Int J Clin Exp Med 2017;10(3):4598-4605
www.ijcem.com /ISSN:1940-5901/IJCEM0042270

Original Article

Efficacy of catalpol and sub-hypothermia therapy on acute severe brain injury and related mechanism

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Received February 24, 2016; Accepted December 15, 2016; Epub March 15, 2017; Published March 30, 2017

Abstract: Acute severe brain injury is difficult for treatment, causing unfavorable prognosis, high morbidity and mortality. Sub-hypothermia may be one of the effective treatments for brain injury, so does catalpol, which has neuroprotective function. The potency and mechanism of combined therapy of catalpol and sub-hypothermia, however, has not been illustrated in patients with diffused brain trauma. Patients with acute and severe brain injury were randomly divided into sub-hypothermia treatment group and catalpol plus sub-hypothermia groups (Combined therapy group). Parameters of patients including intracranial pressure (ICP), blood glucose, lactic acid and oxygen pressure of brain tissues ($P_{br}O_2$) were monitored. Glasgow coma scale (GCS) of patients was used for treatment efficacy evaluation. Acute severe brain injury of rat model was then established and treated with sub-hypothermia only and catalpol plus sub-hypothermia, respectively. Western blotting was used to quantify expressions of Bcl-2 and Bax in rat brain, while caspase 3 activity was measured by test kit. The secretion of inflammatory factors including tumor necrosis factor (TNF)-α and interleukin (IL)-β in rat brain tissues were measured by enzyme linked immunosorbent assay (ELISA). Combined therapy of both sub-hypothermia and catalpol in patients decreased levels of ICP, blood glucose and lactate, and increased $P_{br}O_2$ and GCS score. In rat model, combined treatment decreased caspase 3 activity, Bax expression, secretions of TNF-α and IL-1β, and increased Bcl-2 expression compared with control group ($P < 0.05$). In general, combined therapy had higher efficacy than pure sub-hypothermia therapy. Combined therapy of catalpol and sub-hypothermia could treat acute severe brain injury via mediating the balance between apoptosis and proliferation, as well as decreasing secretions of inflammatory factors, with higher efficacy than pure sub-hypothermia treatment.

Keywords: Catalpol, sub-hypothermia, severe brain injury, apoptosis, inflammatory factor

Introduction

Brain injury has significantly higher mortality and morbidity than any other trauma [1]. Acute severe brain injury (ASBI) is the most severe and frequent sub-type of brain injury. It usually has features including worse prognosis, high morbidity and high mortality. With difficulty in treatment, ASBI may develop into progressive intracranial hypertension, which aggravates primary brain injury, even causing brain hernia, central nervous system failure and death [2, 3]. ASBI thus severely affects the life quality of the public, and brings heavy burdens for the society and family, thus becoming one major public health issue [4]. Sub-hypothermia therapy has significant effects in treating ASBI patients, especially in those with high intracranial pressure (ICP) or brain contusion. Based on the monitor of ICP, sub-hypothermia therapy in appropriate time window can effectively treat ASBI patients without other major complications, by the help of slow warming measures [5, 6]. ASBI is one complicated pathological process involving multiple factors including energy exhausting, glutamate excitotoxicity, free radicals production and inflammation [7, 8]. Sub-hypothermia therapy can alleviate brain edema, decrease the production of free radicals, lower the permeability of blood-brain barrier (BBB) and blood vessels, suppress intracellular acidosis, inhibit body immune response or inflammatory reaction, and decrease energy consumption or alter the metabolic pathway to protect against ASBI for improving patients' prognosis [9-11]. Satisfactory efficacy, however, has not been obtained using pure sub-hypothermia therapy in the treatment of ASBI. Therefore it is

critical to optimize effective drug candidates for treating ASBI.

Catalpol is an effective component extracted from radix rehmanniae, figwort family of traditional Chinese medicine. It is a cyclic enol ether-terpene compound with small molecular weight [12]. Previous study has confirmed its pluripotency including anti-tumor, anti-fugal, anti-viral, anti-Alzheimer's disease, inhibiting permeability of micro-vessels and anti-inflammation [13]. It can also exert protective functions against H_2O_2-induced oxidative stress injury [14]. Its potential function in treating ASBI in conjunction with sub-hypothermia therapy, however, remains unknown yet.

