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Original Research Article

Gastrodia elata powder capsule enhances anti-epileptic effect of carbamazepine by decreasing P-gp expression

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Abstract

Purpose: *To investigate the influence of Gastrodia elata powder capsule (GC) or gastrodin (GTD) on the anti-epileptic effect of carbamazepine (CBZ) on penicillin (PG)-induced epilepsy in rats.*
Methods: *A total 116 rats were used in this study. Rats in the control group (n = 8) were injected with normal saline (NS) in place PG. Epilepsy was induced in the remaining 108 rats on the first day via PG injection. The rats were then divided randomly into six groups (18 rats per group): PG group, CBZ group, CBZ + GC group, CBZ + GTD group, GC group, and GTD group, which were given (p.o.) NS, CBZ (100 mg/kg), CBZ (100 mg/kg.) + GC (350 mg/kg), CBZ (100 mg/kg) + GTD (100 mg/kg), GC (350 mg/kg), and GTD (100 mg/kg), respectively, once a day for 15 days. The behavioral characteristics of the rats were observed and used to assess the anti-epileptic effect of the test drugs. Real-time quantitative reverse transcription-PCR and Western blot assays were employed for the determination of the effect of CBZ, GC and GTD on the expression levels of P-gp.*
Results: *CBZ significantly reduced the symptoms of epilepsy, while GC and GTD enhanced the anti-epileptic effect of CBZ, and reversed the CBZ-induced increases in the protein expressions of mrd1a and P-gp ($p < 0.05$).*
Conclusion: *GC reverses CBZ drug resistance, probably through downregulation of P-gp expression. This finding indicates that GC is a potential anti-epilepsy drug, but it merits further studies.*

Keywords: *Gastrodia elata capsule, Gastrodin, Carbamazepine, Epilepsy, Antiepileptic drugs*

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INTRODUCTION

Epilepsy is one of the most frequent neurological disorders which affects approximately 1-2 % of the world population, with 30 % of epilepsy patients resistant to anti-epileptic drugs (AEDs) [1]. Anti-epileptic drug (AED) resistance is a challenge to the treatment of epilepsy. Once epilepsy patients are resistant to one AED, they will also be resistant to other AEDs, even though the mechanisms of action of these AEDs might be totally different [2]. Furthermore, the mechanism of refractory epilepsy is still not fully understood [3]. Many studies have reported that the expression of multidrug resistance transporter P-gp which is known as drug efflux pump, is significantly increased in refractory epilepsy. Hence, a feasible hypothesis of refractory

epilepsy is that the concentration of AEDs can hardly reach the therapeutic level in the brain because of the drug-induced increase in hippocampal P-gp [4-7]. Therefore, a valid approach to enhance the therapeutic efficacy of AEDs is to reduce the P-gp expression in the hippocampus [8,9].

Traditional Chinese medicine (TCM) had been used for clinical treatment of epilepsy for thousands of years. Epilepsy was first recorded in “Huang Di Nei Jing” which was written in the Qin Dynasty about 2000 years ago. Moreover, there are many experiential and effective recipes such as *Rheum officinale* Bail. Formula, Zhen Heart Pill, and *Gentiana scabra* Bunge. Formula. These are recorded in *Thousand Golden Prescriptions* which was written in the Tang Dynasty by Su Simiao in 652 AD. [10,11]. Studies have shown that some TCMs inhibit the expression of P-gp [12]. Thus, it is possible to treat epilepsy with combination of TCM and antiepileptic drugs.

Gastrodia elata powder capsule (GC) is made from the powder of *Gastrodia elata (Gastrodia elata BL*.), and has been used for a very long time to treat epilepsy in China [13]. It is listed in the Chinese Pharmacopoeia for treating epileptiform convulsion in clinics [14]. Gastrodin (GTD) is the main component of *Gastrodia elata* (Figure 1). It is effective against clonic convulsion and extended latency-time epilepsy induced by pentylenetetrazole [15]. Therefore, in the present study, penicillin (PG)- induced rat epilepsy was chosen as epilepsy model to study whether GC or GTD can improve the curative effect of CBZ on intractable epilepsy. In rodents, two genes, mdr1a and mdr1b encode P-gp [16]. Therefore, real time qRT-PCR was used to investigate the expressions of mdr1a and mdr1b, and Western blot was used to assay the expression of P-gp, so as to unravel the possible molecular mechanism involved in the effect of GC and GTD on epilepsy [17].

