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Research Article
Effect of Co-administration of Compound Danshen Dripping Pills and Valproic Acid on Temporal Lobe Epilepsy

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Abstract

Background and Objective: Temporal Lobe Epilepsy (TLE), accompanied by cognitive impairment, is known for its drug resistance. Compound Danshen Dripping Pills (CDDP), a widely used Chinese Traditional Medicine (TDM), is an effective complementary medicine for the clinical treatment of TLE. The present work was aimed to explore the curative effect of co-administration of CDDP and valproic acid (VPA) in the kainic acid (KA)-induced TLE rat model. **Materials and Methods:** Male Sprague-Dawley (SD) rats were stochastically divided into five groups (n = 60): Saline, Model, CDDP, VPA and VPA+CDDP groups. TLE model was established via stereotactic injections of KA. VPA (189 mg kg^{-1}), CDDP (85 mg kg^{-1}) or combined VPA (189 mg kg^{-1}) and CDDP (85 mg kg^{-1}) were respectively administered to rats in the treatment groups via i.g. for 60 days. Cognitive function was evaluated by the radial-arm maze and surviving neuron cells were observed and counted using Nissl staining. The expression of genes and proteins of the apoptosis factors caspase-3 and caspase-8 was detected by real-time fluorescence quantitative PCR and Western Blot. **Results:** Compared with the Model group, memory and learning skills were improved ($p<0.01$) and neuronal cell death in the CA3 region was alleviated ($p<0.01$) with co-administration treatment of VPA and CDDP. The mRNA and protein expression of caspase-3 and caspase-8 in the VPA+CDDP group was both attenuated. **Conclusion:** VPA combined with CDDP could reduce neuronal damage and cognitive impairment with the possible mechanism to inhibit apoptosis by regulating apoptosis factors like caspase-3 and caspase-8.

Key words: Temporal lobe epilepsy, Chinese traditional medicine, cognitive impairment, neuronal apoptosis, danshen dripping pill, hippocampal neuronal, caspase-3, valproic acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Temporal Lobe Epilepsy (TLE), one of the main types of focal epilepsy, is often medically resistant to currently available treatments. Despite taking Antiepileptic Drugs (AEDs), nearly a third of TLE patients suffer from continuous seizures. Apart from its unpredictable seizures, epilepsy is usually accompanied by a series of frequent complications, especially cognitive impairment and psychiatric comorbidities[1]. In the case of TLE, it is known that there is a syndromic entity such as Hippocampal Sclerosis (HS), deficits in verbal memory performance and cognitive profiles[2,3]. Moreover, many current AEDs polypharmacy can adversely impact cognitive functions when controlling for disease severity and this side effect is exacerbated with increasing dosage and anticonvulsant blood levels[4-6]. Thus, new therapeutic solutions for TLE need to be put forward that can both reduce seizure burden with high efficiency and cognitive impairment, which arises as a comorbidity of TLE or significant side effects of AEDs.

Due to its complex active ingredients and the advantage of multiple targets, Chinese Traditional Medicine (TCM) drug formulation Compound Danshen Dripping Pill (CDDP), including *Salvia miltiorrhiza* Bunge (Labiatae), *Panax notoginseng* (Burk.) F.H. Chen (Araliaceae) and borneol, was reported to have curative clinical effect and safety when combined with conventional therapy in the treatment of heart failure[7]. However, there were few studies about the exact mechanism and clinical efficacy of co-therapy of CDDP and AEDs in treating epilepsy. Previous studies have demonstrated that CDDP was an effective adjuvant drug for controlling seizures, protecting against cognition dysfunction and inhibiting hippocampal neuronal loss when joint with carbamazepine in the KA-induced TLE rat model[8].

Neuronal death can be induced by prolonged seizure or Status Epilepticus (SE) in the brain and the underlying molecular mechanism might be related to apoptosis. Caspases are one of the major family of genes regulating apoptosis with the initiator caspase, caspase-8 and the effector caspase, caspase-3. Caspase-8 and caspase-3 were found to be up-regulated or activated leading to the death-receptor-mediated apoptotic pathway in the AD brain[9]. The clinical finding has reported that well-tolerated TCM possesses potent activity to inhibit caspase[10].

To evaluate the curative effect of the combination of Valproic Acid (VPA) and CDDP, Kainic Acid (KA)-induced TLE rat models were established and given respective medical treatment with CDDP, VPA and their combination, after which cognitive behaviours and neuron cell death were observed. In this study, we found that it could cause less neuronal damage and cognitive dysfunction in the TLE rat model when combining VPA and CDDP and its possible mechanism was to down-regulate apoptosis factors, caspase-3 and caspase-8.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacy, Lanzhou University Second Hospital, Lanzhou, People's Republic of China, between January-November, 2018.