Materials and methods

Research objects

A total of 72 diagnosed ASBI patients from January 2015 to June 2015 were recruited from Weifang People's Hospital. There were 37 males and 35 females, aged between 21 and 72 years (average age = 43.2±11.4 years). All patients were admitted within 24 hours after primary injury, and were diagnosed by clinical symptoms and CT or MRI. GCS in all patients were less or equal to 8 points. No major organ injuries or dysfunctions occurred without tumor or auto-immune diseases.

The experimental protocol has been pre-approved by the ethical committee of Weifang People's Hospital and written consents have been obtained from all patients and healthy volunteers.

Animals

A total of 60 healthy male SD rats (2 month old, SPF grade, body weight 250±20 g) were purchased from laboratory animal center of Weifang Medical university and were kept in an SPF-grade facility there.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Weifang People's Hospital.

Drugs and reagents

Catalpol was purchased from Medicine and Biological Product Institute of China. 10% chloral hydrate was provided in house by the central laboratory of our University. PVDF membrane was purchased from Pall Life Sciences (US). EDTA was obtained from Hyclone (US). Chemical reagents for Western blotting were purchased from Beyotime (China). ECL kit was purchased from Amersham Biosciences (US). Rabbit anti-mouse Bcl-2 and Bax monoclonal antibody, and goat anti-rabbit horseradish peroxidase (HRP) conjugated IgG antibody were obtained from Cell Signaling (US). Caspase 3 activity assay kit was purchased from Pall Life Sciences (US). Enzyme linked immunosorbent assay (ELISA) kit for tumor necrosis factor (TNF)-α and interleukin (IL)-1β were obtained from R&D (US). Animal respirator machine was provided by Harvard (US). Tracheal intubation and carotid intubation tubes were purchased from BD (US).

Patient grouping and treatment

All included patients were randomly divided into two groups, sub-hypothermia group and combined therapy group (N = 36 each). The former group contained 19 males and 17 females, with an average age of 42.5±13.4 years, and average GCS of 5.6±1.8. The combined therapy group consisted of 18 males and 18 females, with an average age of 43.6±11.8 years, and average GCS of 5.8±2.1. No significant differences existed regarding general information between those two groups. In sub-hypothermia therapy group, ventilator (Drager Evita, Germany) was applied after tracheotomy and IV dropping of hibernation-muscle relaxation compound. Body temperature was lowered by water circulation cooling blanket (Medithams, US). 20 mg/kg catalpol was also applied simultaneously with sub-hypothermia therapy in the combined group. Twenty-four hours after the ICP levels were achieved to the normal level, the combined therapy was stopped.

Clinical parameters

ICP was monitored by bedside monitors (HP, US). After therapy, blood glucose and lactate acid levels were quantified in venous blood. Brain oxygen-temperature monitor (GMS, Germany) was employed to determine oxygen pressure of brain tissues ($P_{br}O_2$) level. Seven days after treatment, GCS and other indexes were also evaluated.

ASBI rat model

Rats were fasted 4 hours before surgery. After anesthesia with 10% choral hydrate, rats were fixed in supine position and were sterilized to expose trachea. Intubation was performed inside carotid artery, followed by application of ventilator. Rats were then adapted in a prone position, followed by hair shaving on head and neck. Skull between corona suture and lambdoid suture was exposed. A 450 g metal hammer was fallen from 1.5 m height to hit the mesh on rat head to prepare ASBI model as previously documented [15].

Animal group and treatment

ASBI rats were randomly divided into control, sub-hypothermia and combined therapy groups (N = 20 each). In sub-hypothermia therapy group, water circulation cooling blanket (Medithams, US) was applied to suppress body temperature. In combined therapy group, 20 mg/kg catalpol was also applied simultaneously for 48 h. After treatment, all rats were sacrificed to separate serum, which was stored at -20°C, while leaving brain tissues frozen at -80°C.