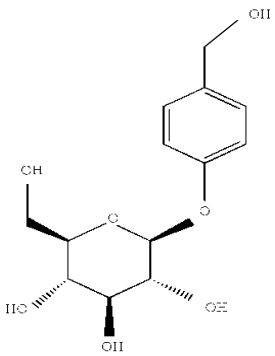

Figure 1: Structure of GTD

EXPERIMENTAL

Animals

Adult male Sprague-Dawley rats weighing 170 - 210 g were purchased from Animal Experiment Center of Lanzhou University (Lanzhou, China). The rats were bred under standard conditions. All experiments were performed between 9:30 a.m. and 3:30 p.m. Ethical approval for the animal studies (B2015-007) was obtained from the Animal Ethics Committee of Lanzhou University, Gansu Science and Technology Office (China). Every effort was made to minimize pain to rats and all experiments were conducted in accordance with the guidelines of Declaration of Helsinki [18].

PG-induced epilepsy rat model

The rat model of epilepsy was established using intraperitoneal injection of penicillin [19] (PG, 760 U/kg, Hayao Pharm, Harbin, China). The intensity of seizure responses was scored according to Racine evaluation system as indicated in Table 1 [20]. Rats showing at least 5 consecutive grade 2 seizures, or 3 consecutive grade 4or 5 seizures, were considered to have PG-induced epilepsy, and these were the only category of rats included in the study. The day the rats were injected PG was recorded as the 1st day.

Table 1: Racine evaluation system

Grade	Intensity of seizure response
0	No response
1	Mouth and facial jerks
2	Nodding or myoclonic body jerks
3	Forelimb clonus
4	Rearing, falling down, hind limb clonus and forelimb tonus
5	Tonic extension of hind limb, status epileptic and/or death

Drug administration

Eight normal SD rats injected with normal saline (NS) were used as the control group (group1). The PG-induced epileptic SD rats were randomly divided into six other groups (18 per group): PG group (group 2), carbamazepine (CBZ) group (group 3), *Gastrodia elata* powder capsule (GC) group (Group 4), gastrodin (GTD) group (group 5), CBZ + GC group (group 6), and CBZ + GTD group (Group 7) (Table 2). Carbamazepine (CBZ), GC and GTD were purchased from China and Western Three-Dimensional Pharmaceutical Company (Shanghai, China); Guizhou, ChengShiLong Pharmaceutical Company (Guizhou, China), and Kunming Pharmaceutical Company (Kunming, China), respectively.

Table 2: Groups, model establishment and drug treatments

No.	Group	Model Establishment	Drug treatment
1	Control	NS	NS
2	PG	PG	NS
3	CBZ	PG	CBZ (100 mg/kg, p.o.)
4	GC	PG	GC (350 mg/kg, p.o.)
5	GTD	PG	GTD (100 mg/kg, p.o.)
6	CBZ+GC	PG	CBZ (100 mg/kg, p.o.) + GC (350 mg/kg, p.o.)
7	CBZ+GTD	PG	CBZ (100 mg/kg, p.o.) + GTD (100 mg/kg, p.o.)

Behavioral tests

On the 1st, 7th and 15th days, the intensity of seizure response, mortality and the incidence of severe seizures were scored as indicated in Table 1. Incidents of severe seizures in grades 3, 4 or 5 were recorded. Mortality in each group was also recorded at each time point.

Sample preservation

On the 1st, 7th and 15th days, 6 rats were randomly selected from each group. After sacrifice, the brain hippocampus of each rat was isolated on ice. The separated hippocampi were immediately weighed and kept in liquid nitrogen for subsequent real time of qRT-PCR, and western blotting analyses.