Animals: Male Sprague-Dawley (SD) rats, weighing 230±20 g, were purchased from the GLP experimental centre of Lanzhou University. Animals had access to get water and food freely to maintain a basic diet and were housed in an air-conditioned room (20±2°C) with a standard 12 hrs light/dark cycle and humidity of 40%. All the protocols and animal experimental procedures in this study were performed following the instructions of the Institutional Animal Care and Use Committee and were approved by the Animal Ethics Committee of Lanzhou University Second Hospital.

Induction of TLE: After anaesthetizing with 10% chloral hydrate (3.5 mL kg^{-1}, i.p.), SD rats were fixed on a brain stereotaxic instrument (#68001, RWD Life Science Co., Ltd., China). TLE was induced using stereotactic injections of KA (1.0 μg μL^{-1}, #K0250; Sigma-Aldrich, St. Louis, MO, USA). The target was the left dorsal hippocampus, 5.60 mm posterior to the bregma, 4.50 mm right lateral from the midline and 5.00 mm deep from the dura. The Saline group was injected with saline at the same dose. After the injection of KA, the rats were given additional care with soft food and comfortable space and were observed for a sequence of behavioural alterations by the same observer to identify whether the rats were successfully ignited according to the Racine level[11] (stage 0, no convulsive behaviour; stage I, facial twitches; stage II, severe facial clonus and head nodding; stage III, unilateral forelimb clonus; stage IV, bilateral forelimb clonus; stage V, rearing and generalized tonic-clonic convulsions).

Drugs treatment: After the injection of KA, the rats which experienced level III-V Racine seizures three times were picked. The TLE model rats were assigned into five groups at random (n = 60): Saline group, Model group, CDDP group, VPA group and VPA+CDDP group. CDDP group was given CDDP by i.g. (85 mg kg^{-1}); VPA group was given VPA by i.g. (189 mg kg^{-1}); and VPA+CDDP group was given VPA (189 mg kg^{-1}) and CDDP (85 mg kg^{-1}) together by i.g. The Saline group and Model group were given the same volume of normal saline

(2.5 mL kg^{-1}). In terms of the Meeh-Rubner formula[8], the clinical dosage was converted into the dose given to rats according to body surface area. VPA (0.1 g/tablet, #170423; Hunan Xiangzhou Pharmaceutical Co., Ltd., China) and CDDP (27 mg/pill, #151201; Tasly Pharmaceutical Co., Ltd., China) were respectively dissolved in normal saline solution in advance. The treatments were administered to all of the rats for consecutive 60 days.

Radial-arm maze test: Radial-Arm Maze (RAM) has been widely used for measuring spatial learning and memory in rodents and it also allows for the evaluation of spatial working and reference memory[12]. Eight arms, the structural constitution of RAM, number orderly from 1-8 (50×15 cm) and extend radially from a central zone through control gates. The food cup and induction apparatus were placed at the end of each arm. Two weeks before RAM, rats were kept on a restricted diet till their body weight declined to 85% of the free-feeding weight. Additionally, rats fasted 24 hrs before the test. The food was initially available in 4 arms (nos. 1, 3, 5 and 7) and rats were set in the central zone. After adapting to the environment for 15 sec, control gates were open and the animals were allowed to explore freely. An arm entry was calculated when the posterior limbs of the rat were within the arm. Two training trials per day within more than 2 hrs interval were given to rats to reach the end of the arms and take food and trials had been lasted for 5 days. On the sixth day, animals foraging was carefully recorded. The number of reference memory errors (enter an arm with no bait) and working memory errors (enter an arm containing baits, but previously entered) were both measured. In addition to the above memory errors to measure, the total time taken to consume all four baits were also recorded. If a rat failed to consume all four baits, 5 min was calculated as time in total.

Nissl staining: Four rats were randomly selected from each group after 60 days' intragastric administration, then narcotized by 10% chloral hydrate intraperitoneal and perfused by 4% paraformaldehyde and saline till stiffness of the limbs via cardiac perfusion. The brain was quickly taken from the head and fixed by paraformaldehyde. The dorsal hippocampal frozen coronal sections were stained with 1% toluidine blue (#G3668; Beijing Solarbio Science and Technology Co., Ltd., China). After gradient alcohol dehydration, the section was transparent using xylene and then sealed with a pack of neutral gum. A microscope (BX-50; Olympus, Tokyo, Japan) was used to observe the damage of hippocampus neurons in each stained section.

