

Curative effects of GM1 in the treatment of severe ischemic brain injury and its effects on serum TNF- α and NDS

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Abstract. The curative effects of monosialotetrahexosyl ganglioside (GM1) in the treatment of severe ischemic brain injury and its effects on tumor necrosis factor- α (TNF- α) and neuropathy disability score (NDS). Sixty patients with severe ischemic brain injury admitted to The First People's Hospital of Jining (Jining, China) from June 2014 to March 2016 were selected. They were randomly divided into the control group (n=30) and the experimental group (n=30). The patients in the control group were treated with routine therapy while those in the experimental group were treated with GM1. The level of TNF- α in the serum was measured by the enzyme-linked immunosorbent assay. The NDS was used to grade the two groups; Pearson's correlation coefficient was applied to analyze the correlation between the content of TNF- α and NDS; the content of superoxide dismutase (SOD) was detected using xanthine oxidase assay, and the content of malondialdehyde (MDA) was detected by thiobarbituric acid method. The clinical recovery time of two groups of patients was recorded. At 14 days after GM1 treatment, the serum TNF- α content and the NDS in the experimental group were significantly lower than those in the control group ($P<0.05$). The content of TNF- α in the patients was positively correlated with the NDS. After treatment, the serum MDA content of patients in the experimental group was lower, while the SOD content was significantly higher than that in the control group ($P<0.05$). After GM1 treatment, hemodynamic parameters of patients in the experimental group were significantly improved compared with those in the control group. The total effective rate of GM1

treatment in the experimental group was higher than that in the control group ($P<0.05$). GM1 has a good clinical significance in the treatment of patients with severe ischemic brain injury and is worthy of clinical promotion and application.

Introduction

Severe ischemic brain damage belongs to a disease type of cerebral infarction and is a global health problem (1). According to statistics, patients with this disease often suffer limb paralysis, fall into a coma or even die. Its mortality rate is 20-35%, and the morbidity rate and recurrence rate are also very high (2,3). At present, the main drugs for the treatment of ischemic brain injury are glutamate receptor antagonists, calcium channel blockers, free radical scavengers, anti-inflammatory and anti-apoptotic drugs (4,5). Although the relevant study has made significant progress in many key mechanisms and processes of injury, clinical trials of neurological protection in patients with ischemic brain injury bring little effect (6). Over the past few years, it was found that the neuroprotective drug monosialotetrahexosyl ganglioside (GM1) promotes growth and repair the neurological impairment. As reported, the exogenous GM1 has been used to promote the nervous system cell regeneration and synapse formation (7,8). However, the role of GM1 in patients with severe ischemic brain injury is rarely reported. This study focused on the systematic evaluation of the curative effect of GM1 in the treatment of patients with severe ischemic brain injury.

Materials and methods

Data of patients. A total of 60 patients with severe ischemic brain injury who were admitted to the Department of Emergency Medicine of Jining Hospital (Jining, China) from June 2014 to March 2016 were selected, and they were diagnosed by head computed tomography (CT) and magnetic resonance imaging (MRI) and received routine laboratory tests (such as erythrocyte sedimentation rate, white blood cell count, urine detection), which are in line with the relevant diagnostic criteria formulated in the Fourth National Cerebrovascular Disease Conference. Under the condition that patients or their family members signed the informed consent, patients were randomly divided into the control group (n=30)

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and the experimental group ($n=30$). In the control group, there were 14 males and 16 females with the average age of 54.9 ± 5.4 years. In the experimental group, there were 18 males and 12 females with the mean age of 52.6 ± 3.9 years. There were no statistically significant differences between the two groups in terms of general data, and the data were comparable. The study was approved by the Ethics Committee of The First People's Hospital of Jining. Written informed consents were signed by the patients and/or guardians.

Experimental grouping. Patients in the control group received routine anti-infection and dehydration treatments to reduce intracranial pressure and symptomatic and supportive treatments were provided, thus preventing complications. Patients in the experimental group were treated with intravenous infusion of GM1 (Sai Dian; National Medicine Permission no. H20093980; 2 ml each one; Beijing Science Sun Pharmaceutical Co., Ltd., Beijing, China) with 2 ml each time and once a day for 14 days on the basis of routine treatments.

