mice, respectively. Afterward, blood samples were collected by the heart punctures at 15 min, 60 min, 90 min, 180 min, and 300 min following drug administration. The samples were kept in tubes with sodium heparin and stored at −20°C.

Table 1: Animals and grouping

Points (min)	LD group	MD group	GD group
15	6	6	6
60	6	6	6
90	6	6	6
180	6	6	6
300	6	6	6

LD group: Low-dose group; MD group: Medium-dose group; HD group: High-dose group

Sample pretreatment

One hundred microliters blood sample, 10 μL internal standard solution (800 μg/mL), and 400 μL ethanol were separately added into EP tube. The mixtures were blended for 30 s and centrifuged for 10 min (3500 r/min). The supernatant was transferred to other 1.5 mL microcentrifuge tube and dried using nitrogen in 37°C water, then dissolved in 200 μL double-distilled water. The mixtures were centrifuged for 10 min (10000 r/min). Therefore, the supernatant was sucked and filtered by using microfiltration membrane. The final sample (10 μL) was submitted to the HPLC system.

Method validation

Analytical validation was confirmed according to the recommendations of the International Conference on Harmonization guidelines.[21] The following characteristics were considered for validation: linearity, precision, accuracy, and robustness.

Linearity was evaluated through calculating a regression line from the plot of peak area versus analyte concentration of the eight standard solutions (0.04, 0.2, 0.4, 2, 4, 20, 40, and 80 μg/mL), using the linear least squares methodology. The calibration curves were constructed using the weighted regression method and defining the peak area ratios as functions of the theoretical concentrations.[22] This method was applied to the standard curve (y = ax + b, where x = concentration, y = peak area ratio, a = slope and b = intercept). The slope-intercept of each standard curve was used to determine the concentration values for unknown samples.

The precision of the method was assessed through repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was determined by analyzing different standard solutions consisting of three levels: low (4 μg/mL), medium (20 μg/mL), and high (40 μg/mL) on the same day. Intermediate precision was evaluated through analyzing these standard solutions on three different days, in triplicate.[22]

Accuracy was measured by the percentage recovery value. The standard drug solution (low: 4 μg/mL; medium: 20 μg/mL; and high: 40 μg/mL) was added to blood sample solution, respectively. The value of accuracy and recovery was tested. Analyses were employed in triplicate.

System suitability test

The system suitability was evaluated by three replicate analyses of GAS standard solutions (including 4, 20, 40 μg/mL). In order to verify the system performance, the suitability parameters were separately investigated under normal temperature and freezing conditions. Briefly, the stability of the solutions was examined over a period of 24 h at room temperature, and the concentration was measured every 6 h (4 times in total), and the relative standard deviation (RSD) was calculated. Moreover, the samples were frozen for 21 h at −20°C, then left at room temperature for 3 h. Therefore, the RSD was calculated.

RESULTS AND DISCUSSION

Method validation

Linearity was assessed using a calibration curve. Then, the ability of the analytical method was checked by obtaining a proportional response to the analyte concentration in the sample. The linearity of the analytical method was evaluated, and a calibration curve was constructed based on eight concentrations from 0.04 to 80 μg/mL. The regression equation of the line was obtained (Y = 49.43 X +0.027), resulting in the correlation coefficient R^2 of 0.999, indicating the quality of curve.

To assess the method precision, GAS standard solutions (4, 20, and 40 μg/mL) were prepared in triplicate and analyzed on the same day (repeatability) and in three different days (intermediate precision), respectively. Tables 2 and 3 separately showed the precision. The maximum RSD value was 7.23%.

Accuracy and recovery were evaluated at three different GAS concentration levels (4, 20, and 40 μg/mL). The percentage of recovery at these three different concentration levels is described in Table 3. The percentage of accuracy and recovery samples was described in Table 4.

System suitability test

The RSD value could reveal the system stability. The results showed that the value of RSD was lower than 7.91% in each group, which indicated that the drug was stable at room temperature. In addition, the RSD value of each group was lower than 5.37%, which suggested that the freeze-thaw stability was good.

The characteristics of pharmacokinetics of different doses gastrodin

The concentrations of GAS in blood samples are shown in Table 5. The pharmacokinetic parameters obtained from 3p97 are shown in Table 6.

The relationship between half-life period/area under the curve and dose of gastrodin

The pharmacokinetic characteristic of GAS was mainly manifest as pharmacokinetic parameters changed accompanied by the dosage alteration. The results showed that the pharmacokinetic parameters, including half-life period and clearance rate, were not constant, which presented in a dose/concentration-dependent manner [Figure 2A]. Moreover, the level of area under the curve (AUC), peak concentration (C_{max}), and cumulative excretion in the urine was not proportional to the GAS dosage [Figure 2B]. Collectively, the non-linear pharmacokinetic parameters of GAS were characterized by plasma concentration, and AUC were not proportional to the GAS dosage, and the elimination half-life time was prolonged associated with the increase of GAS dosage [Figure 2].