Table 1: Primer sequences (5'-3') of caspase-3, caspase-8 and β-actin

Gene	Primer sequences (5'-3')
Caspase-3	F: GAGACAGACAGTGGAACTGACGATG
	R: GGCGCAAAGTGACTGGATGA
Caspase-8	F: TGGTATATCCAGTCACTTTGCCAGA
	R: CTCACATCATAGTTCACGCCAGCT
β-actin	F: GGAGATTACTGCCCTGGCTCCTA
	R: GACTCATCGTACTCCTGCTTGCTG

Real-time fluorescence quantitative PCR: Total RNA, isolated from hippocampus with Trizol reagent according to manufacturer's protocol (#AA7104-1, TaKaRa Bio), was quantified and qualified using a UV spectrophotometer. cDNA was obtained by reverse transcription reaction using a reverse-transcription Kit (#RR036A, TaKaRa Bio). All primers (Sangon Biotech Co., Ltd., China) were synthesized and purified, which were listed in Table 1. Total quantitative PCR reaction system contained iTaqTM Universal SYBR® Green Supermix (10 μL), nuclease-free H2O (6.4 μL), DNA template (2 μL), forward primer (0.8 μL) and reverse primer (0.8 μL). Its reaction conditions were set as follows: initially denatured for 30 sec at 95°C; then denatured for 5 sec at 95°C; followed by 40 cycles of denaturing at 60°C for 30 sec, finally dissociated. All samples were read in triplicate. Relative expression values were normalized to the expression of β-actin and calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blot: RIPA (200 μL) and PMSF (2 μL) solution was used to lyse the hippocampal isolated from the brain and the supernatant was selected from homogenate after centrifuging at 12,000 rpm, 4°C for 4 min. Protein concentrations were determined by a BCA protein assay kit (#PC0020, Beijing Solarbio Science and Technology Co., Ltd., China). Total protein was separated by 10% polyacrylamide gel electrophoresis and transferred onto Polyvinylidene Fluoride (PVDF) membranes (#YA1701, Beijing Solarbio Science and Technology Co., Ltd., China). After incubated in blocking buffer (5% nonfat milk dissolved in Tris-buffered saline and 0.1% Tween-20 (#626NO45; Solarbio Science and Technology Co., Ltd., China)) for 1.5 hrs, the membranes were separately incubated overnight at 4°C with the following primary antibodies: anti-caspase-3 antibodies (1:500, #ZP4127BP27; Boster Biological Technology Co. Ltd, China), anti-caspase-8 antibodies (1:400, #1731243; Boster Biological Technology Co. Ltd, China), anti-β-actin antibodies (1:400, #BST17353873; Boster Biological Technology Co. Ltd, China). After washing with TBST (4×8 min), the blots were treated with HRP-labelled corresponding secondary antibody (1:10000, #9300014001; ABclonal Biotechnology Co. Ltd, China) for 2 hrs. Protein bands were visualized with ECL luminescence imaging and Image J was used for densitometric analysis.

Statistical analysis: The data were presented as means±SEM, statistical analysis was performed using SPSS 19.0 software. The one-way ANOVA followed by Fisher's Least Significant Difference was employed to determine the statistical difference among groups. The value of $p<0.05$ was presented a statistically significant difference.

RESULTS

Effect of the combination of VPA and CDDP on cognitive impairment: After the injection of KA, the rats gradually regained their autonomic activities and then showed chronic epilepsy after the latency period. To investigate the influence of a combination of VPA and CDDP on cognitive impairment, the rats were evaluated in the RAM task at 60 days after drug treatment. Compared with that of the Model group, the results of reference memory errors depicted a declination in the other four groups, whereas it was significantly different in the Saline group ($p<0.01$), VPA group ($p = 0.022$) and VPA+CDDP group ($p = 0.004$) (Fig. 1a). The comparison between VPA and VPA+CDDP group showed a significant difference ($p = 0.034$). The results of the other four groups showed few working memory errors compared with the Model group (Fig. 1b). And there were fewer errors in the VPA+CDDP group compared with the VPA group ($p = 0.031$). For the time taken to consume four baits, rats in the Model group spent more time and those in the VPA+CDDP group spent less time compared with the VPA group ($p = 0.046$) (Fig. 1c). These results inferred that the administration of VPA and CDDP improve learning and memory ability and cognitive impairments in the brain.

Effect of the combination of VPA and CDDP on neuronal protection: According to results of the hippocampal coronal section stained by Nissl (Fig. 2a-b), the hippocampus is structurally intact and the morphology of its neuronal cells in the CA3 region of the hippocampus were dark-stained blue, regular, ordered closely in Saline group. Nevertheless, neurons in the Model group were decreased, disordered and incomplete. Compared with the Model group, there is an increasing number of neuronal cells in the CA3 region of the hippocampus in other groups (Fig. 2c). The number of surviving neuronal cell was 76.20±5.17 in the VPA+CDDP group, which showed a significant difference comparing with the VPA group ($p = 0.011$). These results exhibited that the combination of CDDP and VPA might have a more significant effect on attenuating neuronal injury induced by KA.

