Stellate Ganglion Block Brain Protection During Ischemic Stroke in Diabetes Rats: TLR4/NF-κB Pathway and Inflammation

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Research

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Abstract

Background: Diabetes is an independent risk factor for ischemic stroke, and systemic inflammation may be the principal contradiction that aggravates stroke. The purpose of this study is to explore the effect and mechanism of stellate ganglion block on diabetic stroke.

Methods: Analyze circadian rhythms and blood glucose changes, detect inflammation related signal pathways, and explore long-term neurological outcomes in rats.

Results: Stellate ganglion block is a clinical anesthesia technique that can improve the neurological outcome and prognosis of diabetic rats. Stellate ganglion block improves systemic inflammatory response and inhibits phosphorylation of NF-κB p65. TLR4 agonist abolished the neuroprotective effect of stellate ganglion block, and the neuroprotective effect of TLR4 inhibition was consistent with that of a stellate ganglion block. The effect of stellate ganglion block at night is greatly reduced.

Conclusions: These data optimize the perioperative application of stellate ganglion block in stroke patients, including time and mechanism. It also provides protective perioperative support for diabetic stroke patients to reduce their neurological risks in non-dangerous hyperglycemia and improve long-term neurological outcomes.

Background

Ischemic stroke and diabetes are both debilitating diseases with limited therapies, and both pathophysiological processes are related to inflammation.[1,2] Optimistically, anti-inflammatory measures, including many anti-inflammatory drugs and inhibitors of inflammation-related molecular pathways, have shown multiple salutary benefits in preclinical models of cerebral ischemia.[3–5] At the same time, inflammation is also one of the mechanisms of diabetes.[2] However, these measures may have some limitations in the routine clinical application of perioperative anesthesia. Therefore, a method that is closely related to the perioperative period of anesthesia is needed to treat ischemic stroke in diabetic patients.

Stellate ganglion block (SGB) is a technique unique to the Department of Anesthesia, which plays an unparalleled role in the treatment of many diseases.[6–8] The TLR4/NF-κB pathway is also a classic inflammatory pathway.[9] It has been reported that SGB can significantly reduce the inflammatory response.[10] At the same time, the TLR4/NF-κB pathway also plays a vital role in diabetes and ischemic stroke.[11,12] Given that both diabetes and ischemic stroke bodies are closely related to the TLR4/NF-κB pathway, and evidence suggests that SGB can modulate the inflammatory response,[13] we hypothesized that SGB is associated with diabetic ischemic stroke. The neuroprotective effect is achieved through the inhibition of TLR4/NF-κB pathway. To test this hypothesis, we inhibited and activated the TLR4 through TAK242(3 mg/kg; i. p. HY-11109, MCE, USA) and LPS and examined whether the protective effect of SGB

on diabetic ischemic stroke is related to the TLR4/NF-κB pathway. In addition, for precision and optimization of SGB, we have also explored the application of circadian rhythm in SGB.

Methods

All data supporting the findings of this study are expected to be available within the paper and in the Data Supplement. Additional inquires can be directed to the corresponding author. All procedures were approved by the Harbin Medical University Department of Experimental Animals. Table in the Data Supplement lists the antibodies used. Establish diabetic rats earlier. Middle cerebral artery occlusion (MCAO) in rats was performed as describing. To determine pathway expression, brain was collected and analyzed with western-blot and immunofluorescence. Neurological function score, survival rate, and percentage weight change was used to assess long-term neurological outcome and survival status. Organotypic brain slices TTC staining were utilized to study cerebral ischemia. The inhibitor was injected by tail vein and the agonist was injected intraperitoneally.

Experimental groups

One hundred and fifty-six male Sprague-Dawley (SD) rats weighing 60–80 g were obtained from the Harbin Medical University Department of Experimental Animals. Rats were maintained at a 12-hour light-dark cycle at controlled temperature (21–24 °C) and humidity (40%-70%) with free access to food and water. The rats were randomized into six groups: Sham group (group S, n = 36), MCAO group (group M, n = 36), SGB group (group G, n = 36), LPS group (group L, n = 18), TAK242 group (group T, n = 18) and SGB night group (group N, n = 12). The study was divided into two protocols according to the differences in monitoring indices: (1) Rats were studied for 28 days after surgery to measure neurological function, survival rate and percentage weight change; (2) Rats were sacrificed on 48 hours after injury to harvest samples for the subsequent measurements, including the infarct volume measurement, brain water content, detection of inflammatory cytokines, immunohistochemistry, and western-blot. (Supplemental figure)