Caspase 3 activity assay

Caspase 3 activity in rat brain tissues were detected by assay kit. In brief, cells were firstly digested in trypsin followed by centrifugation at 600 g 4°C for 5 min. Supernatant was discarded, with the addition of tissue lysis buffer for 15-min iced treatment. The mixture was then centrifuged at 20000 g for 5 min at 4°C. 2 mM Ac-DEVD-pNA was then added to detect the optical density (OD) value at 405 nm wave length, reflecting the active level of caspase 3.

Western blotting

Brain tissues were mixed with lysis buffer for iced incubation for 15~30 min. After ultrasound treatment (5 s for 4 times), the mixture was centrifuged at 10000 g for 15 min. The supernatant was saved, quantified, and stored at -20°C for Western blotting assay. Proteins were separated in 10% SDS-PAGE, and were transferred to PVDF membrane by semi-dry method. Non-specific background was removed by 5% defatted milk powder for 2-hour incubation at room temperature. Primary antibody against Bcl-2 or Bax (1:1000 or 1:2000 dilutions) was added with overnight incubation at 4°C. After PBST washing, goat anti-rabbit secondary antibody (1:2000) was then added at dark at room temperature for 30 min. ECL reagent was then used to develop the membrane for 1 min, followed by X-ray exposure and observation. Protein imaging system and Quantity One software was then applied to scan X-ray films and band density. Each experiment was repeated for four times (N = 4).

HE staining

The rat brain samples were fixed with 4% polyoxymethylene, then the samples were embedded with paraffin within 24 h. The HE staining assay was used to observe morphological changes of rat brain tissues under the optical microscope. Finally, we selected 10 high power fields for counting the eosinophils and neutrophils in the tissues.

ELISA

Serum samples were collected from all groups of rats to detect the level of inflammatory factors including TNF-α and IL-1β following the manual instruction of test kits. In brief, 96-well plate was added with 50 μl serially diluted samples, which were used to plot standard curves. 50 μl test samples were then added into test wells in triplicates. After washing for 5 times, liquids were discarded to fill with washing buffer for 30 sec vortex. The rinsing procedure was repeated for 5 times. 50 μl enzyme labelling reagent was then added into each well except blank control. After gentle mixture, the well was incubated for 30 min at 37°C. Chromogenic substrates A and B were sequentially added (50 μl each), followed by 37°C dark incubation for 10 min. The test plate was then mixed with 50 μl quenching buffer as the blue color turned into yellow. Using blank control well as the reference, OD values at 450 nm wavelength were measured by a microplate reader within 15 min after adding quenching buffer. Linear regression model was then plotted based on the concentration of standard samples and respective OD values. Sample concentration was further deduced based on OD values and regression function.

Statistical analysis

At least three independent experiments were performed for each assay of the animal stud-

Table 1. Blood glucose and lactate acid level before and after treatment

Group	Blood glucose (mmol/L)			Blood lactate acid (mmol/L)		
	Before	After	t/*P* value	Before	After	t/*P* value
Sub-hypothermia	11.5±2.3	7.6±2.5*	1.24/0.04	6.7±1.9	2.5±1.4*	2.78/0.026
Catalpol + sub-hypothermia	11.8±1.9	5.2±2.8*,#	1.87/0.03	6.8±1.8	0.9±1.5*,#	3.28/0.013
t/P	1.12/0.073	1.19/0.048		1.06/0.082	2.24/0.021	

Note: *P < 0.05 compared with those before treatment; #P < 0.05 compared to those in sub-hypothermia group.

Table 2. ICP and prognosis of all patients before and after treatment

Group	ICP (mmHg)			GCS		
	Before	After	t/*P* value	Before	After	t/*P* value
Sub-hypothermia	27.2±4.3	21.5±2.7*	1.67/0.044	5.6±1.8	9.3±2.5*	1.54/0.042
Catalpol + sub-hypothermia	28.1±2.9	12.1±3.2*,#	1.94/0.041	5.8±2.1	13.9±2.9*,#	1.69/0.034
t/*P* value	1.23/0.082	1.86/0.036		1.32/0.076	1.76/0.028	

Note: *P < 0.05 compared with those before treatment; #P < 0.05 compared with those in sub-hypothermia group.

ies. SPSS16.0 statistical software was used to process all collected data, in which the measurement data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to compare means across multiple groups. A statistical significance was defined when P < 0.05.

