Am J Transl Res 2022;14(5):3216-3224
www.ajtr.org /ISSN:1943-8141/AJTR0141467

Original Article

Aconitine induces brain tissue damage by increasing the permeability of the cerebral blood-brain barrier and over-activating endoplasmic reticulum stress

Xiaojun Zhang, Xuheng Jiang, Anyong Yu, Haizhen Duan

Department of Emergency, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China

Received January 1, 2022; Accepted March 30, 2022; Epub May 15, 2022; Published May 30, 2022

Abstract: Objective: This study aimed to explore the neurotoxicity of aconitine and its underlying mechanism. Methods: The rats were administered with the aconitine solution intragastrically at different dosages (0.5 mg/kg, 1.5 mg/kg, and 2.5 mg/kg). Evans blue (EB) extravasation and evaluation of tight junction protein expression were performed to determine the permeability of the blood-brain barrier. Cellular damage, apoptosis, and levels of endoplasmic reticulum (ER) stress markers were determined using H&E staining, Tunnel assay, and western blotting. The effects of aconitine on cell viability, apoptosis, and activation of the ER stress signaling in PC12 cells were assessed *in vitro* using the MTT assay, flow cytometry, western blot, and immunofluorescence analysis. Results: Aconitine was observed to significantly increase the murine blood-brain barrier penetrability in a dose-dependent manner. The *in vivo* experimental results revealed that aconitine could stimulate the pathway for endoplasmic reticulum stress. The increase in the endoplasmic reticulum stress in the brain tissue promoted apoptosis, leading to brain damage. Moreover, PC12 cell proliferation was inhibited upon treatment with aconitine in a dose-dependent manner. In addition, cell apoptosis was increased upon treatment with aconitine also in a dose-dependent manner. These findings indicated that aconitine caused damage to PC12 cells via endoplasmic reticulum stress. Conclusion: Aconitine induces brain tissue damage by increasing the penetrability of the blood-brain barrier in the cerebral region and over-activating the endoplasmic reticulum stress.

Keywords: Aconitine, brain injury, endoplasmic reticulum stress, apoptosis

Introduction

Aconitine is a diterpene diester alkaloid derived from the tubers of Aconitum plants belonging to the Ranunculaceae family, which are distributed widely across the world and comprise over 400 species [1]. Aconitine has been used as a traditional herbal drug since ancient times for increasing the peripheral temperature, alleviating pain, and managing cardiovascular and neurological dysfunctions [2]. Modern pharmacological research has also confirmed the following effects of aconitine: pain alleviation, inflammation resistance, anesthesia induction, immune regulation, blood pressure lowering, vascular permeability inhibition, and anti-cancer activity [3, 4]. Currently, aconitine is being applied in the management of various diseases, including rheumatic fever, arthralgia, gastroenteritis, bronchial asthma, apsychia, diarrhea, and dropsy, and also in treating certain endocrine dysregulations and several neoplasms [5, 6]. However, it is frequently reported that the unreasonable application of aconitine has led to aconitine poisoning, several of which are fatal. From 2001 to 2010, Germany, Japan, and China reported nearly 5,000 cases of aconitine poisoning [7]. Previous studies conducted on deciphering the mechanism underlying aconitine poisoning focused mostly on its cardiovascular toxicity, and it was speculated that aconitine acted on the ion channels of cardiomyocytes and inactivated the Na^+ pumps, which lead to arrhythmias [8, 9]. The pharmacological effects of aconitine on the nervous system have, however, been studied scarcely [10, 11]. Since aconitine was detected in the brain tissue during the autopsy of the victims of aconitine

poisoning, it was speculated that aconitine could penetrate the blood-brain barrier into the cerebral tissues, subsequently causing brain injury [12].

Recent years have witnessed an increase in the number of studies concerning neurotoxic drugs damaging the blood-brain barrier (BBB). The existing research suggests injury from oxidative stress as one of the mechanisms underlying the BBB damage. External conditions stimulate the cells and lead to substantial aggregation of the endoplasmic reticulum proteins, either due to unfolding or misfolding of these proteins, and in sequence, a series of stress responses are induced in the cells, which are collectively referred to as the endoplasmic reticulum stress (ERS). Moreover, according to recent studies, apoptosis could represent an important mechanism underlying brain damage [13]. ERS is the initiation pathway for apoptosis, and glucose-regulated protein (GRP78) serves as the marker of ERS [14, 15]. Apoptosis induced via the ERS pathway has become the research hotspot in the field of medicine.

In this context, we aimed to determine if the mechanism underlying the neurotoxicity of aconitine in animals is associated with damage to the blood-brain barrier and whether this association involves endoplasmic reticulum stress.