Effect of the combination of VPA and CDDP on the expression of caspase-3 and caspase-8: The effect of the combination of VPA and CDDP on caspase-3 and caspase-8

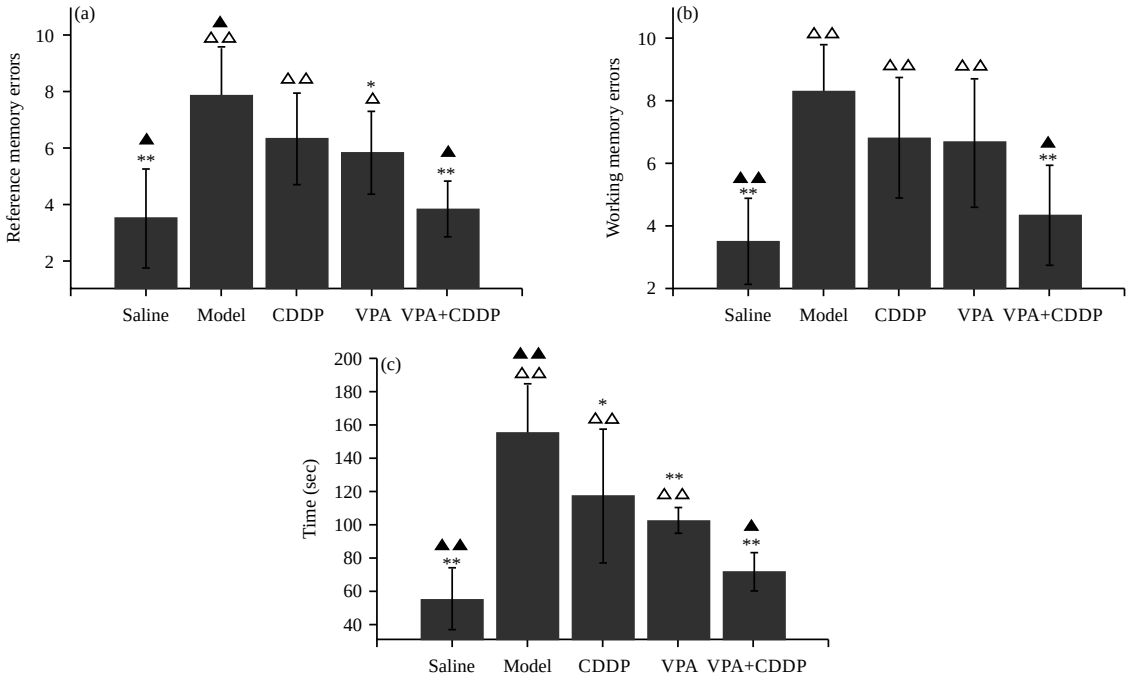

Fig. 1(a-c): Effect of the combination of VPA and CDDP on cognitive impairment

(a) Reference memory errors, (b) Working memory errors and (c) Total time taken to consume all four baits. Results are presented as Mean±SEM. ^p<0.05, ^^p<0.01 vs. Saline group, *p<0.05, **p<0.01 vs. Model group, ▲p<0.05, ▲▲p<0.01 vs. VPA group (n = 8 per group)

