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Original Article

Correlation of α-KGDHC activity with mild hypothermia treatment of acute brain injuries

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Abstract: Objective: The aim of this study was to explore the value of α-ketoglutarate dehydrogenase complex (α-KGDHC) activity in hypothermia treatment of acute brain injuries. Method: Ninety 8-week-old SD rats were randomly divided into three groups (n=30 each). The control group was normally raised. The other two groups underwent acute brain injuries. One group of rats underwent hypothermia treatment at 30-35°C (treatment group), while the other group underwent conventional therapy (model group). Ten rats in each group were sacrificed before injury (T1), 5 days after injury (T2), and 10 days after injury (T3). Middle cerebral artery peak systolic velocity (Vs), pulse wave velocity (Wv) and brain injury markers were measured, including myelin basic protein (MBP), brain-derived neurotrophic factor (BDNF), S-100 protein (S-100B), and serum α-KGDHC expression levels. Pearson's correlation analysis of α-KGDHC, BDNF, and S-100B was performed. Result: At T1, T2, and T3, MBP and S-100B levels in the control group were significantly lower than those in the treatment and model groups (P<0.050). MBP and S-100B in the treatment group were significantly lower than those in the model group (P<0.050). As time went on, MBP and S-100B decreased gradually (P<0.050). At T1, T2, and T3, expression levels of α-KGDHC and α-KGDHC activity in the control group were significantly higher than those in the treatment group and model group (P<0.050). Pearson's correlation analysis showed that BDNF was positively correlated with α-KGDHC activities at T1, T2, and T3 (r=0.863, 0.791, 0.682, P<0.050). There was a negative correlation between S100β and α-KGDHC activity at T1, T2, and T3 (r=-0.842, -0.941, 06-63, P<0.050). Conclusion: Mild hypothermia treatment of acute brain injuries in rats can effectively reduce brain damage and improve nerve repair ability. Monitoring the activity of α-KGDHC in patients can effectively assess the repair of brain damage in patients.

Keywords: Hypothermia, acute brain injury, α-KGDHC, MBP, S-100B

Introduction

There were approximately 1.8 million new brain injury patients, worldwide, in 2016, making this disease the second most common among all parts of the body [1]. The number of patients with brain injuries has increased by approximately 12-fold in the past 10 years. The incidence rate has increased over the same time period [2]. Currently, the pathogenesis of brain injuries is unclear. More than 60% of patients with brain injuries may have had a sudden inflammatory reaction, causing a secondary brain injury, known as acute brain injury [3]. Because of the mechanisms of damage in acute brain injuries, further damage is inflicted upon the central nervous system of the patient. Thus, the prognosis becomes even less optimistic [4]. Notably, the prognosis of patients with acute brain injuries is generally poor. The 5-year survival rate of prognosis is between 30% and 40% [5]. Regarding treatment, it is necessary to repair brain damage, protect brain tissue from stress caused by injury, and avoid the onset of inflammation [6]. Advancements in research have proven that hypothermia treatment has a very high treatment value in cases of brain injuries [7-9]. Mild hypothermia treatment can effectively reduce cerebral oxygen metabolism and oxygen free radical production in the treatment of neurological dysfunction. Furthermore, it can facilitate control of neuronal necrosis and apoptosis [10].

Mild hypothermia treatment has become a popular method for treatment of craniocerebral injury diseases in clinical practice. It has been applied in studies of acute brain injury treat-

ment. It usually takes a long recovery period and multi-testing to determine the patient's recovery. Thus, if there is an effective index that can be used as an indicator of brain injury, this will greatly reduce the recovery cycle. Patients can be better monitored dynamically during treatment. Moreover, α-ketoglutarate dehydrogenase complex (α-KGDHC) is one of the key enzymes in tricarboxylic acid cycle and is also a primary site for the production of reactive oxygen species [11]. Studies have shown that the activity of α-KGDHC is closely related to neuronal damage and oxidative stress response, directly affecting the prognosis of patients with neurodegenerative diseases [12]. Notably, in the acute brain injuries, by restoring the activity of α-KGDHC, it can effectively inhibit the apoptosis of nerve cells and reduce the degeneration of nerve cells after injury [13].