Methods

Chemicals and reagents

Reagents: Ham's F12K medium (ThermoFisher, USA), Horse serum (Sigma, USA), Fetal bovine serum (Gibco, USA), DMSO (Sigma, USA), 0.25% Trypsin-EDTA (Gibco, USA), PageRuler Prestained 10-180 kDa Protein Ladder, and SuperSignal™ West Pico PLUS Chemiluminescence Substrate. The antibodies used were as follows: Anti-GAPDH (#8245, 1:5000, Abcam, USA), Anti-ZO-1 (#190085, 1:1000, Abcam, USA), Anti-claudin 5 (#172968, 1:5000, Abcam, USA), Anti-IRE-1 (#37073, 1:1000, Abcam, USA), Anti-GRP78 (#21685, 1:1000, Abcam, USA), Anti-XBP1 (#37152, 1:1000, Abcam, USA), and Anti-P-IRE1 (#48187, 1:1000, Abcam, USA). The commercial assay kits: the MTT assay kit (Jiangsu Synthgene biotechnology Co., CHINA) and the Pierce™ BCA protein assay kit (ThermoFisher, USA). In addition, Penicillin/Streptomycin solutions (ThermoFisher, USA) were used.

Experimental animals

A total of 32 adult male SD rats (about 200 ± 50 g in body weight) were procured from the Model Animal Research Center, Nanjing University. The animals were randomly assigned to the following four groups: (1) control group (n = 8); (2) low-dose (0.5 mg/kg) group (n = 8); (3) medium-dose (1.5 mg/kg) group (n = 8); and (4) high-dose (2.5 mg/kg) group (n = 8). The animals in all three aconitine groups received the respective concentrations of aconitine via oral administration, and the animals in control group were administered with same amount of normal saline. The animals in each group also received an injection of Evans blue (EB; 2%, 4 mL/kg) at different time points (30 min, 2 h, 6 h, and 12 h after the exposure). One hour later, all animals were sacrificed by performing heart perfusion using a CO_2 euthanasia device (PVC, Wonderful Oasis Biotechnology, China), and the brain tissue samples were obtained. All murine experiments were conducted in strict accordance with the instructions for laboratory animal care and use. The experimental protocols adopted in the present study were approved by the Animal Care Committee, Zunyi Medical University.

Evans blue detection

The brain tissue samples from each group were homogenized by adding 5 mL of formamide followed by stirring. Subsequently, 2 mL of this brain tissue homogenate was placed in a centrifuge tube, heated at 50°C for 48 h, and then centrifuged at 12,000 g for 20 min. An aliquot of 200 μL from the resulting supernatant was transferred into the well of a 96-well plate and absorbance was measured at 620 nm using an ultraviolet spectrophotometer (Beckman Coulter, USA). Accordingly, the content of Evans blue was calculated.

Histological examination

The histological analysis was performed according to a previously reported method. Briefly, the brain tissue samples from each group, extracted at different time points, were subjected to immobilization, dehydration, par-

affin embedding, sectioning (into 4 μm-thick slices), and hematoxylin-eosin (HE) staining. The stained sections were visualized and photographed under an FSX100 microscope.

Immunofluorescence analysis

The brain tissue samples from each group, collected at different time points, were subjected to murine section blockage through sequential incubation with 4% (w/v) BSA, primary antibody GRP78 (overnight incubation), and the corresponding secondary antibody. Afterward, the samples were stained with DAPI and examined under a fluorescence microscope (Nikon, Japan) for counting the cells that were stained positively in each group.

Cell culture

PC12 cells (Shanghai Institute of Biochemistry and Cell Biology) were cultured at 37°C in a humidified chamber with a 5% CO_2 atmosphere. The culture medium used was Ham's F12K medium containing 2 mM of L-glutamine and 1.5 g/L $NaHCO_3$, supplemented with HS (12.5%), an antibiotic-antimycotic (1%), and FBS (2.5%).

MTT assay

Cell viability was evaluated using the MTT assay. Initially, the cells were seeded in the wells of a 96-well plate and cultured until 90% confluency was reached. Next, the cells were exposed to different concentrations (0 μM, 10 μM, 25 μM, and 50 μM) of aconitine for 48 h. After aconitine treatment, MTT buffer (20 μL) was added to each well (containing 200 L of cell culture or medium), followed by another round of incubation at 37°C for 4 h. After crystal formation, the original medium was aspirated gently, dissolved in 150 μL of DMSO, and then gently shaken at room temperature for 5 min to ensure the complete dissolution of the formed crystals. Immediately afterward, absorbance at 490 nm was measured for each well.

TUNEL assay

The TUNEL assay kit was purchased from Synthgene Biotechnology, Jiangsu. The cells were cultured in the wells of 12-well plates until 90% confluence was reached on the coverslips placed in the wells. Subsequently, the cells were exposed to different concentrations (0, 10, 25, and 50 μM) of aconitine for 48 h. After incubation followed by three washes with PBS, the cells were subjected to TUNEL staining performed according to the instructions of the manufacturer. The stained cells were examined under a light microscope using a 40x objective lens to observe the GC apoptosis. The ordinary PC12 cells displayed no morphological changes and blue-violet-stained nuclei. The apoptotic PC12 cells, on the contrary, showed shrinkage, with the nuclei condensed into brown spots.

Apoptosis analysis

In order to detect apoptosis in the cells, Annexin V-FITC/PI staining was performed for 15 min in the dark at room temperature. The stained cells were rinsed, resuspended in binding buffer, and analyzed using a flow cytometer and a fluorescence microscope for the quantification of the apoptotic cells.