Observational indexes

Detection of biochemical indexes. Ten milliliters whole blood of each patient was taken intravenously before and after treatment. The blood was coagulated at room temperature for 1 h. After centrifugation, the serum was stored at 80°C , and the statistical monitoring was conducted for all samples after collection.

Serum tumor necrosis factor- α (TNF- α) was measured by horseradish peroxidase-labeled sandwich immunoassay. In short, antibodies against TNF- α (75 kDa) were coated in each well of a 96-well plate and incubated after the addition of an appropriate amount of serum. The content of enzyme and enzyme-bound TNF- α was determined using tetramethyl-benzidine as the substrate. The optical density (OD) values at the dual wavelengths of 450 and 600 nm were measured under the microplate reader, and the sample concentration was calculated.

The experimental methods of Gao *et al* were used for reference (9). The content of superoxide dismutase (SOD) was determined by xanthine oxidase assay, and the content of malondialdehyde (MDA) was detected by the thiobarbituric acid method.

Observations of haemodynamics. The Medasonic Transpect thermal conductivity detector (TCD) produced in the US was applied. Two megahertz pulsed Doppler ultrasonography was used to detect peak velocity (V_p) and mean velocity (V_m) from the temporal window at the depth of 50–65 mm, and the symmetry [peak velocity difference (DV $_p$) and mean velocity difference (DV $_m$)] of both sides were observed.

Evaluation of clinical curative effects. Study methods of Daousi *et al* were used for reference for neurological deficit score (NDS) (10). The evaluation criteria for the curative effect are based on the relevant criteria formulated in the Fourth National Cerebrovascular Disease Conference (11), and judgments were made combined with clinical symptoms and signs of patients. Total effective rate = obviously effective rate + effective rate.

Statistical methods. The results were analyzed using GraphPad Prism software Version 5.01 (GraphPad Software, San Diego,

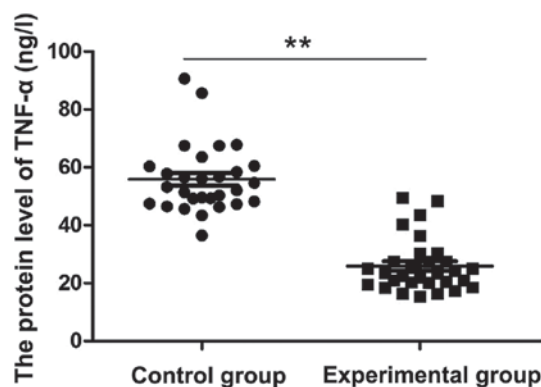


Figure 1. Detection of the contents of serum tumor necrosis factor- α (TNF- α) in two groups of patients by enzyme-linked immunosorbent assay (ELISA). **Compared with control group, $P < 0.05$.

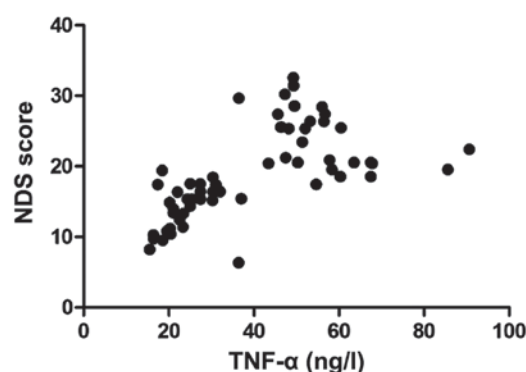


Figure 2. Correlation between the expression level of serum tumor necrosis factor- α (TNF- α) and the NDS in two groups of patients. NDS, neuropathy disability score.

Chile). Measurement data are expressed as mean \pm SD, and differences between indexes were detected using the paired t-test. Count data were detected by the χ^2 test. Pearson's correlation coefficient was used to analyze the correlation between TNF- α and NDS. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Detection of the expression level of serum TNF- α by the enzyme-linked immunosorbent assay (ELISA). As shown in Fig. 1, the serum TNF- α content in the experimental group was 25.89 ± 9.157 ng/l after two weeks of GM1 treatment, while the serum TNF- α content in the control group was 55.83 ± 11.71 ng/l, which was significantly higher than that in the experimental group ($P < 0.05$).

