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Study on the effects of quercetin on brain cell apoptosis and HMGB-1 and TLR-4 level in rats with encephalitis

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Abstract. – OBJECTIVE: To explore the therapeutic effects of quercetin on rats with encephalitis, especially on cell apoptosis, and the levels of HMGB-1 and TLR-4.

MATERIALS AND METHODS: 32 healthy rats were equally assigned into ZC group (healthy group), NY group (encephalitis group), DJ group (60 ml quercetin group), and GJ group (240 ml quercetin group) followed by analysis of cell apoptosis, brain tissue water content, neurons, HMGB-1, TLR-4 and other inflammatory factors.

RESULTS: The ZC group showed normal neuron volume and equitable staining; compared with ZC group, NY group showed neuron volume shrinkage and chromatin condensation; the neuron and color in DJ group were slightly better than NY group; the neuron volume in GJ group increased significantly and chromatin is distributed evenly. TLR-4, IL-4, IL-6, HMGB-1 in ZC and DJ group were significantly lower than those in NY group ($p<0.05$); IL-4, IL-6, HMGB-1 in GJ group significantly decreased compared with DJ group ($p<0.05$); MMP-9 enzyme activity in ZC and DJ group was significantly lower than NY group ($p<0.05$) with lower level in GJ group than DJ group ($p<0.05$). The water content was higher in brain tissue of NY group than ZC group ($p<0.05$) and lower in DJ group than NY group ($p<0.05$) with lower level in GJ group than DJ and NY group (all $p<0.05$). The hippocampal neurons and cortical neurons in ZC and DJ group were higher than those in NY group ($p<0.05$) and elevated in GJ group compared with DJ group and NY group ($p<0.05$).

CONCLUSIONS: Quercetin is effective in treating encephalitis rats possibly through inhibition of neuronal cell apoptosis and level of HMGB-1 and TLR-4.

Key Words:
Quercetin, Encephalitis rats, Apoptosis, HMGB-1, TLR-4.

Introduction

Encephalitis can be classified into viral encephalitis and bacterial encephalitis according to its causes. Bacterial encephalitis is caused by bacterial infection and viral encephalitis is the virus infection of the nervous system. Viral infections can be caused by ordinary virus infection and by spore cells. Viral encephalitis is relevant to over 100 kinds of diseases statistically[1]. Except disability, patients also suffer serious sequelae. The treatment has captured widespread attention in recent years[2]. Viral encephalitis is prone to occur in children because brain has not fully developed, and the viral central nervous system leads to viral encephalitis[3]. For the severity of viral encephalitis, the immediate detection and treatment is critical to reduce its sequelae probability[4]. The common drug for encephalitis is glucocorticoid but having serious side effects, such as reducing the patient's immunity and worsening the disease. Quercetin contains flavonol with anti-inflammatory and antibacterial effects which increases the patient's immunity and depresses cell apoptosis[5,6]. HMGB-1 can activate inflammatory cells and is the only protein that can secrete factors from the cell. We found that HMGB-1 expression is closely related to the pathogenesis of the central nervous system and is highly expressed in brain injury. Abnormal HMGB-1 level will chemoattract neutrophils, causing outside substances to invade epithelial cells, leading to brain damage. Zhang et al[7] found that the decreased HMGB-1 expression in patients can be used for the diagnosis of bacterial meningitis. TLR-4 is composed of 879 amino acids across the cell membrane and can recognize bacterial lipoproteins, promote the inflammatory factors release and increase immune function. TLR can release immune cytokines by recognizing pathogen-related molecular patterns to enhance immune response. When human body is infected by pathogens, TLR-4 increases[8,9]. Whether it has a certain

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effect on inflammation has not yet been fully investigated. Therefore, our study assesses the effect of quercetin on the inflammatory factors (TLR-4 and HMGB-1) in rats with encephalitis and the brain cell apoptosis.