Diabetic rat model

We induced diabetes mellitus (DM) in rats by feeding them a high-fat diet consisting of 20% lard, 20% sucrose, 2.5% cholesterol, and 57.5% standard chow for 4 weeks.37Next, the rats were given free access to water for one night and injected with STZ (Streptozocin) dissolved in 5-citrate buffer (pH 4.5; intraperitoneal, 35 mg/kg; Sigma-Aldrich (Shanghai) Trading Co., Ltd., Shanghai, China). After seven days, we measured non-fasting plasma glucose levels and only animals with levels ≥ 11.1 mmol/l were chosen as diabetic group rats.

MCAO rat model

We established a rat model of middle cerebral artery occlusion (MCAO).38 Experimental rats were given 5% sevoflurane through a face mask for induction of anesthesia, and 2 to 3% sevoflurane was used for

anesthesia maintenance, retaining spontaneous breathing. Skin preparation and disinfection of the surgical area, 1% lidocaine local infiltration anesthesia, a midpoint incision of about 0.5 cm is made at the midpoint of the two ears of the cranial parietal, looking for bregma, the anterior bregma is zero, the cerebral blood flow probe was fixed 4 mm on the left side of the bregma and 1.5 mm on the caudal side, and the cerebral blood flow(CBF) was monitored by laser Doppler flowmetry (TF5000; PRIMED AB; Stockholm; Sweden). Turn over slowly, prepare and disinfect the neck, local infiltration and anesthesia with 1% lidocaine, make an incision at 3 mm parallel to the midline, and separate the subcutaneous tissue with micro forceps bluntly, expose the left sternocleidomastoid muscle, and gradually free the second abdomen muscles, sternocleidomastoid muscle and scapula-hyoid muscle, the left carotid sheath can be seen between the muscles. The common carotid artery, external carotid artery and internal carotid artery was bluntly separated. Electrocoagulation disconnects the communicating branch between the external carotid artery and the internal carotid artery and the collateral branches of the external carotid artery, ligates and disconnects the left external carotid artery, and clamps the proximal end of the common carotid artery and the distal heart of the internal carotid artery with an arterial clip, a suture was inserted from the stump of the external carotid artery, and the cerebral blood flow suddenly dropped, and the decline was greater than 70% of the fundamental value. The suture was fixed and the incision was sutured. Record thread plugs insertion time and cerebral blood flow value. Take out the threaded plug after embolization for 90 minutes. After the operation, the animals were sent to the PACU to wake up and be kept warm.

SGB technique

In this experiment, the left SGB was performed using the percutaneous posterior approach after the MACO model was successfully established,39 and slightly adjusted. The primary operation process is as follows, the modeled MCAO rats used sevoflurane for anesthesia induction and maintenance (3.5% for induction and 2.0% for maintenance), and a warming blanket to maintain body temperature at 37 °C. After induction of general anesthesia, the rats were connected to anesthesia masks to maintain spontaneous breathing and placed in a prone position. Utilizing the cartilage process of rat C7 spinous process as a marker, palpate the structure and connect a 1 mL syringe with a short bevel needle to advance the anterior and posterior direction along the left side of the C7 vertebral body in the median sagittal position. When the needle tip and vertebral body was lost at the time of contact, the needle was withdrawn approximately 5 mm and retraction confirms that there was no blood and cerebrospinal fluid, then a local anesthetic was injected (0.3 mL of 1.0% lidocaine per injection).The above operation is performed by the same experimenter who can be skillfully complete rat SGB at a uniform treatment time point. Completed the nerve block, stop sevoflurane inhalation, the rat wakes up after a few minutes, determines whether the rat has ptosis, and the ptosis changes(Fig. 2d), indicating that the rat SGB success. In addition, we found that in some rats after SGB, the ear capillaries were dilated. (Supplemental figure □)

Measurement of blood sugar level

Thirty minutes before MCAO and forty-five minutes after MCAO/R, the blood glucose of the rat tail vein was measured and recorded.