Western blotting

Proteins were extracted from both cells and rat brain tissue samples using the RIPA buffer. The concentrations of the extracted proteins were determined using the BCA protein assay kit. After separation of the proteins on a 10% SDS-PAGE gel, the desired protein was transferred to a PVDF membrane at a constant flow of 320 mA. After membrane blockage was performed through incubation with 5% non-fat milk for 1.5 h at room temperature, the membrane was incubated overnight at 4°C with the relevant primary antibodies (anti-GAPDH, anti-ZO-1, anti-claudin 5, anti-IRE-1, anti-GRP78, anti-XBP1, and anti-P-IRE1; 1:1000 dilution). The next day, the membrane was washed three times with TBST and then incubated for 1 h at 37°C with the corresponding HRP-conjugated secondary antibody (1:1000 dilution). After another three washes with TBST, chemiluminescence analysis was performed using the ECL reagent. The expression intensity of each protein on the membrane was recorded using a scanner, and the obtained image was analyzed using an image processing program.

Statistical analysis

All experimental results were presented as mean ± SD of the values obtained from a minimum of three repeated experiments, with $P <$

0.05 considered statistically significant. The GraphPad Prism software (version 6.0, GraphPad Prism Inc., San Diego, CA) was used. One-way ANOVA followed by Tukey's post hoc test was applied.

Results

Aconitine exhibited dose-dependent toxicity in mice

At 0.5 h after the administration of different concentrations of aconitine, the rats in the low-dose (0.5 mg/kg) and middle-dose (1.5 mg/kg) groups exhibited mild neurological symptoms, including increased oral secretions, shortness of breath, and unsteady gait. The high-dose (2.5 mg/kg) group exhibited further severe neurological symptoms, including oral secretion disorder, shortness of breath, and weakness in the hind limb. Histological examination revealed that the 0.5 mg/kg group exhibited slight capillary hyperemia and edema of the granular layer cells, while the 1.5 mg/kg group exhibited evident capillary dilatation and congestion, edema at the base of the granular layer cells, and occasional nerve fiber rupture. In the 2.5 mg/kg group, blood capillaries had expanded, neurons were sparse and exhibited arrangement disorder, the neuronal nucleus was shrunken, and the matrix structure was critically swollen (**Figure 1A**). In the 1.5 mg/kg and 2.5 mg/kg treatment groups, the mean area of intercellular fluid in brain tissue increased significantly ($P < 0.05$).

Aconitine increased the permeability of the blood-brain barrier and affected the expression of the blood-brain barrier-related proteins in rats

The permeability of murine BBB was evaluated through Evans blue staining. The rats in the medicated groups exhibited significantly higher ($P < 0.01$) content of Evans blue in their cerebral tissue compared to the control rats, as visible in **Figure 1B**. Next, the expression of the BBB-related proteins was evaluated through western blotting. In the 2.5 mg/kg treatment group, the levels of claudin5 and ZO-1 proteins declined gradually, as illustrated in **Figure 1C**. At 12 h after treatment, the claudin5 and ZO-1 protein levels decreased progressively (**Figure 1D**) with the increase in the medication concentration (0, 0.5, 1.5, and 2.5 mg/kg), and the increases were significant ($P < 0.01$).

Aconitine promoted apoptosis of neuronal cells in rat brain tissue

The toxic effects of aconitine on rat neurons were observed using the TUNEL assay. The degree of damage to rat brain tissue caused by aconitine was observed to increase in a dosage-dependent manner. In the group that received drug administration, the color of the nuclei of the neurons was further intensified, indicating severe apoptosis (**Figure 2A**).

Aconitine increased the expression of the endoplasmic reticulum stress-related proteins in rat brain tissue

Immunofluorescence staining revealed that aconitine aggravated endoplasmic reticulum stress in rat brain tissue. Moreover, with the increase in the drug concentration, the expression of the endoplasmic reticulum stress marker protein GRP78 in the brain tissue was activated gradually (**Figure 2C**, **2D**). At 12 h after treatment (**Figure 2C**, **2D**), the expression of the endoplasmic reticulum stress-related protein GRP78 varied with aconitine concentration (0, 0.5, 1.5, and 2.5 mg/kg), indicating that aconitine causes brain damage by activating the endoplasmic reticulum stress signaling pathway in the brain tissue. Since the induction of apoptosis via the endoplasmic reticulum stress (ERS) signaling pathway is implicated in the development of various diseases, the expressions of other ERS-related markers were also evaluated by western blot analysis as described earlier. It was observed that the expressions of XBP-1, p-IRE-1, and GRP78 in the brain tissue increased with the increase in the drug concentration (**Figure 2E**, **2F**).

Aconitine inhibited the viability of PC12 cells and promoted their apoptosis

The cell injury induced by aconitine was evaluated using the MTT assay. The results revealed that increasing aconitine concentration aggravated the inhibitory effect of aconitine on PC12 cell viability (**Figure 3A**). In addition, the degree of apoptosis induced by aconitine in the PC12 cells, determined through flow cytometry, aggravated with the increase in aconitine concentration (**Figure 3B**, **3C**).