The relationship between elimination half-life/area under the curve and dose of gastrodin

Meanwhile, Figure 3A suggests that the elimination half-life period increased non-linearly, and the growth rate was faster, accompanying with increase of the dose of GAS (i.e., the elimination half-life of GAS was prolonged with the dosage increase.). Figure 3B indicates that AUC showed non-linear growth following the dose increase. Therefore, the results revealed that the characteristics of pharmacokinetics of GAS were non-linear in mice. Moreover, the elimination half-life period extended associated with the dose increase, which demonstrated that the

Table 2: Precision for different levels of Gastrodin

Concentration (μg/mL)	The RSD for the inter-day (%)	The RSD for the intra-day (%)
4	4.19	3.45
20	7.23	2.02
40	5.13	7.11

RSD: Relative standard deviation

Table 3: Repeatability for different levels of Gastrodin

Concentration (μg/mL)	Measured concentration±SD (μg/mL)	RSD (%)
4	3.87	0.54
20	19.23	0.47
40	38.94	0.64

SD: Standard deviation; RSD: Relative standard deviation

Table 4: Accuracy and recovery for the high-performance liquid chromatography method

Concentration (μg/mL)	Concentration before extraction (μg/mL)	Concentration after extraction (μg/mL)	Accuracy (%)	Recovery (%)
4	3.7	3.1	92.5	83.8
20	17.9	15.1	89.5	84.4
40	37.1	34.7	92.8	93.5

Table 5: The concentrations of Gastrodin in blood samples of different group

	Concentrations of GAS (μg/mL)		
Points (min)	LD group	MD group	HD group
15	6.58±1.2019	539.6±58.9291	979.14±113.90
60	1.88±0.7397	56.18±18.9890	131.36±58.409
90	2.04±0.6837	70.58±7.7144	212.67±46.049
180	0.70±0.3961	40.69±10.8750	38.5±6.12649
300	0.29±0.0274	13.35±5.5777	49.28±10.4229

LD group: Low-dose group; MD group: Medium-dose group; HD group: High-dose group; GAS: Gastrodin

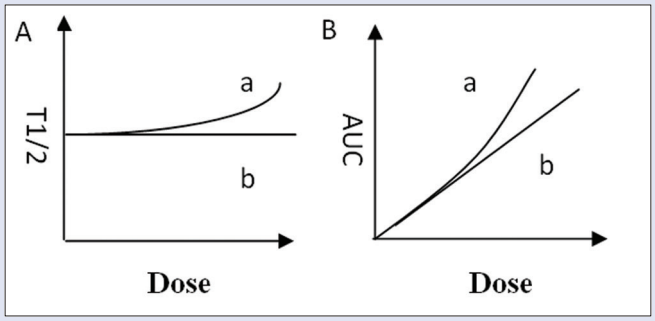

Figure 2: The relationship between the dose of gastrodin and half-life period/area under the curve. (A) The relationship between half-life period and dose of gastrodin. (B) The relationship between area under the curve and dose of gastrodin. a: Non-linear elimination kinetics; b: Linear elimination kinetics

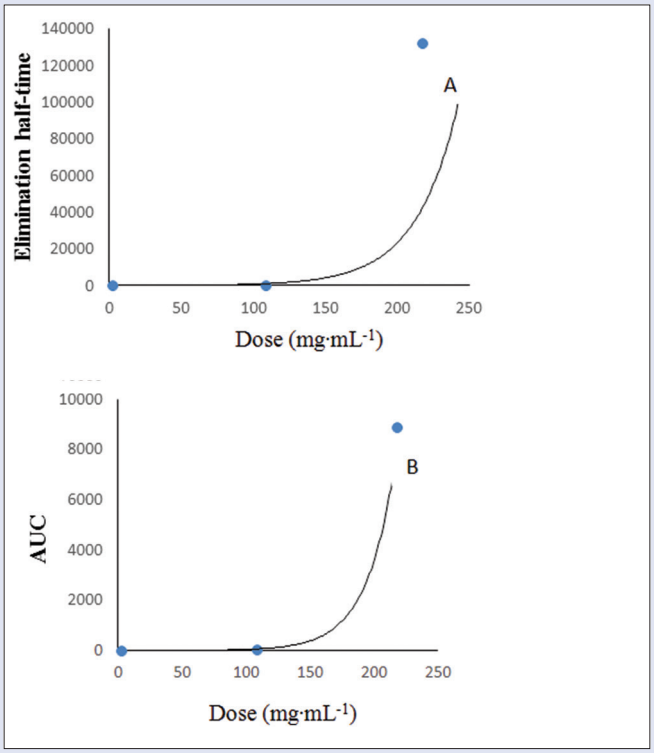

Figure 3: The relationship between elimination half-life/area under the curve and dose of gastrodin. (A) The relationship between elimination half-life period and dose of gastrodin. (B) The relationship between area under the curve and dose of gastrodin

metabolism of GAS was a dose-dependent relationship (i.e., when drug transporter or drug metabolic enzyme had reached saturation, while plasma proteins did not achieve the level of saturation. Consequently, the results indicated that the elimination half-life period was prolonged with the dose increase).