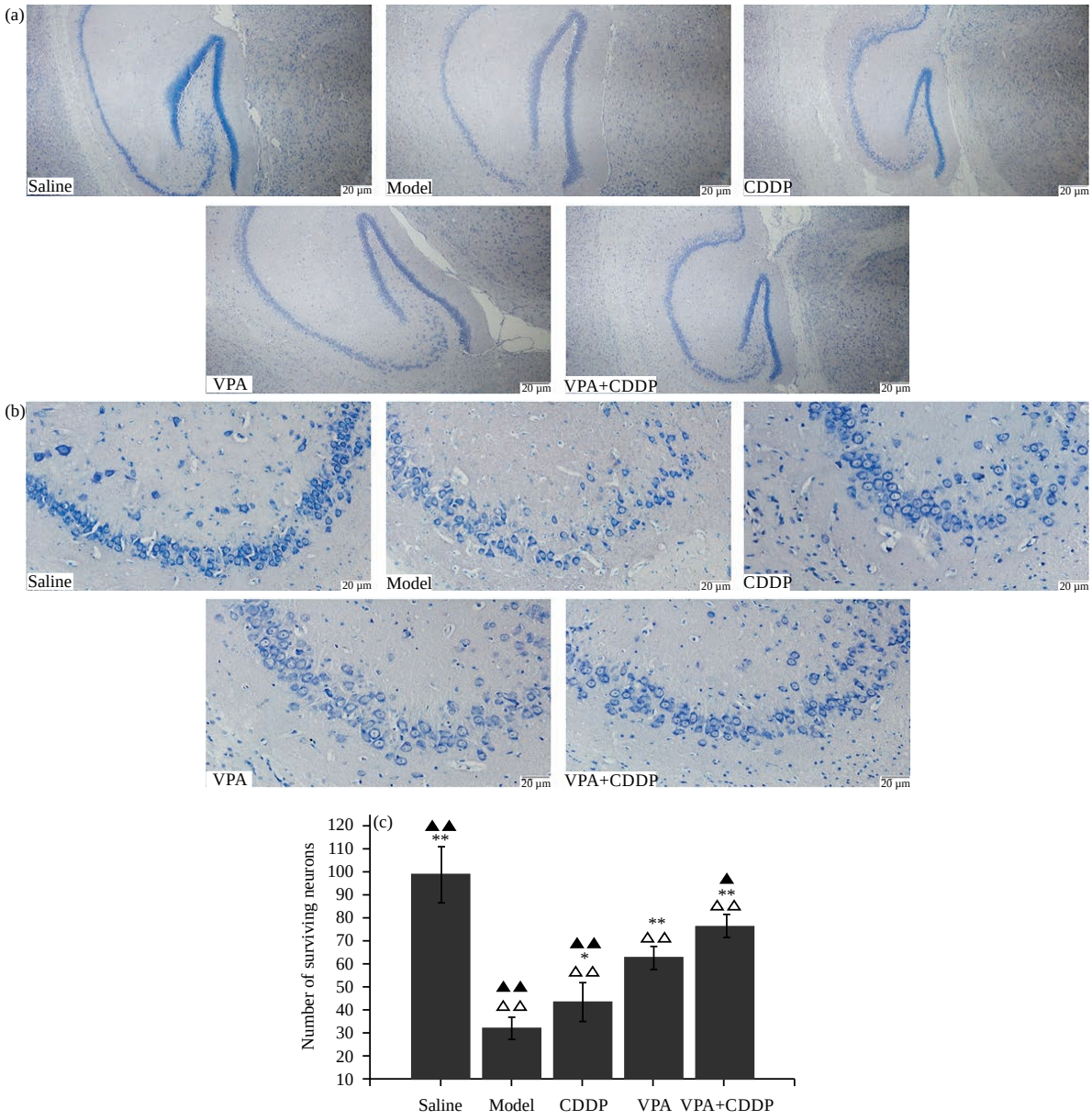

Fig. 2(a-c): Effect of the combination of VPA and CDDP on neuronal protection

(a) Nissl staining of the hippocampus of each group (magnification×40), (b) Nissl staining of the CA3 region in the hippocampus of each group (magnification×200) and (c) The number of surviving neurons in each group. Results are presented as Mean±SEM. ^p<0.05, ^^p<0.01 vs. Saline group, *p<0.05, **p<0.01 vs. Model group, ▲p<0.05, ▲▲p<0.01 vs. VPA group (n = 4 per group)

mRNA expression was investigated by PCR. The level of caspase-3 mRNA in the other four groups was significantly higher than that of the Saline group (p<0.01) (Fig. 3a). Compared with the Model group, the level of caspase-3 mRNA significantly decreased in the VPA+CDDP group (p = 0.001). VPA+CDDP group had a significantly lower level of caspase-3 mRNA than the VPA group (p = 0.017). The level of caspase-8 mRNA all increased in other groups compared with the Saline group (Fig. 3b). The level of caspase-8 mRNA in the VPA+CDDP group was significantly reduced comparing with the Model group (p = 0.028) and was lower than that of the VPA group although there was no statistically significant difference (p = 0.553).

To verify these results, the expression of caspase-3 and caspase-8 was further detected in hippocampus tissues by western blot (Fig. 4a). The expression of caspase-3 and caspase-8 significantly increased in the Model group compared with the Saline group (p<0.01) (Fig. 4b-c).

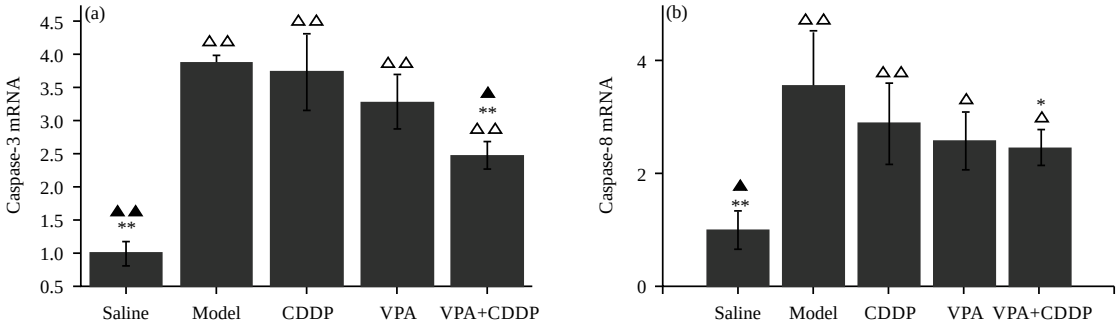

Fig. 3(a-b): Effect of the combination of VPA and CDDP on the relative mRNA expression level of caspase-3 and caspase-8

(a) Caspase-3 and (b) Caspase-8. Results are presented as Mean±SEM. ᵃp<0.05, ᵃᵃp<0.01 vs. Saline group, *p<0.05, **p<0.01 vs. Model group, ▲p<0.05, ▲▲p<0.01 vs. VPA group (n = 6 per group)