Materials and Methods

Laboratory Animals and Grouping

Rats aged 4-8 weeks old, weighted 206-225 g with an average weight (213.25 ±12.37) g were purchased from Yiming Fuxing (Beijing, China) Company. Rats were bred for 7 days 23.3°C-27.2°C, humidity 51%-62%, for 0.5 days. 32 healthy ACL female rats were equally assigned into ZC group (control group), NY group (encephalitis modeling group), DJ group (60mk/kg·d quercetin group), and GJ group (240 mk) / kg·d Quercetin group).

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of First Hospital of Lanzhou University.

Experimental Reagents

Strain: Neisseria meningitidis (Shanghai Seag Biotech Company, Shanghai, China); drug: Quercetin (Jinan Yuncheng Biotechnology Company, Jinan, China); reagents: HMGB-1 Kit (Shanghai Yingxin Laboratory Equipment Company, Shanghai, China); chloral hydrate (Guangzhou Su Ding Pharmaceutical Company, Guangzhou, China); toll-like receptor 4 (TLR-4) PCR primers (Chengdu Chaojiu Ba Biological Company, Chengdu, China). MRI (Shenzhen Mei Rui Company, Shenzhen, China).

Model Establishment of Encephalitis Rats

Rats were anesthetized with 0.22 ml/kg chloral hydrate and punctured brain with a 24-gauge butterfly needle. The NY group was injected with Neisseria meningitidis suspension, and the ZC group was injected with normal saline at 23.5°C. After 10-day inoculation, comatose happened and rat activity was reduced, losing walking ability within 5 seconds then took out 51 μl cerebrospinal fluid for culture. After modeling, the DJ group and GJ group were gavaged with 0.6% [60 mg / (kg·d)] and 2.4% [240 mg / (kg·d)] quercetin solution (20 ml / 200 g) respectively every 24 hours and 7 times. The rat brain was extracted, fixed with paraformaldehyde, dehydrated, embedded, sectioned, and stained with HE.

Detection of Cell Morphology

The cover slide was soaked in ethanol for 5 min and washed with PBS, and then, washed with cell culture solution followed by being stained with HE and observation under the microscope.

Detection of TLR-4 and HMGB-1

An appropriate amount of brain tissue from the encephalitis site was isolated followed by addition of phosphate buffer solution and grinding ultrasonically. After centrifugation, the supernatant was collected to measure the level of TLR-4, IL-4, IL-6, and HMBG1.

Detection of MMP-9 Enzyme Activity

SDS-PAGE gelatin zymography was used to detect the MMP enzyme activity in cerebrospinal fluid.

Detection of Cerebral Edema

72 hours later, the rats were anesthetized with 3.4% chloral hydrate followed by isolation of the brain which was weighed and recorded the wet weight. When the brain was dried, it was weighed and recorded as the dry weight.

Measurement of Cerebral Cortex and Hippocampal Dentate Gyrus Neurons in Rat

Rat brain tissue was fixed in formaldehyde solution, dehydrated, soaked in wax, embedded, sectioned, stained with HE followed by observation under a microscope.

Statistical Analysis

SPSS 17.0 software (SPSS inc., Chicago, IL, USA) analyzed data which were displayed as mean ± SD and were assessed by ANOVA. $p<0.05$ indicates a statistical significance difference.

Results

Morphology of Nerve Cells in the Brain Tissues

ZC group showed normal neuron size and equitable staining; NY group showed smaller neuron volume and chromatin agglutination; compared with NY group, neuron volume in DJ group increased and chromatin was distributed evenly. Compared with the DJ group, the neuron volume in GJ group was significantly increased and chromatin distribution was more equitable. We can see evident effect in DJ group and GJ group with most evident effect in GJ group (Figure 1).