Real time qRT-PCR

Total RNA was isolated from the hippocampus samples utilizing Trizol reagent according to the manufacturer's instructions. The purity of the RNA isolated was determined using UV absorption at 260 nm and 280 nm with SmartSpec plus (Bio-Rad Laboratories, Richmond, CA, USA). The RNA was reverse-transcribed to cDNA using PrimeScript RT Master Mix reverse transcriptase, while PCR was performed on Gene ABI PCR System 7500. The primers used for the cDNA amplifications are shown in Table 3. They were prepared by TaKaRa Reagent Co. Japan. The expression levels of mdr1a and mdr1b were normalized using the expression level of β-actin (internal control), and the data were calculated with v2.0.6 ABI 7500 software (ABI Laboratories, Shanghai, China).

Western blot

Western blot was performed according to the method described previously [21]. Different samples (25 μg protein per sample) were loaded to each lane. The expression level of P-gp (primary monoclonal P-gp antibody, Santa Cruz Biotechnology, 1:200) was normalized to that of β-actin (Beyotime Biological Co. Ltd, Shanghai, China, 1:1000 dilution). The data was quantified with Quantity One Software (Bio-Rad Laboratories, Richmond, CA, USA).

Statistical analysis

Experiments on drug preparation, drug administration and behavioral observation were done by different researchers who were naïve to treatment allocation throughout the experiment. The statistician was also blind to treatments prior to data analysis. The results are presented as mean ± SD of three biological replicates, and were analyzed using ANOVA and Turkey multiple comparison. All analyses were done with SPSS version 17.0. Statistical significance of difference was assumed at $p < 0.05$.

RESULTS

GC and GTD enhanced the antiepileptic effect of CBZ

Intensity of seizure response, incidence of severe seizures and mortality were scored on the 1st, 7th and 15th days. The results showed that on the 1st day, all rats exhibited grade 5 severe seizures which indicated that the PG- induced epilepsy rat model was successfully established. On the 7th and 15th days, the intensity of seizure response was alleviated in all groups and the mortality decreased to zero %. In addition, CBZ treatment significantly attenuated the intensity of seizure responses in CBZ, CBZ + GC and CBZ + GTD groups. The combination of GC and GTD with CBZ resulted in increases in the anti-epilepsy effect of CBZ, especially GC ($p < 0.05$). However, GC or GTD alone had little effect on the progress of PG-induced seizures ($p > 0.05$) (Table 4). These results indicate that CBZ significantly reduced the symptoms of epilepsy, while GC and GTD (especially GC) enhanced the antiepileptic effect of CBZ.

GC and GTD reversed CBZ-induced increase in mrd1a expression

Real Time qRT-PCR was employed to determine the effect of CBZ, GC and GTD on P-gp

Table 3: Primers used for cDNAs amplification

Gene	Length	Forward	Reverse
mdr1a	196bp	5'-AGGGCCTTAACGGAACAGCAG-3'	5'-AGTTCCCAGAGCCATGCACAG-3'
mdr1b	146bp	5'- CCTGAAATCCAGCGGCAGA-3'	5'-ATGTATCGGAGTCGCTTGGTGAG-3'
β-actin	150bp	5'-GGAGATTACTGCCCTGGCTCCTA-3'	5'-GACTCATCGTACTCCTGCTTGCTG-3'

Table 4: Effect of PG-induced seizures in mice

Group	Intensity of seizure response (%)			Incidence of severe seizure (%)			Mortality (%)		
	1st	7th	15th	1st	7th	15th	1st	7th	15th
PG	5.0±0.00 a*	4.3±0.75 a	4.0±1.03 a	100 a	100 a	100 a	5.55	0	0
CBZ	5.0±0.00 a	3.2±1.24 b	1.75±0.24 c	100 a	80 c	25 e	11.11	0	0
CBZ+GC	5.0±0.00 a	2.0±0.97 b	1.4±0.37 c	100 a	54.54 d	0 f	5.55	0	0
CBZ+GTD	5.0±0.00 a	2.6±1.13 b	1.6±0.47 c	100 a	50 d	0 f	11.11	0	0
GC	5.0±0.00 a	3.9±1.27 a	3.25±1.39 a	100 a	90 b	100 a	11.11	0	0
GTD	5.0±0.00 a	4.0±1.89 a	3.4±1.04 a	100 a	100 a	100 a	5.55	0	0

* Letters at top right corner indicate that there were significant differences among groups ($p < 0.05$). PG, CBZ, GC, GTD represent penicillin, carbamazepine, *Gastrodia elata* powder capsule and gastrodin, respectively

expression level, in order to unravel the mechanism involved (P-gp protein is encoded by mdr1a and mdr1b genes in rodents).