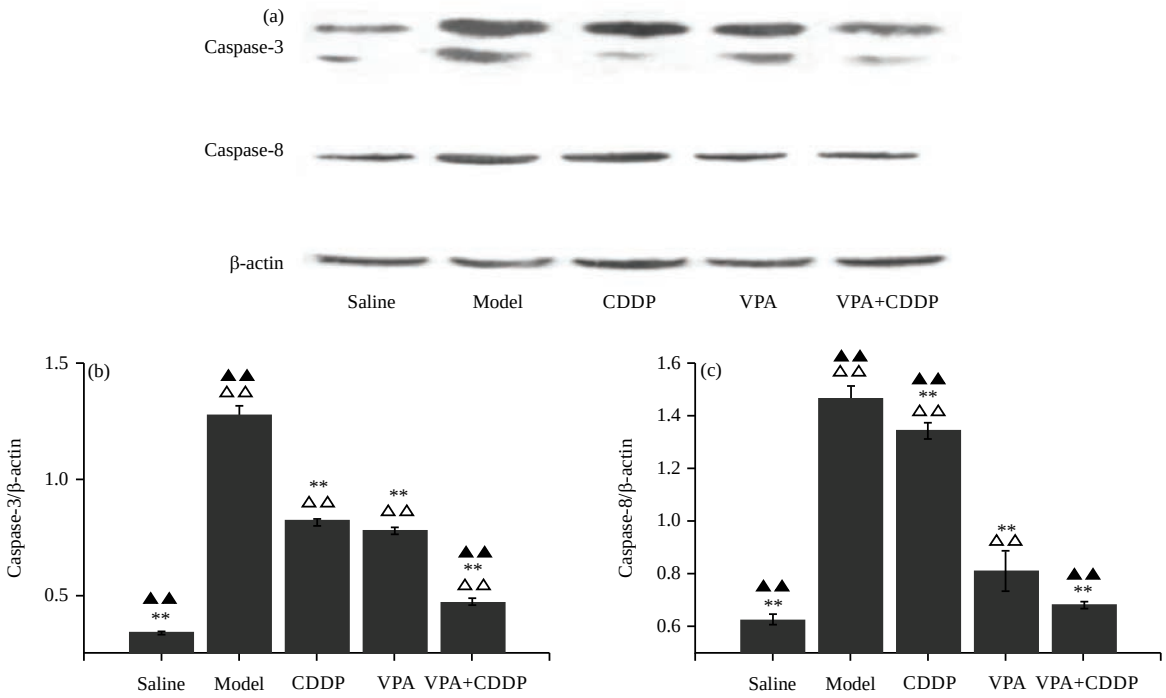

Fig. 4(a-c): Effect of the combination of VPA and CDDP on the expression of caspase-3 and caspase-8

(a) Western blotting protein bands and (b-c) Densitometry analysis was performed with Image J software. Results are presented as Mean±SEM. ᵃp<0.05, ᵃᵃp<0.01 vs. Saline group, *p<0.05, **p<0.01 vs. Model group, ▲p<0.05, ▲▲p<0.01 vs. VPA group (n = 6 per group)

Compared with the Saline group, the expression of caspase-3 in the VPA+CDDP group significantly increased (p<0.01) (Fig. 4b), but the expression of caspase-8 increased with no significant difference (p = 0.153) (Fig. 4c). Additionally, there was a much lower expression of caspase-3 and caspase-8 in the VPA+CDDP group when making a comparison with the VPA group (p<0.01). These results indicated that the expression of caspase-3 and caspase-8 decreased when treated with VPA and CDDP, which might have inhibited neuronal apoptosis.

DISCUSSION

TLE is a serious chronic condition and greatly disruptive to the lives of patients, for which the co-administration of AEDs and TCM is widely used for better clinical treatment. Studies have demonstrated that Songling Xuemaikang Capsules (SXC) and CDDP are more effective in the control of epileptogenesis when combined with Carbamazepine (CBZ)[8,13]. In this study, after 60 days of treatment, neither VPA nor CDDP could effectively improve cognitive function and

neuronal protection alone, yet the combination of VPA and CDDP exerts a better curative effect on neuronal protection and cognitive improvement in the TLE rat model.

Cognitive impairment frequently occurs in epilepsy patients and was affected by multiple factors. Medial TLE is characterized by a set of associated symptoms such as epileptic discharges, focal seizures with medial temporal behavioural signs and Hippocampal Sclerosis (HS) and does harm to the frontotemporal system, of which the neighbouring rhinal cortex and hippocampus are major components[14]. This can be used to clarify the cognition decline in epilepsy. Additionally, treatment of epilepsy, even successful surgeries, affects cognitive profiles and cognitive development should be carefully assessed in patients[15]. The results of RAM showed that TLE rats were suffered declining special memory and learning and cognitive deterioration along with the extension of epileptic seizures. Rats received, respectively, by VPA or CDDP alone behaved worse than those received by the combination of VPA and CDDP, which indicated that the co-administration displayed a better role in ameliorating cognitive competence.