NDS. As shown in Table I, the difference in the NDS between the experimental group and the control group was not statistically significant before the experiment ($P > 0.05$). The NDS was 25.54 ± 5.83 in the control group and 16.34 ± 8.41 in the experimental group after treatment, which was significantly lower than that in the control group ($P < 0.05$).

Correlation between the expression level of TNF- α and NDS. Pearson's correlation coefficient (Fig. 2) showed that the

Table I. Comparison of the NDS between two groups of patients.

Groups	No.	Before experiment	After experiment	t-value	P-value
Control	30	33.12±2.37	25.54±5.83 ^a	1.270	0.0431
Experimental	30	32.58±1.95	16.34±8.41 ^{a,b}	2.032	0.0092
t-value		0.652	1.745		
P-value		0.347	0.0282		

^aP>0.05 compared with that before experiment; ^bP>0.01 compared with that in the control group. NDS, neuropathy disability score.

Table II. Detection of contents of serum MDA and SOD in two groups of patients (mean ± SD).

Detection time	No.	Groups	MDA (nmol/100 mg)	SOD (nmol/100 mg)
Before experiment	30	Control	87.18±5.92	184.25±10.04
		Experimental	90.51±7.33	178.23±8.93
After experiment	30	Control	72.34±6.45 ^a	158.73±12.57 ^b
		Experimental	50.81±10.88 ^{c,d}	120.75±6.82 ^{c,d}

^aP<0.05, ^bP<0.01 and ^cP<0.001 compared with those before experiment; ^dP<0.01 compared with those in the control group. MDA, malondialdehyde; SOD, superoxide dismutase; SD, standard deviation.

Table III. Detection of changes in hemodynamic parameters of two groups of patients before and after GM1 treatment (mean ± SD, mm/sec).

Detection time	No.	Groups	Vp	Vm	DVp	DVm
Before experiment	30	Control	47.36±5.62	32.17±3.57	26.05±3.89	15.04±2.16
		Experimental	48.92±4.77	33.23±3.61	25.58±2.80	14.43±2.22
After experiment	30	Control	67.23±11.40 ^b	38.76±4.03 ^a	15.63±3.52 ^a	7.32±1.42 ^a
		Experimental	85.04±8.68 ^{c,d}	44.37±5.36 ^{a,e}	10.25±4.63 ^{b,e}	3.65±0.77 ^{b,d}

^aP<0.05, ^bP<0.01 and ^cP<0.001 compared with those before experiment; ^dP<0.05 and ^eP<0.01 compared with those in the control group. GM1, monosialotetrahexosylganglioside; SD, standard deviation; mm/sec, millimeter per seconds.

content of TNF- α in the patients was positively correlated with the NDS ($r=4.321$, $P<0.05$).

Contents of serum MDA and SOD in two groups of patients. As shown in Table II, the contents of serum MDA and SOD in two groups of patients were not statistically different before the experiment ($P>0.05$). The content of serum MDA of patient in the experimental group was lower than that in the control group, while the content of SOD was significantly higher than that in the control group after treatment ($P<0.05$).

Changes in hemodynamic parameters before and after GM1 treatment. After GM1 treatment, the Vp and Vm in the blood of patients in the experimental group were significantly increased compared with those in the control group ($P<0.05$), while the DVp and DVm in the experimental group were significantly decreased compared with those in the control group ($P<0.05$) (Table III).

Comparison of the clinical recovery time between two groups of patients. Main clinical manifestations of patients with severe ischemic brain injury are disturbance of consciousness and abnormalities in muscle tension and original reflexes and with the alleviation of the disease, these symptoms will also improve. Results of this study (Table IV) revealed that the reflex recovery time, muscle tension recovery time and consciousness recovery time in the experimental group were significantly shorter than those in the control group ($P<0.05$), and the differences were statistically significant ($P<0.05$).

Comparison of the clinical curative effect between two groups of patients. After 2 months of treatment, the total effective rate of patients in the control group was 66.67% and that of patients in the experimental group was 87.18%. The results (Table V) showed that the clinical curative effect of the experimental group was better than that of the control group ($P<0.05$).

Table IV. Comparison of the clinical recovery time between two groups of patients (mean \pm SD).