Results

Blood glucose and lactate acid levels in patients

All ASBI patients were treated with pure sub-hypothermia or combined therapy. 7 d after treatment, blood glucose and lactate levels were compared between two groups. Results showed that the average continuous treatment time of sub-hypothermia treatment was 58.9± 22.6 hours during 1~7 days, while the average sub-hypothermia time duration in combined therapy group was 57.2±21.7 hours within 1~6 days. The combined therapy thus decreased the time duration of sub-hypothermia treatment but without statistical significance. 7 days after treatment, both treatment methods could decrease the patients' blood glucose and lactate acid levels significantly as compared to those before treatment (P < 0.05). The combined therapy had higher efficacy than pure sub-hypothermia in the treatment of patients with ASBI (**Table 1**).

ICP level and GCS score in patients

We further compared ICP and patient prognosis between two groups. Results showed that either treatment plan effectively decrease ICP as compared to those before treatment (P < 0.05). Combined therapy with catalpol had higher efficacy than pure sub-hypothermia treatment. GCS score of patients after 7 d of the treatment showed significantly elevated performance by combined therapy as compared to pure sub-hypothermia treatment. Both treatments also had better performance than those before treatment (P < 0.05, **Table 2**).

Apoptosis examination in rat model

Test kit was further used to analyze the caspase 3 activity in all groups of model rats. Results showed that after 48 h of treatment, both strategy (sub-hypothermia and combined therapy) effectively decreased caspase 3 activity compared with those before treatment (P < 0.05). However, catalpol plus sub-hypothermia treatment had even lower caspase 3 activity than pure sub-hypothermia treatment (P < 0.05, **Figure 1A**).

Meanwhile, the apoptosis of the ABSI rat model was also examined by TUNEL assay. The results indicated that after 48 h of treatment, both sub-hypothermia treatment and combined therapy obviously decreased TUNEL staining positive cells compared with those before treatment (P < 0.05, **Figure 1B**).

Bcl-2 and Bax protein expression in rat model

Western blotting was further employed to detect the expression of Bcl-2 and Bax proteins in ASBI rat brain tissues after 48 hours. Results

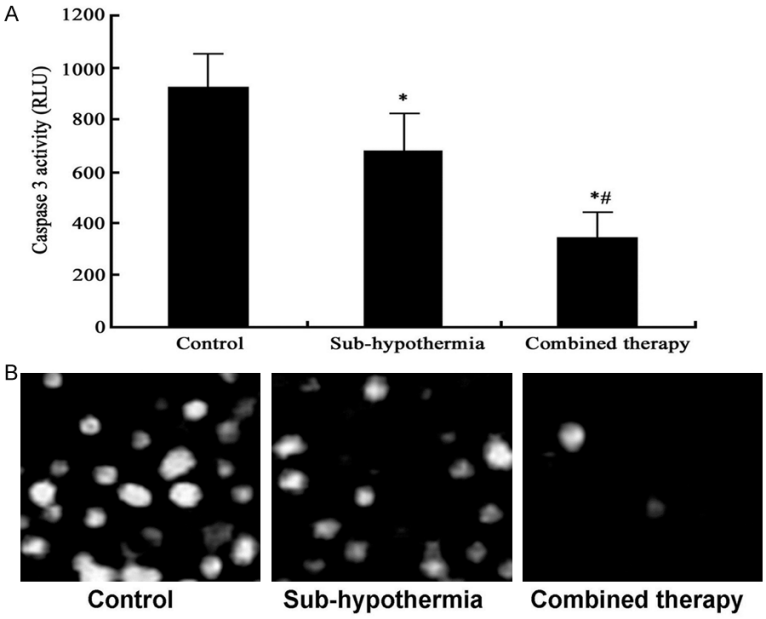

Figure 1. Observation for the apoptosis in ASBI rat model. A. Caspase 3 activity examined by ELISA assay. *P < 0.05 compared with those before treatment; #P < 0.05 compared with those in sub-hypothermia group. B. Apoptosis examined by TUNEL assay. The light stained cells represent the apoptotic cells.