Assessment of neurologic outcome, survival rate, and percentage weight change

Neurological deficits were measured with the modified Neurological Severity Score (mNSS) and Garcia test on days 1, 3, 5, 7, 14, and 28 depending on the length of survival. The 28-day survival rates were also recorded. All the rats were trained twice for adaptation before surgery. The evaluations were conducted by the same investigator who was blinded to the assignment. The weight was monitored daily for 28 days. The percentage of weight change was determined relative to the baseline weight. (n = 6/group)

Measurement of infarct volume

Forty-eight hours after reperfusion, the rats were sacrificed with a lethal dose of sodium pentobarbital. The brains were rapidly removed and sliced into 2-mm coronal sections, stained with 1% 2,3,5-triphenyl tetrazolium chloride (TTC, Biosharp, Beijing, China) for 20 min at 37℃, followed by overnight immersion in 4% paraformaldehyde. Infarct and hemisphere areas were measured using Image software. The ratio of hemispheric infarct was calculated as (contralateral hemisphere area - ipsilateral hemisphere without infarct) /contralateral hemisphere area. (n = 6/group)

Measurement of brain water content

Forty-eight hours after reperfusion, we randomly selected six rats from each group and estimated the brain water content. Briefly, we separated the cerebral cortex from the rest of the brain and recorded the weight (wet weight). Next, each specimen was dried in an electric oven at 80 °C for 48 hours, and the weight was recorded (dry weight). We used the following formula to calculate the brain water content: brain water content = (wet weight - dry weight) / wet weight × 100%. (n = 6/group)

Measurement of inflammatory cytokines

Rats were anesthetized with pentobarbital, and the femoral artery was punctured, and blood samples were placed in heparin-coated tubes and centrifuged (3500 rpm for 20 min at 4 °C). Plasma levels of IL-6, IL-1β, and TNF-α were recorded for 48 hours after reperfusion. We used enzyme-linked immunosorbent assay (ELISA) kit for each cytokine (# Rat IL-6, # Rat IL-1β, and # Rat TNF-α ELISA Kit, Boster, China) according to the manufacturer's instructions. (n = 6/group)

Measurement of immunofluorescence

Forty-eight hours after reperfusion, the rats were killed with a lethal dose of sodium pentobarbital and trans-cardiac perfusion with normal saline and 4% paraformaldehyde. The brains were post fixed in 4% paraformaldehyde at 4 °C for 24 hours. Infarct segments were cut into 10 mm transverse frozen sections. After 0.1% Triton X-100 wash for 20 min, then blocked with 3% BSA for 30 min. The sections were incubated with primary antibody TLR4 (1:100; BS3489, Bioworld technology) and NF-κB (1:200; BMS-

33117M, Bioss) at 4 °C overnight and FITC-conjugated secondary antibody for 50 min sequentially. Finally, the sections were incubated with DAPI for 10 min prior to visualize with an optic microscope (Nikon Eclipse Ti-SR, Japan) at 400 magnification. Images were merged using Image software at the same contrast—the TLR4 in green, NF-κB in red and a blue nucleus. One section per animal was selected. (n = 6/group)

Measurement of western blot

Forty-eight hours after reperfusion, the cerebral cortex was homogenized and centrifuged at 14,000 rpm for 20 min at 4 °C. We collected the supernatants and tested the protein concentrations by the bicinchoninic acid assay (BCA) kit. Then, we parted the protein samples by 10% SDS-PAGE and transferred them onto nitrocellulose membranes. The membranes were blocked with 5% milk powder in 0.1% TBS-T, incubated with primary antibodies TLR4 (1:2000; #35463, SAB), NF-κB p65 (1:2000; #48498, SAB), NF-κB p100 (1:1000; ab191594, Abcam), β-Actin (1:400; PR-0255, ZSGB Bio) and phosphor-NF-κB p65(phosphor Ser536; 1:2000; YP0191, Immunoway) overnight at 4 °C. Subsequently, we washed the membranes with TBS-T and incubated them with goat anti-rabbit horseradish peroxidase-conjugated secondary antibodies (1:5000; ZB-2301, ZSGB Bio) for one hour. The bands were detected with an electrochemiluminescence kit, and a gel imaging system was used only for acquisition and analysis. (n = 6/group)

Statistics

The sample size was based on our previously reported studies. There were no missing or lost data or data that were not included the analysis. We used SPSS (version 21.0) statistical software to analyze the data. The datum is presented as the mean ± standard deviation (SD). Multigroup comparisons were performed with one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. Nonparametric tests were utilized to compare differences among the examined groups. Kaplan-Meier survival curves were comparable using log-rank testing. Repeated measures analysis of variance were used for comparisons within the same group. A p-value < 0.05 was considered statistically significant.