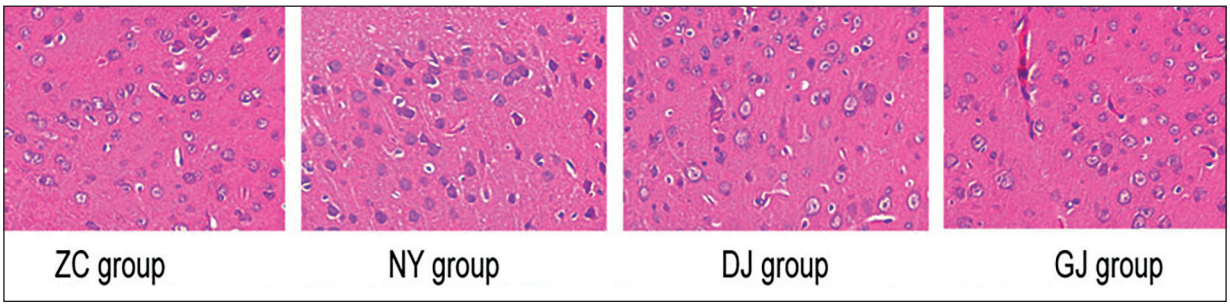

Figure 1. The morphology of nerve cells of brain tissues in four groups (magnification×200).

Table I. Comparison of indicators of inflammatory factors and HMGB-1 in the brain tissue of four groups.

Group	Cases	TLR-4	IL-4	IL-6	HMGB-1
ZC	8	112.15 ± 16.80	109.88 ± 8.78	112.72 ± 13.59	127.83 ± 16.49
NY	8	769.92 ± 6.48*	341.62 ± 27.71*	885.06 ± 18.79*	827.67 ± 28.9*
DJ	8	545.43 ± 46.35*#	277.65 ± 22.18*#	643.12± 24.70*#	599.40 ± 55.40*#
GJ	8	181.04 ± 29.52*#@	134.95 ± 7.21*#@	126.16 ± 17.30*#@	225.10 ± 22.64*#@
f		410.7	287.3	3279	726.2
p		< 0.001	< 0.001	< 0.001	< 0.001

Note: *: $p<0.05$ compared with ZC group; #: $p < 0.05$ compared with NY group; @: $p < 0.05$ compared with DJ group.

Comparison of Indicators of Inflammatory Factors and HMGB-1 in the Brain Tissue

TLR-4 (1.12±0.23), IL-4 (1.10±0.22), IL-6 (1.11±0.35), HMGB-1 (1.28±0.43) in ZC group was significantly lower than NY group (TLR-4: 7.70±0.96, IL-4: 3.39±0.43, IL-6: 8.85±0.94, and HMGB-1: 8.27±1.09) ($p<0.05$); DJ group had lower TLR-4 (5.44±0.67), IL-4 (2.77±0.62)), IL-6 (6.43±0.73), HMGB-1 (5.98±0.82) level than NY group ($p<0.05$); compared with DJ group, GJ group had reduced TLR-4 (1.84± 0.86), IL-4 (1.34±0.5), IL-6 (1.26±0.69), HMGB-1 (2.24±0.69) level ($p<0.05$), indicating that quercetin can inhibit inflammation (Table I, Figure 2).

Comparison of MMP-9 Enzyme Activity in Cerebrospinal Fluid

ZC group had significantly lower MMP-9 enzyme activity (38.23±21.02) than NY group ($p<0.05$). Meanwhile, DJ group also had reduced MMP-9 enzyme activity (601.25±59.26) compared with NY group (1024.32±123.26) ($p<0.05$); the MMP-9 enzyme activity (425.39±60.46) of the GJ group was lower than DJ group with significant differences ($p<0.05$), indicating that Cortin inhibits MMP-9 enzyme activity (Table II and Figure 3).