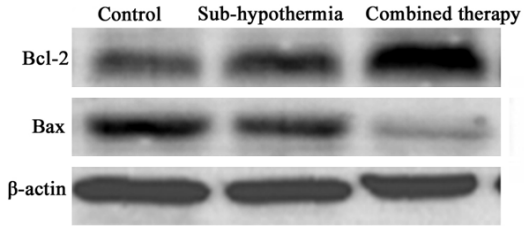

Figure 2. Bcl-2 and Bax protein bands in ASBI rats.

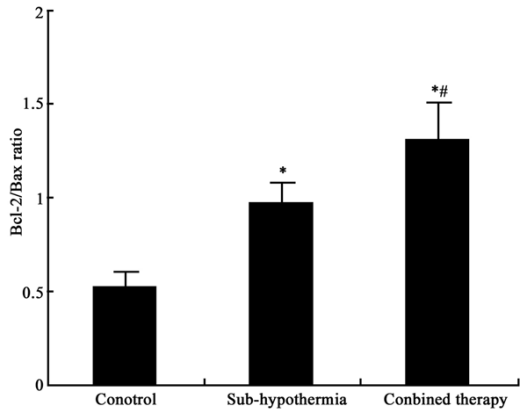

Figure 3. Bcl-2/Bax ratio in ASBI rats. *P < 0.05 compared with those before treatment; #P < 0.05 compared with those in sub-hypothermia group.

found elevated anti-apoptotic protein Bcl-2 and decreased apoptotic protein Bax levels (and elevated Bcl-2/Bax ratio) after treated with sub-hypothermia only or catalpol plus sub-hypothermia) as compared to those before treatment (P < 0.05). The combined therapy obtained higher treatment efficacy regarding Bcl-2/Bax ratio as compared to sub-hypothermia group (P < 0.05, **Figures 2** and **3**).

Inflammatory cell counting in rat model

Morphological changes of ASBI rat brain tissues were examined by HE staining (**Figure 4**). The results showed that many eosinophils and neutrophils were observed in the control group (**Figure 4A**). The Sub-hypothermia treatment obviously decreased the eosinophils and neutrophils levels compared with control group (**Figure 4B**). What's most important is that there were even no eosinophils and neutrophils in the combined therapy group (**Figure 4C**).

Serum TNF-α level under catalpol plus sub-hypothermia therapy in rat model

ELISA was employed to detect the effect of catalpol plus sub-hypothermia therapy on the serum levels of TNF-α in ASBI rats. Results showed that, after 48 h treatment of pure sub-hypothermia or combined therapy, serum TNF-α levels were all significantly decreased in either group as compared to those before treatment (P < 0.05). Moreover, we found that combined therapy had more potent inhibitory effects on the serum TNF-α levels compared with sub-hypothermia group (P < 0.05, **Figure 5**).

Rat serum IL-1β level under hypothermia plus catalpol treatment for rat model

ELISA was employed to detect the secretory level of inflammatory factor IL-1β in ASBI rats from all groups after combined therapy of both

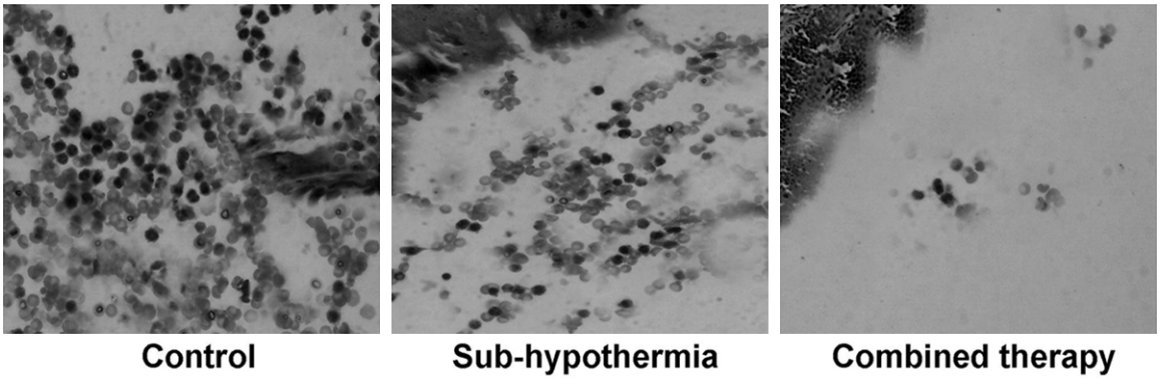

Figure 4. Morphological changes of ASBI rat brain tissues. The brain tissues were stained by using the HE staining. The dark stained cells represent the neutrophils, and gray stained cells represent the eosinophils.