Results

Blood sugar level

During the procedure, the blood glucose level increased significantly. The rapid blood glucose change (group M:18.9 ± 4.03 vs. 23.75 ± 4.23, P < 0.05; group G: 15.2 ± 3.39 vs. 21.4 ± 3.36, P < 0.05) is the leading cause of blood glucose fluctuations in diabetic rats (Table 1).

SGB reduced infarct volume and brain water content

As shown in Fig. 1A, 48 hours after MCAO, rats in group M and G showed prominent infarct areas (42.77 ± 4.79 vs. 24.47 ± 1.61, P < 0.0001), the infarct area was significantly reduced after SGB treatment. Brain

water content showed consistent result (group M vs. group G: 87.53 ± 3.56 vs. 79.14 ± 1.89, P = 0.0009) (Fig. 1B). (Table in the Data Supplement)

Effect of SGB on the neurological outcome, percentage weight change and survival rate

A significant survival benefit was found for the SGB treatment (Fig. 2D). Rats in group M (60%) compared with rats in group G (76.9%) survived until day 28 (P = 0.0213), but not significantly compared with that in group N (63.16%, P = 0.4976, Fig. 4C); this difference was accompanied by neurological deficit scores (Fig. 2A, 2B, 4C), except for the insignificance between group G and S on day 28. With respect to the postoperative weight change (Fig. 2C, 4C), the group G showed a small degree of loss with faster recovery (reached the lowest value on the 3rd day and returned to the preoperative level on the 6th day). The difference was significant compared with the other two groups on each day during evaluation. The weight in group M was at the lowest value on day 5 and recovered to the baseline on day 11, while it was at the lowest value on day 5 and recovered to baseline on day 10 in group N. (Table in the Data Supplement)

Effects of SGB on TLR4/NF-κB pathway

TLR4 and NF-κB content were measured by two methods. Immunofluorescence results, while localization, also quantitatively observed that TLR4 content was much decreased after SGB treatment (1.56 ± 0.57 vs. 1.21 ± 0.44, P = 0.0187). After application of LPS, SGB did not show treatment effect while with more terrible (2.43 ± 0.94 vs. 1.21 ± 0.45, P = 0.0047). Western results of brain tissues at the ischemic semi-dark zone had more consistent judgment (group M vs. group G: 1.51 ± 0.03 vs. 1.03 ± 0.05, P < 0.0001). Immunofluorescence results localization showed that NF-κB was mainly present in the nucleus in group M, we think because that maybe there is more NF-κB transfer to the nucleus in group M. Western-blot results showed that group M phosphorylation was enhanced, while SGB inhibited NF-κB p65 phosphorylation (1.18 ± 0.14 vs. 0.18 ± 0.15, P = 0.0022). In addition, except for group T, NF-κB p100 all didn’t show a significant difference.

Effects of SGB on inflammation

The IL-1β and TNF-α level in group G significantly decreased, different from that in group M (IL-1β: 67.9 ± 5.66 vs. 23.01 ± 6.3, P < 0.0001; TNF-α: 371.69 ± 29.66 vs. 213.77 ± 3.4, P < 0.0001). Additionally, compared with group G, the IL-1β and TNF-α level were much lower in group T (IL-1β: 23.01 ± 6.3 vs. 9.06 ± 0.91, P < 0.0001; TNF-α: 213.77 ± 3.4 vs. 121.09 ± 17.93, P < 0.0001), and much higher in group L (IL-1β: 131.76 ± 13.58 vs. 23.01 ± 6.3, P = 0.0006). The TNF-α level in group N significantly decreased (371.69 ± 29.66 vs. 221.23 ± 29.16, P < 0.0001) while IL-6 level was no significant difference (60.05 ± 5.63 vs. 54.23 ± 1.25, P = 0.0785) (Fig. 3B, 4B). (Table in the Data Supplement)

Discussion

This study found that SGB can effectively improve long-term neurological function after ischemic stroke in diabetic rats, and its mechanism is related to TLR4/NF-κB pathways. At the same time, there are diurnal differences in SGB treatment

This experiment uses the diabetic rat MCAO model. There are a lot of diabetic patients and they are prone to ischemic stroke. The mortality and long-term adverse outcomes are significantly higher than those of the general population.[14–16] Moreover, the primary method of clinical perioperative preparation is still hypoglycemic, but a multi-center significant data study found that merely controlling blood sugar cannot effectively improve the long-term neurological function of diabetic patients after stroke.[15] Therefore, it is particularly important to find an effective perioperative control method for diabetic stroke patients. This experiment utilizes the diabetic rat MCAO model to study from two aspects of treatment and molecular mechanism. In addition, during the MCAO modeling process, blood glucose was measured on the morning of the operation day to ensure the stability of the diabetic rat model, and blood glucose was monitored after the modeling was completed to rule out that the blood glucose was too high or too low to affect the survival of MCAO rats. At the same time, in order to ensure consistency of the MCAO models in each group, after the model was established, rats with a Longa score,[17] of 2 were selected, and rats with other score levels were excluded from the experiment.