Comparison of Water Content in Brain Tissue

The brain tissue water content of NY group (81.96±1.46) was higher than ZC group (71.03±0.97) and DJ group (77.42±1.28) ($p<0.05$). The brain water content of GJ group (75.12±1.02) was significantly lower than DJ

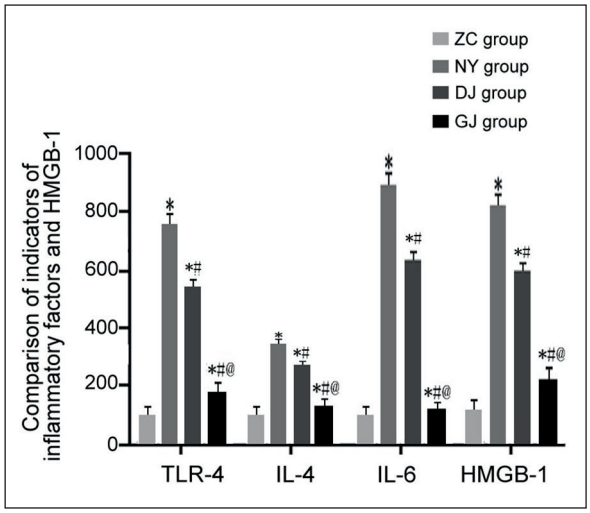

Figure 2. Comparison of indicators of inflammatory factors and HMGB-1 in the brain tissue of four groups. Note: *: $p<0.05$ compared with ZC group; #: $p<0.05$ compared with NY group; @: $p<0.05$ compared with DJ group.

Table II. Comparison of MMP-9 enzyme activity in cerebrospinal fluid of four groups.

Group	Cases	MMP-9 enzyme activity
ZC	8	38.34 ± 7.23
NY	8	234.51 ± 31.54*
DJ	8	126.25 ± 19.71*#
GJ	8	72.29 ± 7.96*#@
f		157.1
p		< 0.001

Note: *: $p < 0.05$ compared with ZC group; #: $p < 0.05$ compared with NY group; @: $p < 0.05$ compared with DJ group.

Table III. Comparison of water content in brain tissue of four groups.

Group	Cases	Brain water content (%)
ZC	8	71.04 ± 0.72
NY	8	81.95 ± 0.83*
DJ	8	77.41 ± 0.80*#
GJ	8	73.11 ± 0.82*#@
f		298.2
p		< 0.001

Note: *: $p < 0.05$ compared with ZC group; #: $p < 0.05$ compared with NY group; @: $p < 0.05$ compared with DJ group.

group and NY group (both $p<0.05$), indicating that quercetin was effective on cerebral edema (Table III, Figure 4).

Comparison of the Number of Hippocampal and Cortical Neurons

Hippocampal neurons and cortical neurons in the ZC group (152.36±16.25 and 184.25±26.12) and DJ group (110.23±12.36 and 142.35±12.23) were higher than NY group (98.25±24.56) and (129.56±22.38) ($p<0.05$). The hippocampal neurons (126.21±18.12) and cortical neurons in the GJ group (159.56±16.42) was increased compared with DJ group and NY group ($p<0.05$); indicating that quercetin can promote the regeneration of the above indicators (Table IV and Figure 5).

Discussion

Bacterial encephalitis is caused by bacterial infection[10]. It is the lower respiratory tract that is often infected. It has been one of the world's top three high-risk diseases with 10% death rate