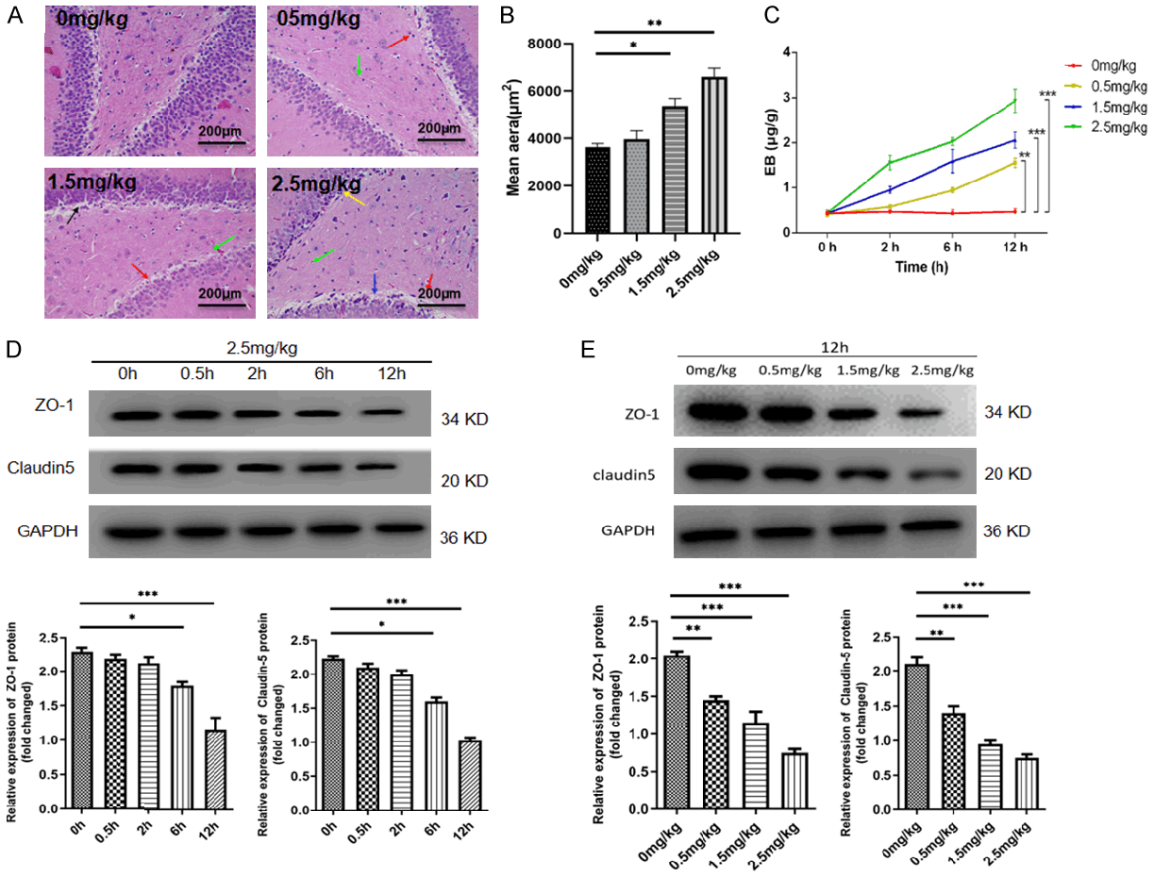

Figure 1. The blood-brain barrier was partially disrupted upon aconitine administration. (A) The effects of aconitine on neuronal cells (H&E staining results): capillary hyperemia (green arrows), edema at the base of granulosa cells (red arrows), nerve fiber fracture (black arrows), the disorder of neurons (yellow arrows), and neuronal nucleus pyknosis hyperchromatism (blue arrows); scale bar = 200 μm. (B) Mean area of intercellular fluid in brain tissue after treatment with different concentrations of aconitine. $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA followed by Tukey's post hoc test (n = 4). (C) Evans blue content was positively correlated with aconitine concentration in rat brain tissues. $^{**}P < 0.01$, $^{***}P < 0.001$; one-way ANOVA followed by Tukey's post hoc test (n = 4). (D) Expressions of ZO-1 and claudin5 at different time points after treatment with 2.5 mg/kg aconitine (Western blotting results). Histogram summarizes the results in (D). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; one-way ANOVA followed by Tukey's post hoc test (n = 4). (E) Expressions of ZO-1 and claudin5 proteins in the 12 h after treatment with different concentrations of aconitine (Western blotting results). Histogram summarizes the results in (E). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; one-way ANOVA followed by Tukey's post hoc test (n = 4).

Aconitine activated endoplasmic reticulum stress in PC12 cells

Immunofluorescence staining revealed that aconitine aggravated endoplasmic reticulum stress in PC12 cells. Moreover, the expression of the endoplasmic reticulum stress marker protein GRP78 in the PC12 cells was increased along with the increase in aconitine concentration (**Figure 3D**, **3E**).