In experiments with rats as experimental subjects, the commonly used methods of SGB include surgical exposure (cervical sympathetic nerve trunk transaction, stellate ganglion catheterization, direct injection of medicine),[18,19] percutaneous injection (anterior approach, posterior approach, lateral approach).[39,20] The surgical exposure method directly dissected the stellate ganglion, and the effect was definite, but it required invasive operation on the rat, which would cause inevitable damage to the rat body. The percutaneous injection method is less traumatic, simple to operate, less painful, and reproducible. However, because it is blind typing, the success rate will vary depending on the proficiency of the operator. Ultrasound guidance, visual operation, precise block location, exact effect can better avoid the occurrence of related complications, but the size of rats is minimal, it is relatively difficult to apply ultrasound. To the best of our knowledge, no one has used ultrasound guidance for SGB in rats. This experiment is to complete the SGB on the basis of the rat MCAO model. During the establishment of the rat MCAO model, the neck has been dissected, and re-exposure of the neck area will be more difficult and will increase the body damage of the rat. The size of rats is small, and the use of ultrasound-guided nerve blocks is a relatively tricky and challenge to learn. In the percutaneous injection method, the anterior approach is relatively more likely to damage the neck structure, while the lateral approach requires the use of intravenous anesthetics, which will increase the mortality of the rat MCAO model. Based on the above reasons, this experiment used the percutaneous posterior approach was used to complete rat SGB.

Our data indicate that SGB has an excellent therapeutic effect on the acute phase of ischemic stroke in diabetic rats. The recovery of stroke is usually assessed clinically for weeks and months later.[21] Therefore, clinical improvement after intervention should also be evaluated over a relatively long observation period. Our study found that the long-term functional advantages of SGB are very prominent,

and it has more comprehensively demonstrated its salutary effect on 28-day long-term neurological outcome and weight recovery of diabetic rats. But at the same time, we also concluded that the high mortality rate after stroke in diabetic rats far exceeds clinical data. This may be linked to the blood glucose of rats. In our study, we found that rats with blood glucose more expressive than 25 mmol/L had much higher mortality than other diabetic rats, which may be correlated with their significantly higher postoperative blood glucose, more generous than 33.3 mmol/L. To prevent the influence of blood glucose in this study, we limited the blood glucose of diabetic rats in the range of 15–25 mmol/L. In addition, starting the second day after MCAO, nutritional support (dry granules on the cage floor, oral jelly food and jelly food in Petri dishes) could reduce the 14-day mortality rate from 59–15%.[22] In our experiments, rats had free access to food and water after surgery without additional nutritional support.

The TLR4/NF-κB pathway was first discovered in tumor immunity,[23] and in the following 22 years, it has been discovered to play an essential role in the pathophysiology of many diseases.[10,24,25] Previous studies have shown that this pathway plays a vital role in the progression of inflammation.[26,27] As a part of the cell membrane surface involved in innate immune response, TLR4 is closely related to microglia, which is a kind of brain macrophage, activates transcription factor NF - κ B, which regulates cytokines and chemokines related to inflammation.[28–30] Choi, I. et al. study showed that microglial activation and autophagy were near related to the TLR4/NF-κB pathway.[28,31] Autophagy and activation of microglia are critical pathological processes and pathophysiological mechanisms of ischemic stroke.[31–33] In order to better explore the molecular mechanism of SGB, improving the long-term neurological function of ischemic stroke, we explored the TLR4/NF-κB pathway. Our study found that SGB treatment can slow down the process of NF - κ B p65 to nuclear transfer, reduce the level of NF - κ B p65 phosphorylation and reduce the inflammatory reaction. However, during this process, we found that the level of IL-6 did not change significantly, which may be directly related to the time point of 48 hours. The level of IL-6 increased rapidly after infection and reached a peak at 2 h.[34,35] In this study, blood sampling time was 48 hours after the operation, which may miss the peak value of IL6, leading to no significant difference.