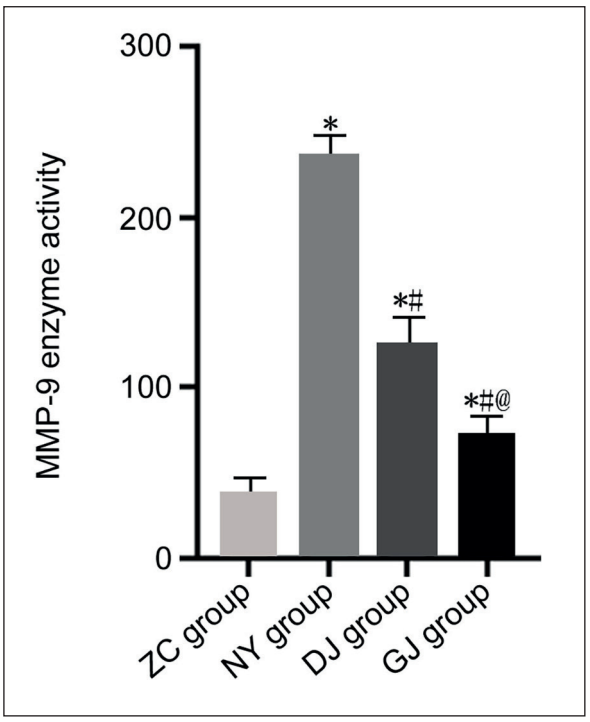

Figure 3. Comparison of MMP-9 enzyme activity in cerebrospinal fluid of four groups. Note: *: $p<0.05$ compared with ZC group; #: $p<0.05$ compared with NY group; @: $p<0.05$ compared with DJ group.

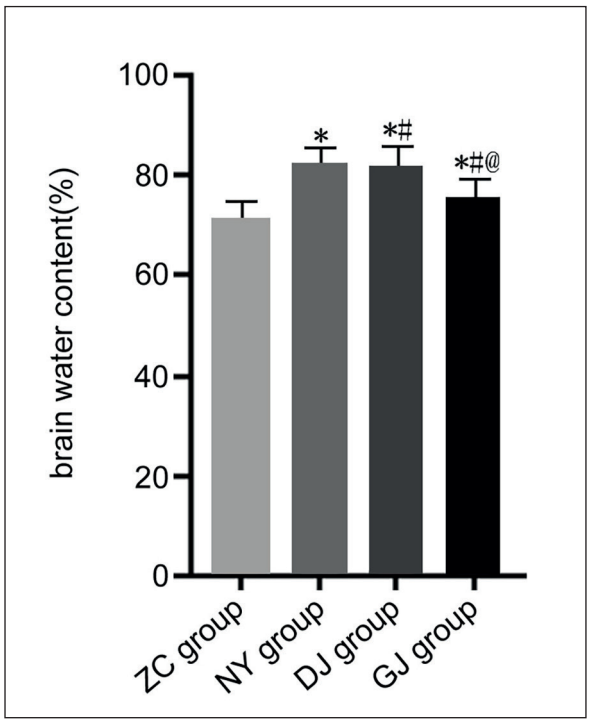

Figure 4. Comparison of water content in brain tissue of four groups. Note: *: $p<0.05$ compared with ZC group; #: $p<0.05$ compared with NY group; @: $p<0.05$ compared with DJ group.

Table IV. Comparison of the number of hippocampal and cortical neurons in the four groups.

Group	Cases	Neurons	
		Hippocampal neurons	Cortical neurons
ZC	8	152.38 ± 15.97	184.00 ± 16.12
NY	8	98.13 ± 10.06*	129.38 ± 20.33*
DJ	8	110.25 ± 13.55*#	142.00 ± 7.65*#
GJ	8	126.38 ± 14.15*#@	159.63 ± 18.35*#@
f		23.77	16.79
p		< 0.001	< 0.001

Note: *: $p < 0.05$ compared with ZC group; #: $p < 0.05$ compared with NY group; @: $p < 0.05$ compared with DJ group.

among various diseases. At present, bacterial brain inflammation is the main cause of death in the low-developing countries[11]. The main component of quercetin is flavonoids, which can protect the nervous system, resist inflammation, and regulate cell damage and apoptosis. Meanwhile, quercetin is also effective in treating epilepsy, inhibiting tumors with anti-inflammatory effects[12].