As is well known, Hippocampal Sclerosis (HS) is a typical pathological symptom of TLE and appears to be one of the causes leading to cognitive impairment, which is essentially the loss of hippocampal neuron. CA1 cell loss was found in medial TLE patients with mental disorders and such progressive neuronal loss in CA1 and CA3 region might result in cognitive impairment[16,17]. Severe neuronal loss, especially in the CA3 region was found in the KA-treated group in this study. CA3 region of the hippocampus mediates the acquisition and rapid encoding of new spatial information within short-term memory[18]. This might account for the spatial learning and memory deficits in the present RAM task. Improvement of neuronal loss was found in the CA3 region both in CDDP and VPA group and even more neuron cells were discovered in the VPA+CDDP group. Concerning this, VPA combined with CDDP plays a more effective role in reversing neuron cell death in the hippocampus and further preventing cognitive impairment.

Neuronal apoptosis is a complex intrinsically programmed process executed by enzymes and proteins. Some studies demonstrated that it contributes to neuronal cell loss[19,20]. Caspases, a family of aspartate-specific cysteine proteases, are the key factor in the process of cell apoptosis. Caspase-3 is the main effector factor of cell apoptosis and plays a crucial role in death signalling that regulate apoptosis[21]. Abundant activated caspase-3 was found to be located exclusively in neurons and areas with strong expression of caspase-3 displayed an extensive loss of neuronal cell[20]. As an apical caspase, caspase-can inhibit Vacuolar H^+-ATPase (V-ATPase) activity and initiate Lysosomal Membrane Permeabilization (LMP) to activate lysosome-associated cell death[22]. Several active components of CDDP have been reported, which are correlated with caspases. The active ingredient of Salvia miltiorrhiza, including salvianolic acid B, tanshinone IIA and tanshinone IIB could suppress cleaved caspase-3 and its proteins for neuroprotective via inhibition of apoptosis[23-25]. Borneol reversed neuronal injury by depressing the expression of the caspase family-related apoptotic signalling pathway[26]. PCR data showed that neither VPA or CDDP failed to depress caspase-3 and caspase-8, whereas Western Blot data showed differently. However, the results of PCR and Western Blot consistently showed that VPA with co-administered CDDP could significantly decrease the expression of caspase-3, caspase-8 and their mRNAs and subsequently inhibited cell apoptosis and reversed neuronal damage in the hippocampus.

CONCLUSION

In summary, the present study provides evidence for the complementary role of CDDP when combined with VPA in the KA-induced TLE rat model. Co-administration of VPA and CDDP exerts more effective efficacy on neuronal cell protection and cognitive improvement with the possible underlying mechanism of apoptosis inhibition through down-regulated caspase-3 and caspase-8.

SIGNIFICANCE STATEMENT

This study discovers an effective co-administration of VPA and CDDP that can be beneficial for exerting a neuronal protective effect on the KA-induced TLE rat model. This study will provide a promising theoretical basis for clinical medication on TLE.

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REFERENCES

1. Verrotti, A., D. Carrozzino, M. Milioni, M. Minna and M. Fulcheri, 2014. Epilepsy and its main psychiatric comorbidities in adults and children. J. Neurol. Sci., 343: 23-29.