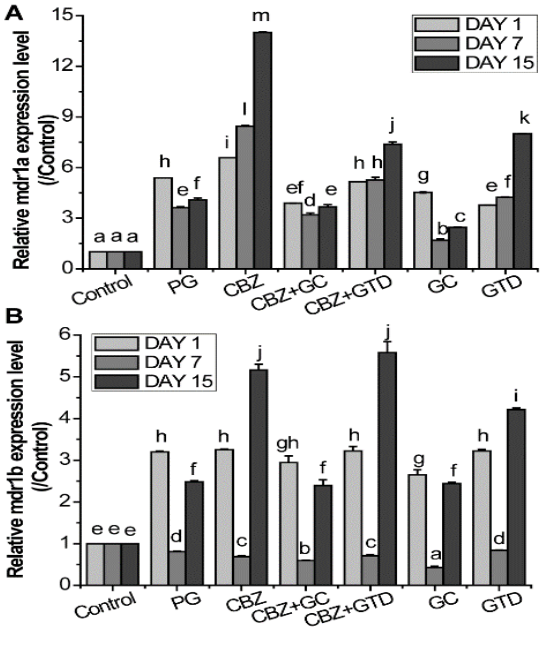

Figure 2: GC and GTD reversed the CBZ-induced increase in mrd1a expression. (A) Relative expression level of mrd1a, (B) relative expression level of mrd1b. Data are presented as mean ± SD of at least three independent experiments. Bars with different letters indicate significant differences ($p< 0.05$) among groups. PG, CBZ, GC and GTD represent penicillin, carbamazepine, *Gastrodia elata* powder capsule and gastrodin, respectively

On the 1st day, the results showed that mdr1a and mdr1b expression levels were significantly increased in all groups, when compared with the control group ($p < 0.05$; Figure 2). The results also indicated that PG induced the expressions of mdr1a and mdr1b genes. On the 7th and 15th days, the mdr1a expression level in the CBZ group was further increased, relative to the PG group. Results obtained for the CBZ + GC and CBZ + GTD groups showed that GC and GTD significantly decreased the mdr1a expression level of CBZ ($p < 0.05$). However, on the 7th and 15th days, the trend in mdr1b expression changed as a result of the expression level of mdr1a (Figure 2 B). Hence, Western blot method was employed to determine the P-gp protein expression level.

GC reversed the CBZ-induced increase in P-gp protein expression

The results revealed bands of 170 kD and 40 kD, corresponding to P-gp and β-actin. As showed in Figure 3, on the 1st day, the expression level of P-gp in PG- induced epilepsy rat hippocampus in all groups increased about two-fold, when compared to control group, indicating that PG treatment significantly induced the expression of P-gp. On the 7th and 15th days, with the metabolism of PG, the P-gp level of PG group was lower than that on the 1 st day. However, CBZ, CBZ+GTD and GTD groups still had higher expression levels of P-gp, when compared to PG group ($p < 0.05$). Moreover, GC significantly decreased the expression level of P-gp, and reversed the effect of CBZ on P-gp ($p < 0.05$). The P-gp expression level in CBZ + GC and GC groups decreased to normal levels, indicating that GC reversed the increase in P-gp expression induced by CBZ.

DISCUSSION

Most seizures can be controlled effectively through timely use of anti-epilepsy drugs [22].