Discussion

Aconitine is the major medicinal constituent in the Aconitum plants that are native to China. Aconitine is generally used in the management of diseases such as rheumatism. However, the therapeutic dosage range of aconitine is narrow, and ingestion of even 0.2 mg of aconitine may cause poisoning. This is because of the strong neurotoxicity of aconitine, which often leads to aconitine poisoning or even death due to the misuse of this drug [16-18]. In recent years, few studies have been conducted across the world to decipher the mechanism underlying the central nervous system toxicity caused by aconitine [12]. Moreover, the majority of the existing studies are limited to ion channels, neurotransmitters, energy metabolism,

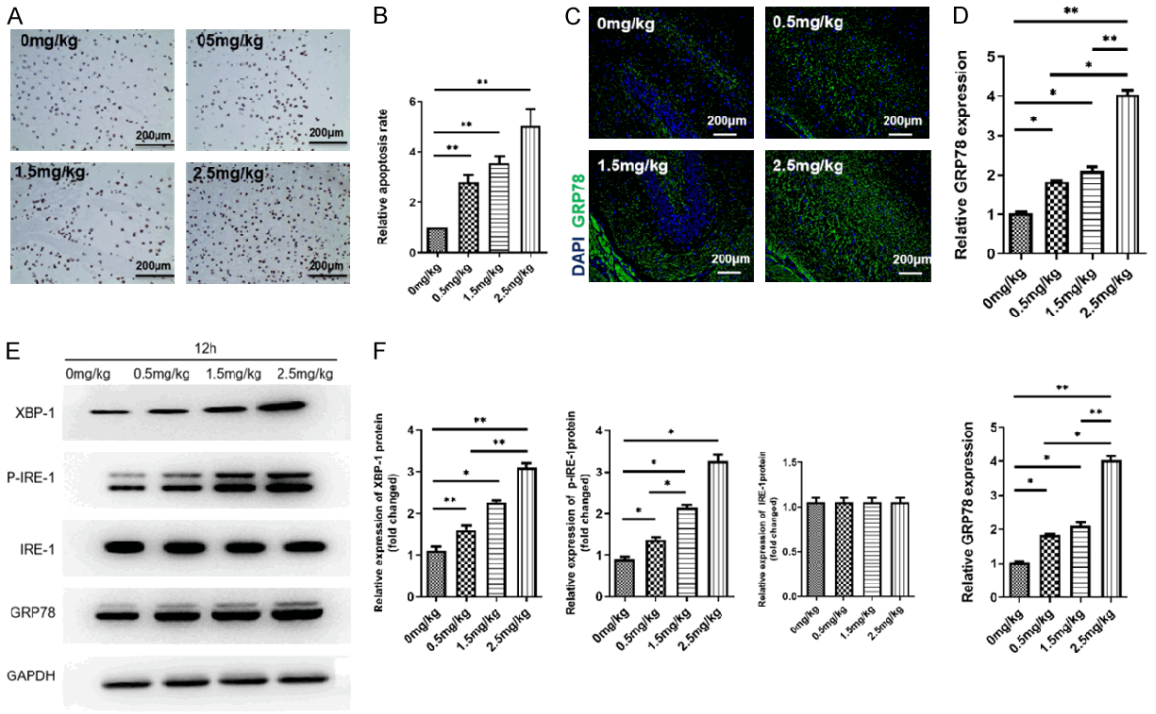

Figure 2. Neuronal apoptosis and activation of endoplasmic reticulum stress in rat brain tissues upon aconitine administration. (A) Apoptosis of neuronal cells in rat brain tissues at 12 h after treatment with different concentrations of aconitine, determined using the Tunnel assay (scale bar = 200 μm). (B) Quantitative analysis of the apoptosis rate. $^{**}P < 0.01$; one-way ANOVA followed by Tukey's post hoc test (n = 4). (C) Immunofluorescence staining results illustrating the accumulation of GRP78 (green) gradually increasing with the increase in drug concentration. Cell nuclei (blue) were counterstained with DAPI. Scale bar = 200 μm. (D) Bar graph summarizing the immunofluorescence results for the accumulation of GRP78 presented in (C). $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA followed by Tukey's post hoc test (n = 4). (E) Bands representing the expressions of XBP-1, p-IRE-1, IRE-1, and GRP78 proteins at 12 h after treatment with different drug concentrations (western blotting results). GAPDH was used as the internal control. (F) Histogram summarizing the results for the expressions of XBP-1, p-IRE-1, IRE-1, and GRP78 presented in (E). $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA followed by Tukey's post hoc test (n = 4).

and nerve cell damage, while studies on the roles of gene regulation and the blood-brain barrier in the central nervous system toxicity caused by aconitine are scarce [19-21]. Therefore, to further deepen the understanding of the mechanism underlying the neurotoxicity caused by aconitine, the present study aimed to determine whether the mechanism of brain damage caused by aconitine is related to the blood-brain barrier damage. Under physiological conditions, these tight connections restrict paracellular transport, thereby effectively preventing the toxic substances from entering the brain parenchyma. The molecular weight of the Evans blue (EB) dye is close to that of albumin present in the blood, and EB is capable of binding to albumin [23]. Therefore, upon injury to the central nervous system, the permeability of the BBB increases, and the albumin-bound Evans blue is able to cross the BBB smoothly and enter the brain tissue. This phenomenon forms the basis of the quantitative detection of BBB damage [24]. In the present study, it was revealed that the expressions of ZO-1 and Claudin5 proteins decreased gradually with the prolongation of aconitine poisoning and the increase in the medication concentration. The decrease in the expression of tight junction protein was most evident at 12 h after treatment, which was consistent with the EB exudation detected in the brain tissue at 12 h after aconitine administration. Therefore, it was inferred that acute aconitine poisoning may lead to the breakdown of the BBB.