Therefore, this study was undertaken to apply sub-hypothermia for intervention in a rat model of acute brain injury. The aim of this study was to analyze the correlation of α-KGDHC activity with rat brain tissue injury markers during treatment, providing reference and guidance for future clinical application of hypothermia in the treatment of acute brain injuries.

Materials and methods

Animal data

Ninety clean-grade 8-week-old SD rats, weighing 140-250 g, were purchased from Shanghai KaiXue Science and Technology Co., Ltd. They were raised at a temperature of 24.00±2.00°C and humidity of 50.00±5.00%, under natural light.

Methods

Ninety rats were randomly divided into three groups (n=30 each). The control group was normally raised. The other two groups underwent acute brain injuries, in accordance with the methods of Xu et al. [14] (Feeney's free fall hitter). Rats were fed individually 3 days before surgery and fasted 8 hours before surgery. They were intraperitoneally injected with 10% chloral hydrate, at a dose of 350 mg/kg, as an anesthetic. After anesthesia, the rats were placed prone on a brain stereotactic apparatus. After iodophor disinfection, the rat periosteum was dissected. A bone window (5.0 mm in diameter) was drilled using a dental drill at 1.5 mm posterior to the right parietal crown of the rat and 2.5 mm beside the midline. A ram was placed on the dura mater. Next, a 20-g weight was applied by free fall along the peripheral catheter, beginning 30 cm from the rat brain. After impacting the dura mater, the right parietal lobe was contused with an impact force of 600 g/cm. Four to five drops of 4×10^4 U gentamicin-sulfate were instilled at the impact site. The bone window was closed with bone wax to suture the rat scalp. All model rats were treated with conventional treatment for intracranial hypertension, blood circulation, dehydration, and other brain injuries for a total of 10 days.

A group of randomly selected rats was selected as the mild hypothermia treatment group. The rats were placed on the cooling blanket during each treatment. Brain temperatures and anus temperatures were adjusted to 33-34°C within 30 minutes using heating lamps and cold air. They were maintained at a 33-34°C body temperature for 4 hours. After completion of treatment (once per day), the rats were rewarmed to a normal body temperature within 1.5 hours and returned to the feeding room. The other group was used as a model group and was not treated with hypothermia. Those rats were returned to the breeding room after modeling was complete. Ten rats in each group were selected before injury (T1), 5 days after injury (T2), and 10 days after injury (T3). Continuous monitoring of tissue blood flow was performed using laser Doppler techniques. The scanning blood flow imaging system uses a highly sensitive CCD camera to capture the dynamic scattered light signal at high speed, then generates a blood flow image through computer analysis. After blood flow parameter testing was completed, the rats were sacrificed by cervical dislocation and the blood of the brain artery was obtained. α-KGDHC expression levels were measured using an automatic biochemical analyzer and an α-KGDHC ELISA kit (Shanghai Caiyou Industrial Co., Ltd., YS04518B). Intact brain tissues were obtained from each rat. The cortex surrounding the wound, the hippocampus, and the cerebral region were separated and placed on ice. Tissues were stored in liquid nitrogen at -190°C. Activity of α-KGDHC was detected by using a 96-well multi-plate (purchased from China Thermo Fisher Scientific Co., Ltd.). Brain tissue was prepared as 2% tissue homogenate in lysis buffer. The supernatant was collected after cryopreservation and

Table 1. Comparison of Vs among rat groups (cm/s)

	Therapy group	Model group	Control group	F	P
T1	79.86±7.54	78.45±8.04	127.83±12.42$^{\Delta,\nabla}$	85.972	<0.001
T2	93.24±8.07*	84.37±7.66$^{*,\Delta}$	128.17±12.84$^{\Delta,\nabla}$	55.731	<0.001
T3	107.49±7.68$^{*,\#}$	93.36±8.34$^{*,\#,\Delta}$	127.98±12.59$^{\Delta,\nabla}$	29.431	<0.001

Note: *represents compared with the same group at T1, P<0.050; $^{\#}$represents compared with the same group at T3, P<0.050; $^{\Delta}$represents compared with the treatment group at the same time, P<0.050; $^{\nabla}$represents compared with the model group at the same time, P<0.050.