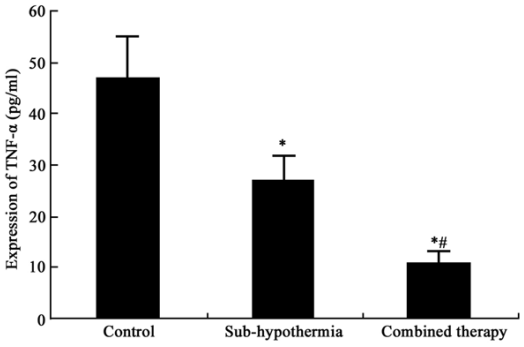

Figure 5. Serum TNF-α levels in ASBI rats. *P < 0.05 compared with those before treatment; #P < 0.05 compared with those in sub-hypothermia group.

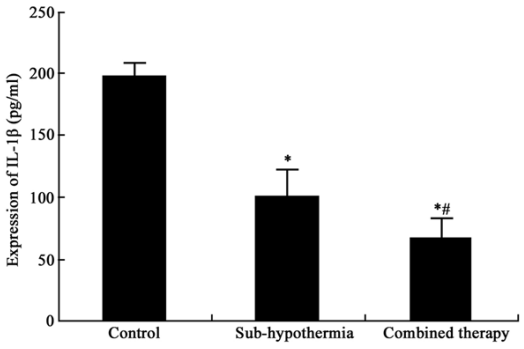

Figure 6. Serum IL-1β levels in ASBI rats. *P < 0.05 compared with those before treatment; #P < 0.05 compared with those in sub-hypothermia group.

catalpol and sub-hypothermia. Results showed that after 48 h of both treatments, serum IL-1β levels in both groups were significantly decreased compared with control group (P < 0.05). Catalpol plus sub-hypothermia treatment, however, had more potent inhibitory effects on the serum IL-1β levels as compared to sub-hypothermia treatment (P < 0.05, **Figure 6**).

Discussion

ASBI was usually caused by head trauma in clinics, and is often manifested as brain tissues damage, cerebrovascular injury, which further cause interruption of neuron structure and function, local tissue ischemia, imbalance of oxygen and glucose metabolism, and eventually neuron death [16, 17]. Sub-hypothermia treatment is one physical approach to decrease glucose and oxygen metabolic levels in brain tissues, alleviate brain edema, lower the accumulation of glutamate excitotoxicity, thus having significant treatment efficacy on ASBI patients [18]. Continuous sub-hypothermia treatment, however, may cause brain injury by itself or complications such as hypotension, electrolytes imbalance, heart arrhythmia, blood clotting disorder and immune suppression [19]. The development of effective drug candidates to protect neurons in a synergistic function of sub-hypothermia was thus of critical importance for improving prognosis of patients. Catalpol is extracted from traditional Chinese medicine and has multiple pharmaceutical activities. It plays an important role in anti-inflammation, mediating oxidation-reduction balance, and neuron protection in diseases such as Alzheimer's and Parkinson's disease [20]. Whether catalpol provides protective role against ASBI in conjunction with sub-hypothermia and related mechanisms, however, remains unclear yet.

This study demonstrated that the combined therapy of catalpol and sub-hypothermia could

shorten the treatment time of ASBI patients, thus may alleviate the adverse effects of sub-hypothermia therapy to certain extents. Hyperglycemia and hyperlactatemia are major symptoms during acute phase of ASBI, and affect patients' prognosis [21]. The combined therapy between catalpol and sub-hypothermia and pure sub-hypothermia both can decrease hyperglycemia and hyperlactatemia reactions, thus inhibiting acute response of ASBI. Combined therapy had higher treatment efficacy and prognosis compared with sub-hypothermia only.