Furthermore, cell morphology is reported to play a crucial role in the maintenance of cellular function [25]. After apoptosis, the corresponding function of the tissue is inevitably affected, eventually leading to the dysfunction of the

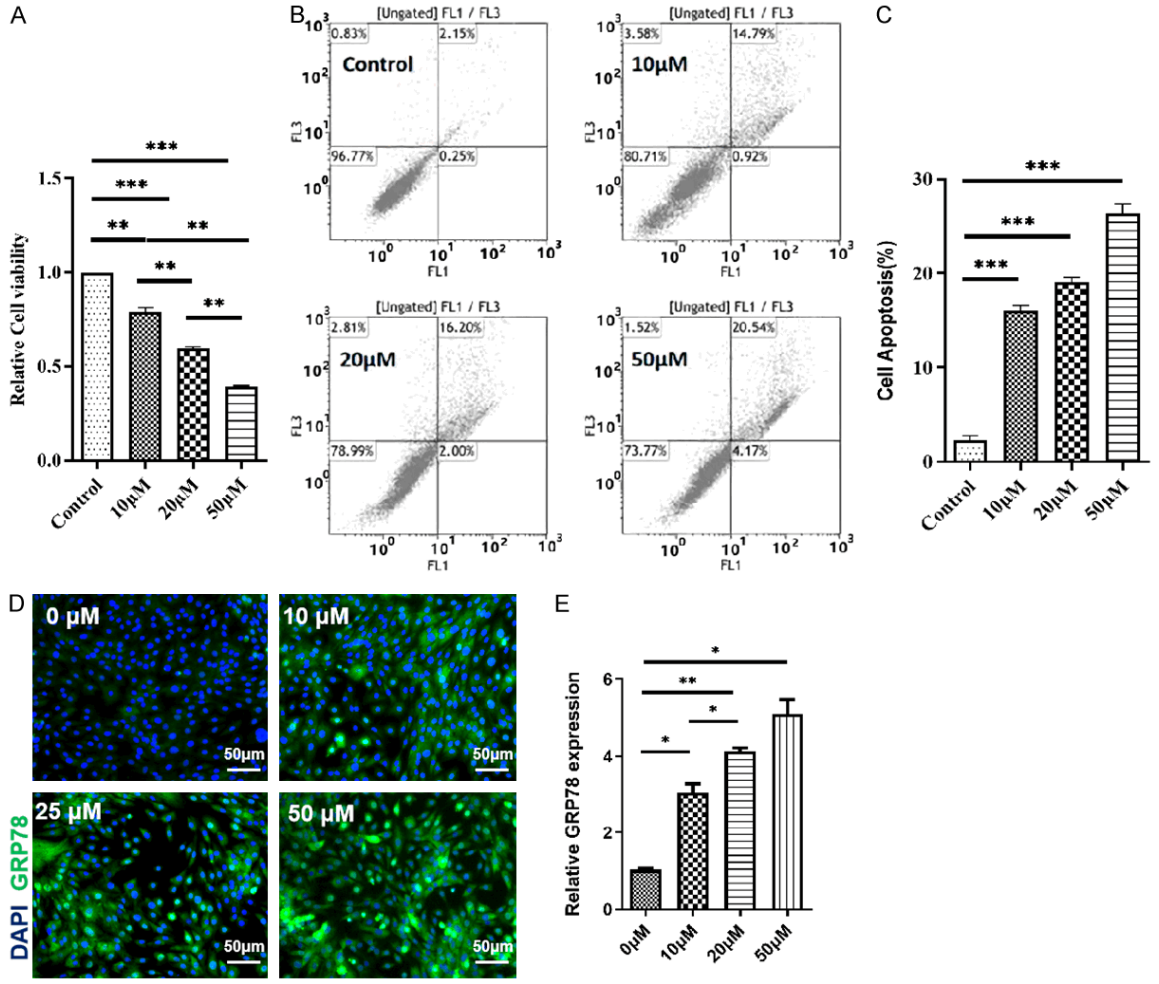

Figure 3. Neuronal apoptosis and activation of endoplasmic reticulum stress upon aconitine administration *in vitro* in PC12 cells. (A) The effects of different concentrations of aconitine on PC12 cell viability, determined through the MTT assay. $^{**}P < 0.01$, $^{***}P < 0.001$; one-way ANOVA followed by Tukey's post hoc test (n = 5). (B) The effects of different concentrations of aconitine on PC12 cell apoptosis determined through flow cytometry. (C) Bar graph summarizing the flow cytometry results for PC12 cell apoptosis presented in (B). $^{***}P < 0.001$; one-way ANOVA followed by Tukey's post hoc test (n = 5). (D) Immunofluorescence staining results illustrating the accumulation of GRP78 (green) gradually increasing with the increase in drug concentration. Cell nuclei (blue) were counterstained with DAPI. Scale bar = 50 μm. (E) Bar graph summarizing the immunofluorescence results for the accumulation of GRP78 presented in (D). $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA followed by Tukey's post hoc test (n = 5).