Table 2. Comparison of Wv among rat groups (m/s)

	Therapy group	Model group	Control group	F	P
T1	11.84±1.84	12.04±1.96	27.96±2.83$^{\Delta,\nabla}$	168.511	<0.001
T2	16.73±1.59*	14.87±1.31$^{*,\Delta}$	27.17±3.07$^{\Delta,\nabla}$	96.472	<0.001
T3	21.52±2.04$^{*,\#}$	16.94±1.84$^{*,\#,\Delta}$	27.63±2.62$^{\Delta,\nabla}$	55.068	<0.001

Note: *represents compared with the same group at T1, P<0.050; #represents compared with the same group at T3, P<0.050; $^{\Delta}$represents compared with the treatment group at the same time, P<0.050; $^{\nabla}$represents compared with the model group at the same time, P<0.050.

stored at -80°C. α-KGDHC activity was determined based on reduced NADH production. After reacting the reactant mixture for 5 minutes, α-KGDHC pure enzyme and 10 μL of 0.1 mmol/L α-ketoglutaric acid were added to the reaction system (0.8 mL, pH=8.0). The system reacted for 10 minutes to prepare a reaction standard curve. The reaction mixture was quickly added to protein samples (60 μg per sample). Optical density values of emitted fluorescence at 460 nm, after excitation at a wavelength of 340 nm, were recorded and the activity of α-KGDHC was calculated.

Outcome measures

Observations were made for several parameters. Cerebral blood flow parameters included peak flow velocity (Vs) of the middle cerebral artery and pulse wave velocity (Wv). Brain injury markers included myelin basic protein (MBP), brain-derived neurotrophic factor (BDNF), S-100 protein (S-100B), α-KGDHC expression levels in serum, α-KGDHC activity in brain tissue, and time dependence of α-KGDHC activity with hypothermia treatment.

Statistical methods

Data were analyzed and processed using SPSS 24.0 statistical software (Beijing Sitron Weida Information Technology Co., Ltd.). Results are expressed as mean ± standard deviation. Comparisons among multiple groups were performed using ANOVA. Post hoc pairwise comparisons were performed by LSD t-test. Correlations were analyzed using Pearson's analysis. P<0.050 indicates statistical significance.

Results

Brain injury modeling results

Two of the 60 model rats died. Thus, the modeling success rate was 96.67%. There were 30 control rats, 29 model rats, and 29 treatment rats. At T1 and T2, 10 rats were sacrificed in each group. At T3, 10 rats in the control group were sacrificed, while 9 rats were sacrificed in the model and treatment groups.

Comparison of cerebral blood flow parameters

Vs and Wv of the three groups were statistically different (P<0.001). At T1, T2, and T3, Vs and Wv of control group were significantly higher than the treatment group and model group (P<0.050). There were no significant differences in Vs and Wv between the control group at different points (P>0.050, **Tables 1** and **2**).

Comparison of brain injury markers

Differences in MBP, BDNF, and S-100B in the three groups of rats were statistically significant (P<0.001). At T1, T2, and T3, MBP and S-100B of control group were significantly lower than that of treatment group and model group. BDNF of the control group was significantly higher than that of the treatment group and model group (P<0.050). There were no significant differences in MBP, S-100B, and BDNF between the control group at different time points (P>0.050) (**Tables 3**-**5**).

Serum α-KGDHC expression levels

Serum α-KGDHC levels at T1, T2, and T3 in the treatment group were 307.25±49.57 ng/g, 411.34±82.36 ng/g, and 486.50±67.34 ng/g, respectively. In the model group, serum α-

Table 3. Comparison of MBP levels among rat groups (mmol/L)

	Therapy group	Model group	Control group	F	P
T1	21.98±2.31	21.42±2.64	2.14±0.87$^{\Delta,\nabla}$	293.243	<0.001
T2	14.81±1.53*	16.94±1.94$^{*,\Delta}$	2.21±0.94$^{\Delta,\nabla}$	272.132	<0.001
T3	8.67±0.86$^{*,\#}$	13.83±1.14$^{*,\#,\Delta}$	2.19±0.88$^{\Delta,\nabla}$	347.618	<0.001

Note: *represents compared with the same group at T1, P<0.050; $^{\#}$represents compared with the same group at T3, P<0.050; $^{\Delta}$represents compared with the treatment group at the same time, P<0.050; $^{\nabla}$represents compared with the model group at the same time, P<0.050.