Groups	No.	Reflex recovery time (day)	Muscle tension recovery time (day)	Consciousness recovery time (day)
Control	30	10.34 \pm 1.87	10.50 \pm 1.42	9.47 \pm 1.37
Experimental	30	6.83 \pm 3.26 ^b	8.62 \pm 2.73 ^a	5.22 \pm 1.99 ^a
t-value		3.34	1.85	4.36
P-value		0.028	0.041	0.023

^aP<0.01 and ^bP<0.01 compared with those in the control group. SD, standard deviation.

Table V. Comparison of the clinical curative effect between two groups of patients.

Groups	No.	Obviously effective	Effective	Ineffective	Total effective rate
Control	30	12	9	11	63.33%
Experimental	30	24	4	2	93.33% ^a
t-value					3.61
P-value					0.017

^aP<0.05 compared with those in the control group.

Discussion

A pathological study has shown that ischemic injury can lead to different types of neuronal cell primary and necrotic cell death (11). The hippocampal CA1 pyramidal neuron is found to be the most vulnerable and most common injury, which is often the leading cause of memory impairment in patients (12,13), and the cerebellar Purkinje cell injury leads to torso ataxia of patients (14). With the prolongation of ischemic time, the pyramidal cell layer, mitral neurons and striatum neurons of the thalamus are damaged to varying degrees (15,16). In this study, the NDS of patients was significantly decreased after GM1 treatment, and the reflex recovery time, muscle tension recovery time and consciousness recovery time were significantly shorter than those in the control group, which suggested that GM1 significantly protects the nervous system of patients with severe cerebral ischemic injury.

Under normal physiological conditions, oxygen free radicals (oxygen free radicals, H₂O₂ and OH) in the cells are produced in the cytoplasm and mitochondria, and then are rapidly removed under the action of endogenous anti-oxidants (SOD) (17). During the hypoxia-ischemia, the amount of generated oxygen free radicals is more than the nervous system can protect, so they cause brain injuries by attacking polyunsaturated fats. If oxygen free radicals cannot be removed in time, they will cause lipid peroxidation, thus leading to the formation of lipid peroxides (MDA) so as to further damage brain tissues (18). In the present study, it was found that GM1 significantly increased the level of SOD in patients while reducing MDA content, indicating that GM1 can restore the balance of oxygen free radical reaction and lipid peroxidation in patients.

Inflammatory mediators play a key role in the pathogenesis of severe ischemic brain injury (19,20). TNF- α is a proinflammatory cytokine. In previous studies, TNF- α

messenger ribonucleic acid (mRNA) has been confirmed to be expressed in the brain at 1-4 h after hypoxia-ischemia. TNF- α at high concentration causes neuronal apoptosis by mediating the caspase-8 pathway, but reducing TNF- α receptors can decrease neuronal injuries (21,22). The inhibition of TNF- α is beneficial in maintaining neurological function and protecting nerve cells from neurotoxicity. Consistent with these findings, the results of this study showed that the level of serum TNF- α in the experimental group was significantly decreased compared with that in the control group and was positively correlated with the NDS, suggesting that GM1 inhibits the expression of TNF- α and alleviates the neurological function of ischemic brain injury, and TNF- α is also a clinical index to evaluate the neurological function of patients with ischemic brain injury.

In conclusion, this study revealed that good clinical benefits were achieved using GM1 in the treatment of severe ischemic brain injury. Other studies have shown that GM1 inhibits TNF- α level in patients, reduces systematic NDS, improves patient blood flow, regulates the balance of oxygen free radical responses and lipid peroxidation in patients and significantly shortens the clinical recovery time of patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