body [26]. According to staining results obtained in the present study, the degree of damage to the rat brain tissue caused by aconitine was further deteriorated as aconitine concentration increased. Neuronal cell edema and vacuolar degeneration were observed, while the vascular space was significantly widened, and the apoptosis was worsened. The results of the *in vitro* experiments in PC12 cells revealed that proliferation decreased, and apoptosis increased in these cells treated with increased aconitine concentration. According to previous studies, apoptosis induction via the endoplasmic reticulum (ER) signaling pathway is implicated in the development of various diseases [27-29]. Therefore, the next step in the present study was to determine whether the endoplasmic reticulum stress (ERS) was involved in the apoptosis induced by aconitine. ER is the most important Ca^{2+} reservoir of cells, with the concentration of Ca^{2+} in the ER being thousands of times higher than that in the cytoplasm [51]. The experimental results of the present study revealed that the expressions of the ERS-related proteins XBP-1, p-IRE-1, and GRP78 increased gradually with the increase in aconi-

tine concentration. It is reported [30] that the toxic effects of aconitine on nerve cells could be related to the inhibition of ion channel activity, intracellular calcium accumulation, and calcium balance-imbalance activation. Therefore, it was inferred that the activation of the ERS signaling pathway in the brain tissue could be related to intracellular calcium overload and calcium imbalance.

In the past decade, nearly 5,000 cases of aconitine poisoning have been reported worldwide, with the affected individuals often exhibiting symptoms involving the central nervous system [31]. Although several relevant studies have attempted to describe the mechanism underlying the central nervous system toxicity caused by aconitine, the precise mechanism of this toxicity remains to be elucidated. In this context, the present study, through both *in vitro* and *in vivo* experiments, demonstrated that aconitine may cause critical brain damage via two pathways: 1) by reducing the BBB penetrability through the down-regulation of ZO-1 and claudin5 proteins; 2) through significant upregulation of the p-IRE-1, XBP-1, and GRP78 proteins, thereby causing brain tissue damage via the over-activation of the ERS levels in the brain tissue. These findings may broaden the scope of understanding the mechanism underlying the central nervous system toxicity caused by aconitine and establish a strong theoretical foundation for further research on developing aconitine neurotoxicity antagonists for the clinical management of aconitine poisoning.

In conclusion, the neurotoxic mechanism of aconitine may be related to the destruction of BBB and trigger of endoplasmic reticulum stress. However, the concrete ERS signaling pathways remain to be fully elucidated, which is the limitation and the future work of our study.

Acknowledgements

The present work was supported by the Project of Zunyi Science and Technology [Project No. (2019) 89 and (2021) 56] and the Project of Guizhou Administration of Traditional Chinese Medicine [Project No. QZYY-2019-012].

Disclosure of conflict of interest

None.

Address correspondence to: Haizhen Duan, Department of Emergency, The Affiliated Hospital of Zunyi Medical University, No. 149, Dalian Road, Huichuan District, Zunyi 563003, Guizhou, China. Tel: +86-851-28608260; E-mail: haizhenduan@126.com

References

[1] Zhang Y, Zong X, Wu JL, Liu Y, Liu Z, Zhou H, Liu L and Li N. Pharmacokinetics and tissue distribution of eighteen major alkaloids of Aconitum carmichaelii in rats by UHPLC-QQQ-MS. J Pharm Biomed Anal 2020; 185: 113226.

[2] Gao X, Hu J, Zhang X, Zuo Y, Wang Y and Zhu S. Research progress of aconitine toxicity and forensic analysis of aconitine poisoning. Forensic Sci Res 2020; 5: 25-31.

[3] He Y, Wei Z, Ci X, Xie Y, Yi X, Zeng Y, Li Y and Liu C. Effects of liquorice on pharmacokinetics of aconitine in rats. Xenobiotica 2019; 49: 1485-1493.

[4] Zhou W, Liu H, Qiu LZ, Yue LX, Zhang GJ, Deng HF, Ni YH and Gao Y. Cardiac efficacy and toxicity of aconitine: a new frontier for the ancient poison. Med Res Rev 2021; 41: 1798-1811.

[5] Li X, Gu L, Yang L, Zhang D and Shen J. Aconitine: a potential novel treatment for systemic lupus erythematosus. J Pharmacol Sci 2017; 133: 115-121.

[6] Tai CJ, El-Shazly M, Wu TY, Lee KT, Csupor D, Hohmann J, Chang FR and Wu YC. Clinical aspects of aconitum preparations. Planta Med 2015; 81: 1017-1028.

[7] Lu G, Dong Z, Wang Q, Qian G, Huang W, Jiang Z, Leung KS and Zhao Z. Toxicity assessment of nine types of decoction pieces from the daughter root of Aconitum carmichaeli (Fuzi) based on the chemical analysis of their diester diterpenoid alkaloids. Planta Med 2010; 76: 825-830.