Table 4. Comparison of BDNF levels among rat groups (pg/mL)

	Therapy group	Model group	Control group	F	P
T1	12.88±1.74	13.04±1.86	43.62±3.07$^{\Delta,\nabla}$	590.834	<0.001
T2	20.53±2.07*	16.54±2.41$^{*,\Delta}$	44.12±3.42$^{\Delta,\nabla}$	305.913	<0.001
T3	29.34±3.62$^{*,\#}$	21.54±2.77$^{*,\#,\Delta}$	43.76±3.19$^{\Delta,\nabla}$	118.254	<0.001

Note: *represents compared with the same group at T1, P<0.050; $^{\#}$represents compared with the same group at T3, P<0.050; $^{\Delta}$represents compared with the treatment group at the same time, P<0.050; $^{\nabla}$represents compared with the model group at the same time, P<0.050.

Table 5. Comparison of S-100B levels among rat groups (pg/mL)

	Therapy group	Model group	Control group	F	P
T1	11.62±1.24	11.87±1.42	0.17±0.02$^{\Delta,\nabla}$	377.124	<0.001
T2	5.67±0.73*	8.61±1.04$^{*,\Delta}$	0.16±0.03$^{\Delta,\nabla}$	341.683	<0.001
T3	2.81±0.46$^{*,\#}$	6.14±0.93$^{*,\#,\Delta}$	0.16±0.02$^{\Delta,\nabla}$	246.152	<0.001

Note: *represents compared with the same group at T1, P<0.050; $^{\#}$represents compared with the same group at T3, P<0.050; $^{\Delta}$represents compared with the treatment group at the same time, P<0.050; $^{\nabla}$represents compared with the model group at the same time, P<0.050.

KGDHC levels at T2, T2, and T3 were 309.54±52.33 ng/g, 366.24±60.58 ng/g, and 408.69±68.30 ng/g, respectively. In the control group, serum α-KGDHC levels were 614.33±86.34 ng/g, 616.27±90.24 ng/g, and 615.68±87.39 ng/g, respectively. At T1, T2, and T3, α-KGDHC levels in the control group were significantly higher than those in the treatment and model groups (P<0.050) (**Figure 1**).

Comparison of α-KGDHC activity

Activities of α-KGDHC at T1, T2, and T3 in the treatment group were 3.69±0.67, 9.54±2.08, and 14.59±1.37, respectively. α-KGDHC activities at T1, T2, and T3 in the model group were 3.58±0.81, 6.41±1.08, and 10.35±0.76, respectively. α-KGDHC activities at T1, T2, and T3 in the control group were 24.82±2.24. At T1, T2, and T3, α-KGDHC activity of the control group was significantly higher than that of the treatment and model groups (P<0.050) (**Figure 2**).

Pearson's correlation analysis

Pearson's correlation analysis showed that BDNF was positively correlated with α-KGDHC activities at T1, T2, and T3 (r= 0.863, 0.791, 0.682, P<0.050). There was a negative correlation between S100β and α-KGDHC activity at T1, T2, and T3 (r=-0.842, -0.941, 06-63, P<0.050) (**Table 6**).

Discussion

Craniocerebral injury is one of the most common injuries in neurosurgery, but its pathogenesis remains unclear. Some studies have suggested that it may be mediated by collective oxygen free radical damage and abnormal energy metabolism [15]. Currently, clinical treatment of acute brain injuries mainly consists of early intervention and comprehensive treatment. The effects of mild hypothermia on brain protection are extremely prominent. By reducing brain metabolism, reducing damage to brain tissue from excitatory amino acids and oxygen free radicals, and regulating apoptotic factors, nerve damage can be treated [16]. Studies have shown that the application of mild hypothermia treatment on cerebrovascular disease can greatly improve patient nerve function and reduce harm caused by oxidative stress [17]. For treatment of acute brain injuries, a similar treatment mechanism is applicable. α-KGDHC is a rate-limiting enzyme in the mitochondrial matrix of the tricarboxylic acid cycle. Its activity plays an important role in mitochondrial energy metabolism and generation of reactive oxygen species [18]. Mitochondria are the main sites for oxidation and energy conversion in human tissues, but tricarboxylic acids in the mitochondrial matrix are extremely fragile and susceptible to oxidative damage by free radicals. When human brain tissue is damaged, it will directly cause the destruction of tricarboxylic acids in the mitochondria, generating a large amount of reactive oxygen species and causing apoptosis of