GAS is the principal bioactive component extracted from *G. elata*, which has been used as a TCM to treat various diseases including cardiovascular diseases, dizziness, headache, and dementia for centuries in our country.[23] In 1978, two laboratories simultaneously reported that GAS's formula is $C_{13}H_{18}O_7$, and its molecular weight is 286 Da,[24] and GAS is easy to dissolve in methanol, ethanol, and water. From then on, the biological actions of GAS were extensively investigated and accumulating evidence documented that numerous pharmacological activities had been attributed to GAS comprising sedative/hypnotic, anti-vertigo, analgesic, anti-epileptic,[25] antidepressant,[26] memory-improving,[27] anti-aging, lowering blood pressure,[28] and so on. For now, multidose administration was rarely used to investigate the pharmacokinetic characters of GAS. Consequently, in order to further clarify the pharmacokinetic characters of GAS, GAS administration with different doses by intravenous injection was performed, then blood samples were collected from cardiac puncture at different time points and subsequently detected using HPLC.

The main characteristics of non-linear elimination kinetics are mainly embodied in that some pharmacokinetic parameters change accompanied by the dose alteration, that is, dose-dependent pharmacokinetics. Some pharmacokinetic parameters such as clearance and half-life often manifest as a dose-dependent/concentration-dependent relationship. Simultaneously, the AUC, C_{max}, and the urinary excretions of GAS are not in direct proportion to the GAS dose. Our results revealed that the elimination half-life of GAS and AUC was increased non-linearly with the increasing of GAS dose. Therefore, the pharmacokinetic character of GAS is non-linear in mice. Moreover, the elimination half-life of GAS prolonged with the increasing dose demonstrated that the metabolism of GAS was also in a dose-dependent pattern.

Moreover, the value of K_{12} is higher than K_{21} value, and the deviation value is gradually large with the increasing of GAS dose. This means that the diffusion rate of drug distribution from the central chamber to the surrounding chamber was increased with the increase of dose. As

Table 6: Pharmacokinetic parameters of high-, mid-, low-dose groups

Parameters	Unit	LD group	MD group	HD group
A	ng/L	10.0015±3.55	34,801.5028±20.74	2833.7005±23.37
Alpha	L/min	0.0832±0.11	0.2213±0.12	0.0584±0.01
B	ng/L	1.2958±0.76	127.8175±6.18	46.4737±12.33
Beta	L/min	0.0043±0.00	0.0071±0.00	0.0004±0.00
V (c)	mg/kg/mg/mL	0.4268±0.91	0.1524±0.21	0.6076±0.16
T1/2alpha	min	17.7805±10.61	4.5343±3.81	12.2477±2.57
T1/2beta	min	83.8071±2.10	106.6640±22.49	131,717.97±18.93
K_{21}	L/min	0.0074±0.00	0.0096±0.00	0.0013±0.00
K_{10}	L/min	0.0523±0.081	0.1856±0.12	0.0054±0.01
K_{12}	L/min	0.0278±0.03	0.0332±0.02	0.0520±0.02
AUC	(mg/mL) × min	547.16105±11.26	48,542.6609±23.96	8,895,976.286±13.88
CL (s)	mg/kg/min (mg/mL)	0.0089±0.01	0.0112±0.01	0.0041±0.01

AUC: Area under the curve; K_{12}: Transfer rate constant from one-compartment to two-compartment; K_{21}: Transfer rate constant from two-compartment to one-compartment; A: Total amount of drugs in the body; V(c): Apparent volume of distribution; T1/2: Half-life; CL(s): Clearance

a result, pharmacokinetic characteristics of GAS in mice could be in accordance with open bicameral model.

K_{10} is the constant for eliminating the rate of drugs in the central chamber. With the increasing of dose, the values of K_{10} first increase and then decreases. In this study, the values of K_{10} in MD group are higher than that of in LD group, while which of in HD group are significantly lower than MD group.

This illustrated that the eliminating rate of GAS was obviously slowed down in central chamber with the increase of drug dose when once the drug level went beyond the middle concentration, which may induce by the metabolism of the tissue of the central chamber (such as liver and kidney) reduced slowly.

The plasma protein binding rate plays a pivotal role in the process of drug biotransformation. The higher plasma protein binding rate would contribute to the increase of the concentration of binding drug, and the concentration of free drug is relatively lower. However, many related researches showed that the plasma protein binding rate of GAS is only 4%–7%, so the plasma protein binding rate has little impact on the elimination of GAS.

CONCLUSION

Taken together, the pharmacokinetics of GAS in mice were consistent with the two-compartment model. With the increasing of dose, the elimination half-life was gradually increased. It indicates that plasma protein binding rate of GAS is not saturated in low and middle doses. With the increase of dose, the transporter or metabolic enzymes is gradually saturated, so the elimination half-life is gradually increased.

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Conflicts of interest

There are no conflicts of interest.

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