The mass propagation of encephalitis virus leads to cell apoptosis. The virus can enter the cell through the thinned cell membrane. The cell apoptosis can be induced by specific reasons, including intrinsic signals and the external signal[13]. The study shows that quercetin can inhibit the expression of inflammatory factors TLR-4, IL-4, IL-6, and HMGB-1. The cerebrospinal fluid HMGB-1 level in patients with encephalitis is significantly higher[14-16]. It is believed that the cerebrospinal fluid HMGB-1 level can be used as an indicator to identify encephalitis. Previous studis[17,18] showed that the expression of TLR-4 in colitis is higher than that in normal tissues and inhibition of TLR4, TNF-α, and IL-1β is beneficial for the treatment. In addition, reduced HMGB-1 level was found in the serum of patients with cerebral infarction after treatment[19]. According to relevant studies[20], the regulation of TLR-4 on inflammation has been done through nuclear factor processing NF-kB. Increased inflammation can cause serious brain damage and nerve cell apoptosis. The damage to the brain tissue is consistent with the degree of the inflammation caused by the disease, and TLR-4 level is positively related with the brain tissue damage.

This study found that Quercetin can reduce the activity of MMP-9. The group of low doses showed no differences, but the change of high dose group is extremely evident. Quercetin can reduce the water content of brain tissue in encephalitis rats. The effect of GJ group is more evident than that of DJ group, indicating that quercetin is effective in the treatment of cerebral edema. Cell death has been shown to be promoted through MMP-9[21]. MMP-9 is overexpressed in cerebral ischemia and after inhibition of JNK, the condition of cerebral ischemia was relived[22]. MMP-9 is highly expressed in patients with meningitis and inhibiting MMP-9 and IL-1β can protect the brain of patients[23]. Barillari[24] has shown that MMP-9 is highly expressed in human myeloma and quercetin can inhibit its expression in the treatment of human myeloma. It has been found that MMP-9 can open the cerebrospinal fluid barrier. When inflammation occurs, the le-

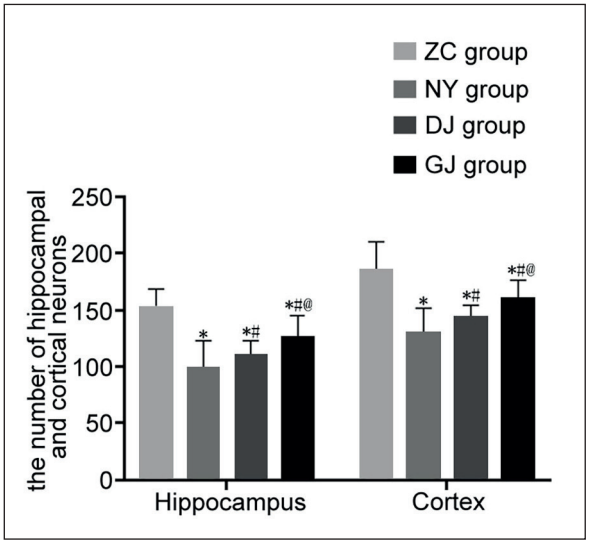

Figure 5. Comparison of the number of hippocampal and cortical neurons in the four groups of rats. Note: *: $p<0.05$ compared with ZC group; #: $p<0.05$ compared with NY group; @: $p<0.05$ compared with DJ group.

vel of MMP-9 is higher than normal, which causes the immune cells invasion to nerve tissue and elevated MMP-9 will bring more serious sequelae to patients[25]. A certain dose of quercetin can improve the water content of brain tissue in patients with encephalitis and helpful for the treatment of patients with encephalitis[26]. The study found that after the treatment, the number of hippocampal and cortical neurons was effectively increased, indicating that quercetin can increase the number of cerebral cortex and hippocampal neurons. By the relevant medical researchers, hippocampal cells were damaged during the examination of patients with bacterial meningitis[27], which confirms that bacterial encephalitis can damage the hippocampus. Injury of hippocampus tissue will cause the memory loss and bring more serious sequelae to the patient.

Conclusions

Quercetin is effective in the treatment of encephalitis possibly through inhibition of apoptosis of brain nerve cells and secretion of inflammatory factors TLR-4 and HMGB-1. This is the first time showing the protective effect of quercetin on encephalitis, which is the novelty of our study, indicating that quercetin might be used for treating encephalitis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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