2. Mathon, B.. L.B. Ulvin, C. Adam, M. Baulac and S. Dupont *et al*., 2015. Surgical treatment for mesial temporal lobe epilepsy associated with hippocampal sclerosis. Rev. Neurologique, 171: 315-325.
3. Sarkis, R.A., S. McGinnis, S.N. Rushia, S. Park, E.E. Ansari and K.C. Willment, 2018. Growing older with drug-resistant epilepsy: Cognitive and psychosocial outcomes. J. Neurol., 265: 1059-1064.
4. Quon, R.J., M.T. Mazanec, S.S. Schmidt, A.S. Andrew and R.M. Roth *et al*., 2020. Antiepileptic drug effects on subjective and objective cognition. Epilepsy Behav., Vol: 104. 10.1016/j.yebeh.2020.106906.
5. Operto, F.F., G.M.G. Pastorino, R. Mazza, M. Carotenuto and M. Roccella *et al*., 2020. Effects on executive functions of antiepileptic monotherapy in pediatric age. Epilepsy Behav., Vol: 102. 10.1016/j.yebeh.2019.106648.
6. Witt, J.A., C.E. Elger and C. Helmstaedter, 2015. Adverse cognitive effects of antiepileptic pharmacotherapy: Each additional drug matters. Eur. Neuropsychopharmacol., 25: 1954-1959.
7. Lin, S.S., C.X. Liu, J.H. Zhang, X.L. Wang and J.Y. Mao, 2020. Efficacy and safety of oral Chinese patent medicine combined with conventional therapy for heart failure: An overview of systematic reviews. Evidence Based Complementary Altern. Med., Vol. 20. 10.1155/2020/8620186.
8. Jia, C., S. Han, L. Wei, X. Dang and Q. Niu *et al*., 2018. Protective effect of compound danshen (*Salvia miltiorrhiza*) dripping pills alone and in combination with carbamazepine on kainic acid-induced temporal lobe epilepsy and cognitive impairment in rats. Pharm. Biol., 56: 217-224.
9. Rohn, T.T., E. Head, W.H. Nesse, C.W. Cotman and D.H. Cribbs, 2001. Activation of caspase-8 in the alzheimer`s disease brain. Neurobiol. Dis., 8: 1006-1016.
10. Fattorusso, R., S. Frutos, X. Sun, N.J. Sucher and M. Pellecchia, 2006. Traditional Chinese medicines with caspase-inhibitory activity. Phytomedicine, 13: 16-22.
11. Phelan, K.D., U.T. Shwe, D.K. Williams, L.J. Greenfield and F. Zheng, 2015. Pilocarpine-induced status epilepticus in mice: A comparison of spectral analysis of electroencephalogram and behavioral grading using the Racine scale. Epilepsy Res., 117: 90-96.
12. Kim, H., J.Y. Park and K.K. Kim, 2018. Spatial learning and memory using a radial arm maze with a head-mounted display. Psychiatry Invest., 15: 935-944.
13. Yang, H., R. Zhang, C. Jia, M. Chen, W. Yin, L. Wei and H. Jiao, 2019. Neuronal protective effect of songling xuemaikang capsules alone and in combination with carbamazepine on epilepsy in kainic acid-kindled rats. Pharm. Biol., 57: 21-27.
14. Elger, C.E., C. Helmstaedter and M. Kurthen, 2004. Chronic epilepsy and cognition. Lancet Neurol., 3: 663-672.
15. Sveikata, L., N. Kavan, A.J. Pegna, M. Seeck and F. Assal *et al*., 2019. Postoperative memory prognosis in temporal lobe epilepsy surgery: The contribution of postictal memory. Epilepsia, 60: 1639-1649.
16. Di, J., L.S. Cohen, C.P. Corbo, G.R. Phillips, A. El Idrissi and A.D. Alonso, 2016. Abnormal tau induces cognitive impairment through two different mechanisms: Synaptic dysfunction and neuronal loss. Sci. Rep., Vol. 6, 10.1038/srep20833.
17. Lu, J., H. Huang, Q. Zeng, X. Zhang and M. Xu *et al*., 2019. Hippocampal neuron loss and astrogliosis in medial temporal lobe epileptic patients with mental disorders. J. Integr. Neurosci., 18: 127-132.
18. Kesner, R.P., 2007. Behavioral functions of the CA3 subregion of the hippocampus. Learn. Mem., 14: 771-781.
19. Xu, S., Q. Pang, Y. Liu, W. Shang, G. Zhai and M. Ge, 2007. Neuronal apoptosis in the resected sclerotic hippocampus in patients with mesial temporal lobe epilepsy. J. Clin. Neurosci., 14: 835-840.
20. Weise, J., T. Engelhorn, A. Dörfler, S. Aker, M. Bähr and A. Hufnagel, 2005. Expression time course and spatial distribution of activated caspase-3 after experimental status epilepticus: Contribution of delayed neuronal cell death to seizure-induced neuronal injury. Neurobiol. Dis., 18: 582-590.
21. Cao, X., P. Wen, Y. Fu, Y. Gao, X. Qi *et al*., 2019. Radiation induces apoptosis primarily through the intrinsic pathway in mammalian cells. Cell. Signalling, Vol. 62. 10.1016/j.cellsig.2019.06.002.
22. Zhong, B., M. Liu, C. Bai, Y. Ruan and Y. Wang *et al*., 2020. Caspase-8 induces lysosome-associated cell death in cancer cells. Mol. Ther., 28: 1078-1091.
23. Yu, X.Q., C.C. Xue, Z.W. Zhou, C.G. Li and S.F. Zhou, 2008. Tanshinone IIB, a primary active constituent from *Salvia miltiorrhiza*, exerts neuroprotective effect via inhibition of neuronal apoptosis *in vitro*. Phytother. Res., 22: 846-850.
24. Li, H., W. Han, H. Wang, F. Ding and L. Xiao *et al*., 2017. Tanshinone IIA inhibits glutamate-induced oxidative toxicity through prevention of mitochondrial dysfunction and suppression of MAPK activation in SH-SY5Y human neuroblastoma cells. Oxid. Med. Cell. Longevity, Vol. 2017. 10.1155/2017/4517486.
25. Yu, X., Q. Guan, Y. Wang, H. Shen, L. Zhai, X. Lu and Y. Jin, 2019. Anticonvulsant and anti-apoptosis effects of salvianolic acid B on pentylenetetrazole-kindled rats via AKT/CREB/BDNF signaling. Epilepsy Res., 154: 90-96.
26. Liu, R., L. Zhang, X. Lan, L. Li, T.T. Zhang, J.H. Sun and G.H. Du, 2011. Protection by borneol on cortical neurons against oxygen-glucose deprivation/reperfusion: Involvement of anti-oxidation and anti-inflammation through nuclear transcription factor κappaB signaling pathway. Neuroscience, 176: 408-419.