Further study regarding the mechanism of combined therapy including catalpol and sub-hypothermia revealed the participation of apoptotic protein Bax and anti-apoptotic proten Bcl-2. The increase of Bax and decrease of Bcl-2 expression initiate cell apoptotic signal transduction for facilitating cell apoptosis. Cell death was inhibited vice versa. When cells start programmed death, caspase 3 is one factor inducing apoptotic activity [22, 23]. Neuronal injury can release large amounts of inflammatory factors, which further interrupt the body balance between anti-inflammatory and pro-inflammatory, thus aggravating neuronal damage in a positive feedback manner [24]. Therefore this study established rat ASBI model, on which the effect of catalpol combined with sub-hypothermia therapy on apoptosis-anti-apoptosis homeostasis. Results showed decreased caspase 3 activity, elevated Bcl-2 expression, decreased Bax expression and secretions of inflammatory factors TNF-α and IL-1β after the treatment of both sub-hypothermia and combined therapy with the latter having higher treatment efficacy.

In summary, the combined therapy of catalpol and sub-hypothermia could alleviate ASBI via mediating the balance between apoptosis and anti-apoptosis, and decreasing inflammatory factors secretions, with higher efficacy than sub-hypothermia treatment only. This study thus provides new insights for novel treatment approaches for brain injury, along with optimal choices of strategy.

Disclosure of conflict of interest

None.

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References

[1] Kelly ML, Roach MJ, Banerjee A, Steinmetz MP and Claridge JA. Functional and long-term outcomes in severe traumatic brain injury following regionalization of a trauma system. J Trauma Acute Care Surg 2015; 79: 372-377.

[2] Bosarge PL, Shoultz TH, Griffin RL and Kerby JD. Stress-induced hyperglycemia is associated with higher mortality in severe traumatic brain injury. J Trauma Acute Care Surg 2015; 79: 289-294.

[3] Hammond FM, Barrett RS, Shea T, Seel RT, McAlister TW, Kaelin D, Ryser DK, Corrigan JD, Cullen N and Horn SD. Psychotropic medication use during inpatient rehabilitation for traumatic brain injury. Arch Phys Med Rehabil 2015; 96: S256-253, e214.

[4] Alhelali I, Stewart TC, Foster J, Alharfi IM, Ranger A, Daoud H and Fraser DD. Basal skull fractures are associated with mortality in pediatric severe traumatic brain injury. J Trauma Acute Care Surg 2015; 78: 1155-1161.

[5] Godbolt AK, Stenberg M, Jakobsson J, Sorjonen K, Krakau K, Stalnacke BM and Nygren DeBoussard C. Subacute complications during recovery from severe traumatic brain injury: frequency and associations with outcome. BMJ Open 2015; 5: e007208.

[6] Magnoni S, Mac Donald CL, Esparza TJ, Conte V, Sorrell J, Macri M, Bertani G, Biffi R, Costa A, Sammons B, Snyder AZ, Shimony JS, Triulzi F, Stocchetti N and Brody DL. Quantitative assessments of traumatic axonal injury in human brain: concordance of microdialysis and advanced MRI. Brain 2015; 138: 2263-2277.

[7] Feldman KW, Sugar NF and Browd SR. Initial clinical presentation of children with acute and chronic versus acute subdural hemorrhage resulting from abusive head trauma. J Neurosurg Pediatr 2015; 16: 177-185.

[8] Sporns PB, Zimmer S, Hanning U, Zoubi T, Wolfer J, Herbort M, Schwindt W and Niederstadt T. Acute tonsillar cerebellar herniation in a patient with traumatic dural tear and VAC therapy after complex trauma. Spine J 2015; 15: e13-16.

[9] Choi HA, Badjatia N and Mayer SA. Hypothermia for acute brain injury--mechanisms and practical aspects. Nat Rev Neurol 2012; 8: 214-222.