A recent study found that the change in circadian rhythm is the reason for the failure of the transformation of rodent-related research to clinical.[36] There is some diurnal variation in SGB treatment. Interestingly, we found that during the day, SGB was effective in improving ischemia and long-term neurological function, promoting weight recovery, reducing mortality, and reducing inflammation; however, during the night, SGB was not effective in improving ischemia and reducing mortality, but it was able to improve some neurological function and weight recovery while reducing inflammation. This suggests that SGB can exert anti-inflammatory effects both during the day and night, which are near related to long-term neurological function and body weight recovery. As for the failure of ischemia to improve, there may be a correlation with sympathetic nerve activity. During the daytime sympathetic overexcitability, SGB could play useful influential role. This also serves as a theoretical basis for the application of SGB precision and optimization of treatment plans for clinical patients. On the other hand, diabetes is an independent risk factor for stroke, and the pathway agonist LPS can markedly activate

inflammation-related pathways, leading to inflammatory storms, leading to high mortality in group L (Table 2).

SGB, as a clinical treatment of a variety of complex diseases, can play a useful role, and rapid onset, exact effect, small side effects, repeatable operation, with the popularization of ultrasound-guided, its safety is more guaranteed than before, can be effectively used in patients with ischemic stroke. Different from previous experiments, this experiment contains the following innovations. First of all, there were few studies on diabetic stroke in the past. We studied the effect of SBG on this compound model, which is of great significance to promote the development of the discipline. Secondly, we take the lead in the study of circadian rhythm, which provides a new concept and theoretical basis for the optimization of relevant treatment measures in clinical application. Moreover, previous studies on SGB were still relatively simple, but our research explores SGB from the molecular and mechanism levels, making the mechanism of SGB more precise and clearer. Finally, we found the best control range of blood glucose. When blood glucose exceeded 25.0 mmol / L, the mortality of the MCAO model would increase significantly, which provided some reference for related basic research. In addition, we found that in some rats after SGB, the ear capillaries were dilated. (Fig. 2 in the Data Supplement)

Our research also has certain limitations. The ischemia time selected in this study is 90 minutes, which is the time window from the onset of the disease to the recanalization treatment for most patients in the hospital. However, in clinical practice, some patients are transferred from outside the hospital to the hospital, and the treatment time window will be different. In addition, the SGB technique is a minimally invasive blocking technique that can only be implemented by skilled anesthesiologists.

Conclusions

Our study shows that SGB is effective in improving long-term neurological outcome and quality of life after ischemic stroke in diabetic rats, and the mechanism is mainly related to inhibition of TLR4/NF-κB specific pathway activation. We do not exclude any autonomic role in this process, but the long-term neurological function is mainly influenced by inflammation by circadian rhythm comparison. In addition, SGB is widely used in a variety of clinical diseases, and this study provides a more profound basis for the precise optimization of strategies in the perioperative period.

List Of Abbreviations

CBF cerebral blood flow

DM diabetes mellitus

ELISA enzyme-linked immunosorbent assay

LPS Lipopolysaccharides

MCAO middle cerebral artery occlusion

MCAO/R MCAO /reperfusion

mNSS modified Neurological Severity Score

PACU postanesthesia care unit

SGB Stellate ganglion block

STZ Streptozocin

TTC 2,3,5-triphenyl tetrazolium chloride

Declarations

Ethics approval and consent to participate

The Institutional Animal Care Committee approved the experimental protocols of Harbin Medical University, and all procedures were conducted in strict accordance with the guidelines for the care and use of laboratory animals of Harbin Medical University as well as the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines for animal research.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Qiang Wan, Xin Zhang, Yuan Xiao and Xiang-nan Liu. The first draft of the manuscript was written by Ting-ting Li and Qiang Wan, Wan-Chao Yang review and editing and all

authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table -1

Blood glucose level of each group of rats

Group	M	G
preoperative	18.9 ± 4.03*	15.2 ± 3.39*
intraoperative	23.75 ± 4.25	21.4 ± 3.36
*p < 0.05		

Table -2

Survival of group G, L and T

Group	G	L	T
survive	12*	12	12
total	17	26	16
48 h-survival rates	70.59%	46.15%	75%
*p < 0.05 vs. group L.			