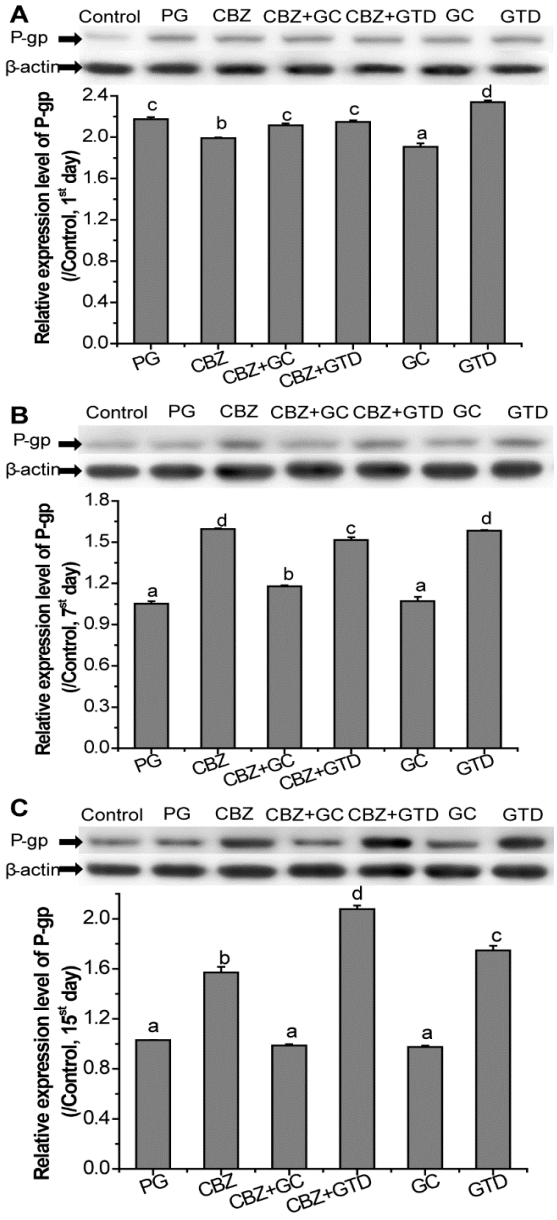

Figure 3: GC decreased P-gp expression and reversed the CBZ-induced increase in P-gp expression. Relative expression level of P-gp measured on 1st day (A), 7th day (B) and 15th day (C). Data are presented as mean ± SD of at least three independent experiments. Bars with different letters indicate significant differences (p< 0.05) among groups. PG, CBZ, GC and GTD represent penicillin, carbamazepine, *Gastrodia elata* powder capsule and gastrodin, respectively

However, it has been reported that 20 - 30 % of seizure conditions are uncontrollable due to drug resistance caused by multiple dosing [23]. Thus, a cocktail therapy is usually used for combating drug resistance so as to achieve improved curative effects [24]. A previous study showed that P-gp is overexpressed in intractable epilepsy patients and in the brain of rat model of refractory epilepsy [25]. Thus, it is reasonable to conclude that the basis of resistance in patients with intractable epilepsy is P-gp over-expression. Therefore, the potential relationship between P-gp expression level and the curative effect of antiepileptic drug needs to be unraveled.

In the present study, CBZ significantly decreased the intensity of seizure responses, mortality and the incidents of severe seizures associated with epilepsy. Moreover, GC and GTD enhanced the anti-epileptic effect of CBZ, implying that they changed the expression level of P-gp. Thus, real time qRT-PCR and Western blot assays were employed to determine the effect of CBZ, GC and GTD on the expression level of P-gp. The results showed that CBZ significantly increased the expression levels of mrd1a and P-gp, while GC significantly decreased the expression of P-gp and reversed the GBZ-induced increase in mrd1a. In addition, GTD induced the expression of P-gp, and increased the expression level of P-gp. This may be due to differences in the molecular mechanisms of action of GC and GTD.

It is known that traditional Chinese medicines and their bioactive components may have similar pharmacological functions [26]. However, in this study, GTD which is the main active component of *Gastrodia elata* did not produce the same treatment effect as GC. The differences between the effects of GC and GTD on the expression of P-gp may due to their different mechanisms of action.

CONCLUSION

The results obtained in this study indicate that GC reverses CBZ drug resistance most probably due to downregulation of the expression of P-gp. Thus, GC is a promising and potent anti-epilepsy drug candidate. This study provides data and theoretical support for the clinical application of GC as adjuvant treatment for epilepsy.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Xiangji Dang and Haisheng Jiao conceived and designed the study, Xiangji Dang, Pei Zhao and Long Qin collected and analyzed the data, while Yan Liu wrote the manuscript.

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