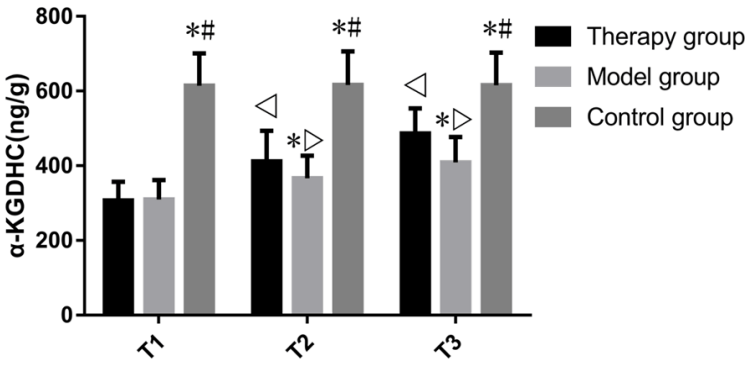

Figure 1. Serum α-KGDHC expression levels in the three groups of rats. *represents the expression level of α-KGDHC in the same treatment group, P<0.050; #represents the expression level of α-KGDHC in the same period model group, P<0.050; Δrepresents the expression level of α-KGDHC in the same group T1, P<0.050; ▽represents the expression level of α-KGDHC compared with the same group T2, P<0.050.

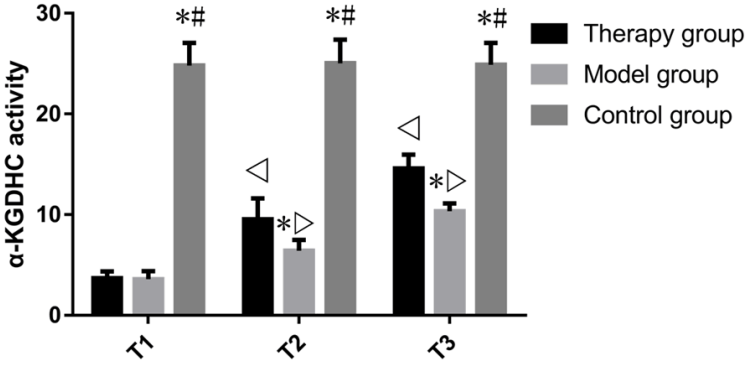

Figure 2. α-KGDHC activity in the three groups of rats. *represents a comparison of α-KGDHC activity in the same treatment group, P<0.050; #represents a comparison with the α-KGDHC activity of the simultaneous model group, P<0.050; Δrepresents the α-KGDHC activity compared with the same group T1, P<0.050; ▽represents a comparison with α-KGDHC activity in the same group T2, P<0.050.

Table 6. Pearson's correlation analysis

	Time	r	P
BDNF	T1	0.863	0.001
	T2	0.791	0.006
	T3	0.682	0.030
S100β	T1	-0.842	0.002
	T2	-0.941	<0.001
	T3	-0.663	0.037

cells. If not sufficiently controlled, the generation of reactive oxygen species can exceed the limited concentration that can be tolerated in brain tissue, thereby causing high levels of oxidative stress in the tissue. These factors may lead to more serious diseases, such as brain necrosis [19]. Studies have shown that, when studying the effects of α-KGDHC activity in patients with Alzheimer's disease, changes in α-KGDHC activity were found to be closely related to the severity of patient conditions [20]. Thus, α-KGDHC activity may provide an extremely sensitive marker for future reaction to brain injuries. However, there are few references concerning the relationship between α-KGDHC activity and brain damage. In this experiment, a rat model of acute brain injury was established and treated with mild hypothermia. Differences in brain injury indexes and α-KGDHC activity were measured among the three groups, aiming to explore the value of hypothermia therapy in acute brain injuries and the significance of α-KGDHC activity in brain injuries.