FL and YiZ designed, conducted the study and analyzed the data. FL and YiZ wrote the manuscript. FL and YuZ collected and analyzed the fundamental data of patients. XS interpreted the biochemical indexes, haemodynamics and evaluation of clinical curative effects. GZ conducted the measurements of serum TNF- α and YL evaluated the neurological deficit score (NDS). All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The First People's Hospital of Jining (Jining). Written informed consents were signed by the patients and/or guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Broughton BRS, Reutens DC and Sobey CG: Apoptotic mechanisms after cerebral ischemia. *Stroke* 40: e331-e339, 2009.
- Kahlert P, Knipp SC, Schlamann M, Thielmann M, Al-Rashid F, Weber M, Johansson U, Wendt D, Jakob HG, Forsting M, *et al*: Silent and apparent cerebral ischemia after percutaneous trans-femoral aortic valve implantation: A diffusion-weighted magnetic resonance imaging study. *Circulation* 121: 870-878, 2010.
- Beck H and Plate KH: Angiogenesis after cerebral ischemia. *Acta Neuropathol* 117: 481-496, 2009.
- Hossmann KA: Treatment of experimental cerebral ischemia. *J Cereb Blood Flow Metab* 2: 275-297, 1982.
- Maher J and Hachinski V: Hypothermia as a potential treatment for cerebral ischemia. *Cerebrovasc Brain Metab Rev* 5: 277-300, 1993.
- Ginsberg MD: Current status of neuroprotection for cerebral ischemia: Synoptic overview. *Stroke* 40 (Suppl 3): S111-S114, 2009.
- Ledeer RW and Wu G: The multi-tasked life of GM1 ganglioside, a true factotum of nature. *Trends Biochem Sci* 40: 407-418, 2015.
- Wang Q, Song YH, Tang Z, Wang ZP, Xu Q and Bao N: Effects of ganglioside GM1 and neural growth factor on neural stem cell proliferation and differentiation. *Genet Mol Res*: Aug 5, 2016 (Epub ahead of print). doi: 10.4238/gmr.15038376.
- Gao M, Ding H, Zhong G, Lu J, Wang H, Li Q and Wang Z: The effects of transrectal radiofrequency hyperthermia on patients with chronic prostatitis and the changes of MDA, NO, SOD, and Zn levels in pretreatment and posttreatment. *Urology* 79: 391-396, 2012.
- Daousi C, Benbow SJ, Woodward A and MacFarlane IA: The natural history of chronic painful peripheral neuropathy in a community diabetes population. *Diabet Med* 23: 1021-1024, 2006.
- Wu X: Summary of the Fourth National Cerebrovascular Disease Conference. *Stroke Nerv Dis*: 4: 105-109, 1997.
- Niizuma K, Yoshioka H and Chen H, Kim GS, Jung JE, Katsu M, Okami N and Chan PH: Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. *Mol Basis Dis* 1802: 92-99, 2010.
- Wang JY, Xia Q, Chu KT, Pan J, Sun LN, Zeng B, Zhu YJ, Wang Q, Wang K and Luo BY: Severe global cerebral ischemia-induced programmed necrosis of hippocampal CA1 neurons in rat is prevented by 3-methyladenine: A widely used inhibitor of autophagy. *J Neuropathol Exp Neurol* 70: 314-322, 2011.
- Zhang F and Chen J: Leptin protects hippocampal CA1 neurons against ischemic injury. *J Neurochem* 107: 578-587, 2008.
- Lee JJ, Li L, Jung H and Zuo Z: Postconditioning with isoflurane reduced ischemia-induced brain injury in rats. *Anesthesiology* 108: 1055-1062, 2008.
- del Zoppo GJ and Zoppo G: Inflammation and the neurovascular unit in the setting of focal cerebral ischemia. *Neuroscience* 158: 972-982, 2009.
- Wei L, Fraser JL, Lu ZY, Hu X and Yu SP: Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiol Dis* 46: 635-645, 2012.
- Niatetskaya ZV, Sosunov SA, Matisiukovich D, Utkina-Sosunova IV, Ratner VI, Starkov AA and Ten VS: The oxygen free radicals originating from mitochondrial complex I contribute to oxidative brain injury following hypoxia-ischemia in neonatal mice. *J Neurosci* 32: 3235-3244, 2012.
- Kumar A, Mittal R, Khanna HD and Basu S: Free radical injury and blood-brain barrier permeability in hypoxic-ischemic encephalopathy. *Pediatrics* 122: e722-e727, 2008.
- Candelario-Jalil E, Yang Y and Rosenberg GA: Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia. *Neuroscience* 158: 983-994, 2009.
- Iadecola C and Alexander M: Cerebral ischemia and inflammation. *Curr Opin Neurol* 14: 89-94, 2001.
- Günther C, Buchen B, He GW, Hornef M, Torow N, Neumann H, Wittkopf N, Martini E, Basic M, Bleich A, *et al*: Caspase-8 controls the gut response to microbial challenges by TNF- α -dependent and independent pathways. *Gut* 64: 601-610, 2015.



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