[10] Lee HC, Chuang HC, Cho DY, Cheng KF, Lin PH and Chen CC. Applying cerebral hypothermia and brain oxygen monitoring in treating severe

traumatic brain injury. World Neurosurg 2010; 74: 654-660.
[11] Yan Y, Tang W, Deng Z, Zhong D and Yang G. Cerebral oxygen metabolism and neuroelectrophysiology in a clinical study of severe brain injury and mild hypothermia. J Clin Neurosci 2010; 17: 196-200.
[12] Xue B, Ma B, Zhang Q, Li X, Zhu J, Liu M, Wu X, Wang C and Wu Z. Pharmacokinetics and tissue distribution of aucubin, ajugol and catalpol in rats using a validated simultaneous LC-ESI-MS/MS assay. J Chromatogr B Analyt Technol Biomed Life Sci 2015; 1002: 245-253.
[13] Wang JM, Yang LH, Zhang YY, Niu CL, Cui Y, Feng WS and Wang GF. BDNF and COX-2 participate in anti-depressive mechanisms of catalpol in rats undergoing chronic unpredictable mild stress. Physiol Behav 2015; 151: 360-368.
[14] Xu Z, Zhang L, Li X, Jiang Z, Sun L, Zhao G, Zhou G, Zhang H, Shang J and Wang T. Mitochondrial fusion/fission process involved in the improvement of catalpol on high glucose-induced hepatic mitochondrial dysfunction. Acta Biochim Biophys Sin (Shanghai) 2015; 47: 730-740.
[15] Rubenstein R, Chang B, Davies P, Wagner AK, Robertson CS and Wang KK. A novel, ultrasensitive assay for tau: potential for assessing traumatic brain injury in tissues and biofluids. J Neurotrauma 2015; 32: 342-352.
[16] Dietrich WD and Bramlett HM. Therapeutic hypothermia and targeted temperature management in traumatic brain injury: clinical challenges for successful translation. Brain Res 2016; 1640: 94-103.
[17] Li X, Ji Z, Gu Y, Hu Y, Huang K and Pan S. Mild hypothermia decreases the total clearance of glibenclamide after low dose administration in rats. Neurosci Lett 2016; 614: 55-59.
[18] Murtha LA, McLeod DD, Pepperall D, McCann SK, Beard DJ, Tomkins AJ, Holmes WM, McCabe C, Macrae IM and Spratt NJ. Intracranial pressure elevation after ischemic stroke in rats: cerebral edema is not the only cause, and short-duration mild hypothermia is a highly effective preventive therapy. J Cereb Blood Flow Metab 2015; 35: 2109.
[19] Yu T, Yang Z, Li H, Ding Y, Huang Z and Li Y. Short duration combined mild hypothermia improves resuscitation outcomes in a porcine model of prolonged cardiac arrest. Biomed Res Int 2015; 2015: 279192.
[20] Zhu J, Chen X, Wang H and Yan Q. Catalpol protects mice against renal ischemia/reperfusion injury via suppressing PI3K/Akt-eNOS signaling and inflammation. Int J Clin Exp Med 2015; 8: 2038-2044.
[21] Rabow L, DeSalles AF, Becker DP, Yang M, Kontos HA, Ward JD, Moulton RJ, Clifton G, Gruemer HD, Muizelaar JP, et al. CSF brain creatine kinase levels and lactic acidosis in severe head injury. J Neurosurg 1986; 65: 625-629.
[22] Borghetti G, Yamaguchi AA, Aikawa J, Yamazaki RK, de Brito GA and Fernandes LC. Fish oil administration mediates apoptosis of Walker 256 tumor cells by modulation of p53, Bcl-2, caspase-7 and caspase-3 protein expression. Lipids Health Dis 2015; 14: 94.
[23] Liao H, Bao X, Zhu J, Qu J, Sun Y, Ma X, Wang E, Guo X, Kang Q and Zhen Y. O-Alkylated derivatives of quercetin induce apoptosis of MCF-7 cells via a caspase-independent mitochondrial pathway. Chem Biol Interact 2015; 242: 91-98.
[24] Zhang W, Xing B, Yang L, Shi J and Zhou X. Icaritin attenuates myocardial ischemia and reperfusion injury via anti-inflammatory and anti-oxidative stress effects in rats. Am J Chin Med 2015; 43: 1083-1097.