Results of this experiment showed that cerebral blood flow parameters and brain injury markers in injured rats treated with mild hypothermia were not equivalent to those of normal rats. They were, however, effectively improved with respect to indicators in the model group. Present results suggest that hypothermia has a high treatment value in cases of acute brain injuries. Vs and Wv are indicators that reflect cerebral blood flow velocity. Increased expression levels have been associated with faster cerebral blood flow velocity and stronger blood supply to the brain [21]. Hypothermia may cause dilation of cerebral blood vessels by increasing the local nitric oxide content in the brain tissues of rats. Cerebral blood vessels are dilated and cerebral blood flow increases accordingly. Neuronal damage, apoptosis, and necrosis, caused by insufficient blood supply to cerebral blood vessels, are effectively improved. This is an important basis for the treatment of acute brain injuries. Damage to brain tissue and nerve function are the most important manifes-

tations of acute brain injuries. Notably, the severity of a patient's condition is often reflected in brain damage markers [22]. Of these markers, MBP is primarily present in oligodendrocytes and is primarily a component of central nervous system myelin [23]. BDNF is a major source of nutrients for neuronal mitosis and neuro-restoration. Its expression level determines whether patients have secondary brain damage [24]. S-100B is a classical brain injury marker, widely found in nerve tissue. When brain tissue is damaged, it often releases a large volume of cerebrospinal fluid into the blood, which allows S-100B to serve as a sensitive and active brain damage marker [25]. Rats in the treatment group showed significantly improved levels of many markers, compared with the model group, indicating that hypothermia treatment can effectively reduce brain damage and improve nerve repair ability. This method is worthy of widespread application in clinical settings. Serum α-KGDHC content, in treated rats, was significantly higher than in the model group, suggesting that the mitochondrial oxidative phosphorylation ability of rats in the treatment group was significantly improved. Thus, brain damage appeared to be effectively improved. The significant increase in α-KGDHC activity in the treatment group was presumably related to the intervention of hypothermia therapy. The hypothermia treatment process can effectively reduce metabolic byproducts of anaerobic glycolysis and tricarboxylic acid cycle [26]. Thus, nicotinamide adenine dinucleotide (NADH) can be greatly reduced, resulting in a significant decrease in the ratio of NADH/NAD+ and an increase in the activity of α-KGDHC. In the sub-low temperature environment, the concentration of superoxide dismutase in brain tissue can also be improved. By increasing the concentration of superoxide dismutase, the concentration of hydrogen peroxide in brain tissue can be reduced. Therefore, the ability of hydrogen peroxide to inhibit α-KGDHC activity is greatly reduced, enabling increased α-KGDHC activity. This mechanism of action requires further experimental research. α-KGDHC is a rate-limiting enzyme involved in the tricarboxylic acid cycle in the mitochondrial matrix. It plays an important role in maintaining the balance of redox reactions in brain tissue [27]. It is speculated that when patients suffer acute brain injuries, a large amount of active oxygen is produced in the brain. At this time, a severe oxidative stress reaction will occur in the brain tissue, greatly affecting the activity of α-KGDHC. This causes a large number of mitochondria to release cytochrome C in the brain tissue, resulting in a series of nerve damage and neuronal necrosis. Similarly, Pearson's correlation analysis showed that BDNF was positively correlated with α-KGDHC activity, while S100β was negatively correlated with α-KGDHC activity at T1, T2, and T3. Results suggest that higher α-KGDHC activity leads to higher levels of the neuroprotective factor BDNF and lower levels of S100β, further indicating that α-KGDHC activity is closely related to brain damage in patients. This experiment was performed by establishing a rat model of acute brain injury and applying mild hypothermia. Differences in brain injury indexes and levels of α-KGDHC activity were analyzed among the three groups of rats. There were limitations, however, due to experimental conditions. Mechanisms of the effects of mild hypothermia on the activity of α-KGDHC requires further research. There are differences between the animal model and actual human disease.

In summary, mild hypothermia treatment of acute brain injuries in rats can effectively reduce brain damage and improve nerve repair ability. Monitoring the activity of α-KGDHC, it is possible to effectively judge the repair of brain damage in patients.

Disclosure of conflict of interest

None.

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