[8] Bonanno G, Ippolito M, Moscarelli A, Misseri G, Caradonna R, Accurso G, Cortegiani A and Giarratano A. Accidental poisoning with Aconitum: case report and review of the literature. Clin Case Rep 2020; 8: 696-698.

[9] Rao S and Sikdar SK. Modification of alpha subunit of RIIA sodium channels by aconitine. Pflugers Arch 2000; 439: 349-355.

[10] Ameri A. Age-dependent differences in sensitivity to aconitine of rat hippocampal slices. Neurosci Lett 1996; 216: 13-16.

[11] Voss LJ, Voss JM, McLeay L and Sleigh JW. Aconitine induces prolonged seizure-like events in rat neocortical brain slices. Eur J Pharmacol 2008; 584: 291-296.

[12] Niitsu H, Fujita Y, Fujita S, Kumagai R, Takamiya M, Aoki Y and Dewa K. Distribution of Aconi-

tum alkaloids in autopsy cases of aconite poisoning. Forensic Sci Int 2013; 227: 111-117.
[13] Xiang BQ, Gao H, Chen XW, Dai YY and Wang WT. Excessive endoplasmic reticulum stress mediates brain damage in hypoxia hypercapnia induced pulmonary hypertension rats. Sheng Li Xue Bao 2017; 69: 413-421.
[14] Ryu S, Lim W, Bazer FW and Song G. Chrysin induces death of prostate cancer cells by inducing ROS and ER stress. J Cell Physiol 2017; 232: 3786-3797.
[15] Casas C. GRP78 at the centre of the stage in cancer and neuroprotection. Front Neurosci 2017; 11: 177.
[16] Lin CC, Chan TY and Deng JF. Clinical features and management of herb-induced aconitine poisoning. Ann Emerg Med 2004; 43: 574-579.
[17] Chan TY. Aconitum alkaloid poisoning related to the culinary uses of aconite roots. Toxins (Basel) 2014; 6: 2605-2611.
[18] Wei S, Zhang H, Li B, Ji J and Shao X. Insecticidal effect of aconitine on the rice brown planthoppers. PLoS One 2019; 14: e0221090.
[19] Lou J, Wu H, Wang L, Zhao L, Li X, Kang Y, Wen K and Yin Y. Taurine-magnesium coordination compound, a potential anti-arrhythmic complex, improves aconitine-induced arrhythmias through regulation of multiple ion channels. Toxicol Appl Pharmacol 2018; 356: 182-190.
[20] Zhu L, Lu L, Guo E, Wu J, Wang Y, Hu M and Liu Z. The influences of aconitine, an active/toxic alkaloid from aconitum, on the oral pharmacokinetics of CYP3A probe drug buspirone in rats. Drug Metab Lett 2014; 8: 135-144.
[21] Gao X, Zhang X, Hu J, Xu X, Zuo Y, Wang Y, Ding J, Xu H and Zhu S. Aconitine induces apoptosis in H9c2 cardiac cells via mitochondriamediated pathway. Mol Med Rep 2018; 17: 284-292.
[22] Obermeier B, Daneman R and Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nat Med 2013; 19: 1584-1596.
[23] Lin Y, Pan Y, Wang M, Huang X, Yin Y, Wang Y, Jia F, Xiong W, Zhang N and Jiang JY. Blood-brain barrier permeability is positively correlated with cerebral microvascular perfusion in the early fluid percussion-injured brain of the rat. Lab Invest 2012; 92: 1623-1634.
[24] Seiffert E, Dreier JP, Ivens S, Bechmann I, Tomkins O, Heinemann U and Friedman A. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. J Neurosci 2004; 24: 7829-7836.
[25] Chengappa P, Sao K, Jones TM and Petrie RJ. Intracellular pressure: a driver of cell morphology and movement. Int Rev Cell Mol Biol 2018; 337: 185-211.
[26] D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 2019; 43: 582-592.
[27] Yan M, Shu S, Guo C, Tang C and Dong Z. Endoplasmic reticulum stress in ischemic and nephrotoxic acute kidney injury. Ann Med 2018; 50: 381-390.
[28] Zhang L, Cheng X, Xu S, Bao J and Yu H. Curcumin induces endoplasmic reticulum stress-associated apoptosis in human papillary thyroid carcinoma BCPAP cells via disruption of intracellular calcium homeostasis. Medicine (Baltimore) 2018; 97: e11095.
[29] Al-Bahlani SM, Al-Bulushi KH, Al-Alawi ZM, Al-Abri NY, Al-Hadidi ZR and Al-Rawahi SS. Cisplatin induces apoptosis through the endoplasmic reticulum-mediated, Calpain 1 pathway in triple-negative breast cancer cells. Clin Breast Cancer 2017; 17: e103-e112.
[30] Peng C, Zheng T, Yang F, Li YX and Zhang DK. Study of neurotoxic effects and underlying mechanisms of aconitine on cerebral cortex neuron cells. Arch Pharm Res 2009; 32: 1533-1543.
[31] Chan TY. Incidence and causes of aconitum alkaloid poisoning in Hong Kong from 1989 to 2010. Phytother Res 2015; 29: 1107-1111.