Figures

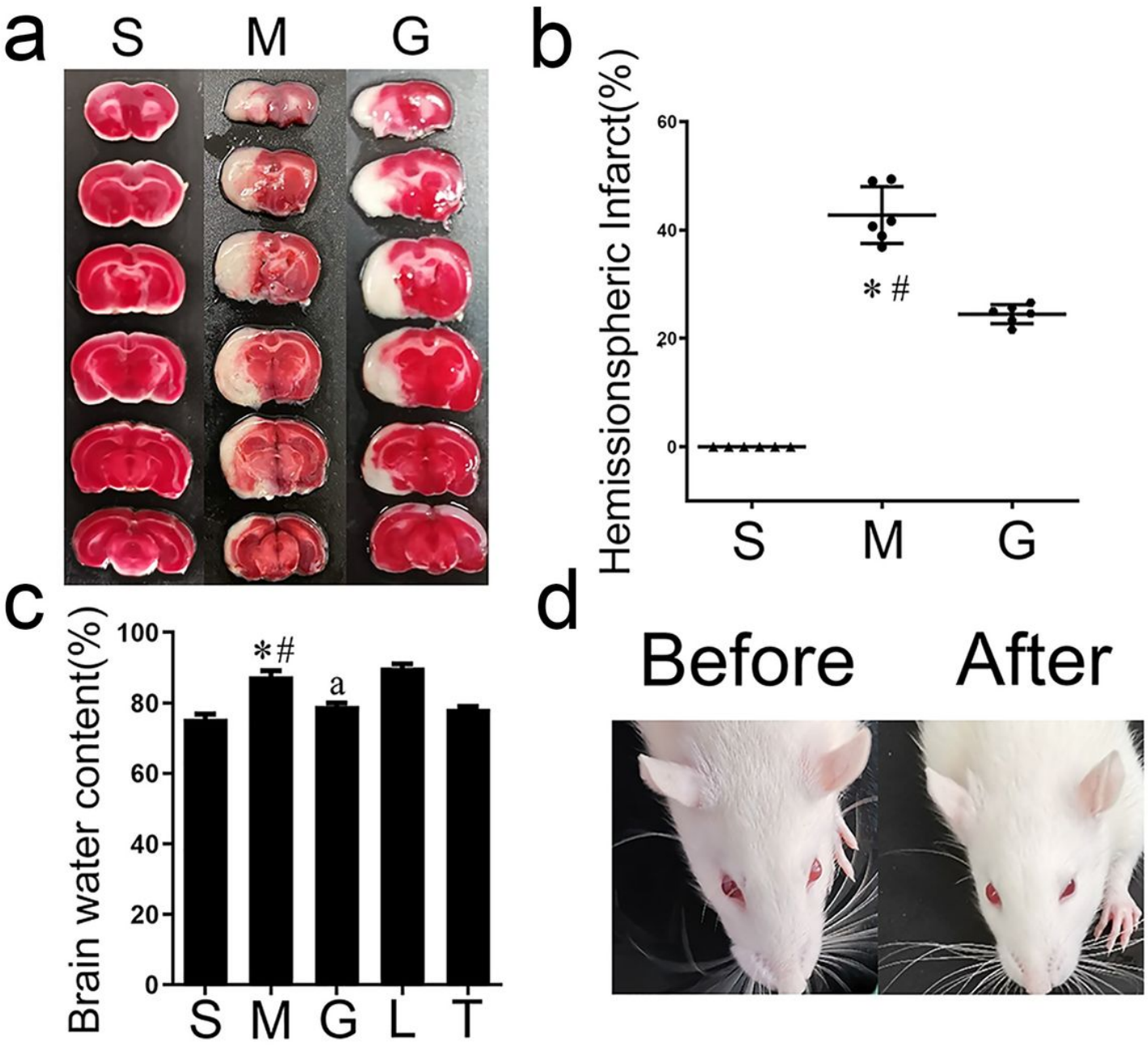

Figure 1

Effect of SGB on infarct volume and eyes change. A, Representative TTC staining in comparable sections from the three groups. B, Quantification of the ratio of the hemispheric infarct. C, The brain water content of three groups. D, Comparison of the eyes changes before and after SGB. (n=6). The data represent the mean ± SD. Difference among groups were analyzed by using one-way ANOVA followed by the Student–Newman–Keuls post hoc test. *p<0.05 vs. group S. #p<0.05 vs. group G. ap<0.05 vs. group L.

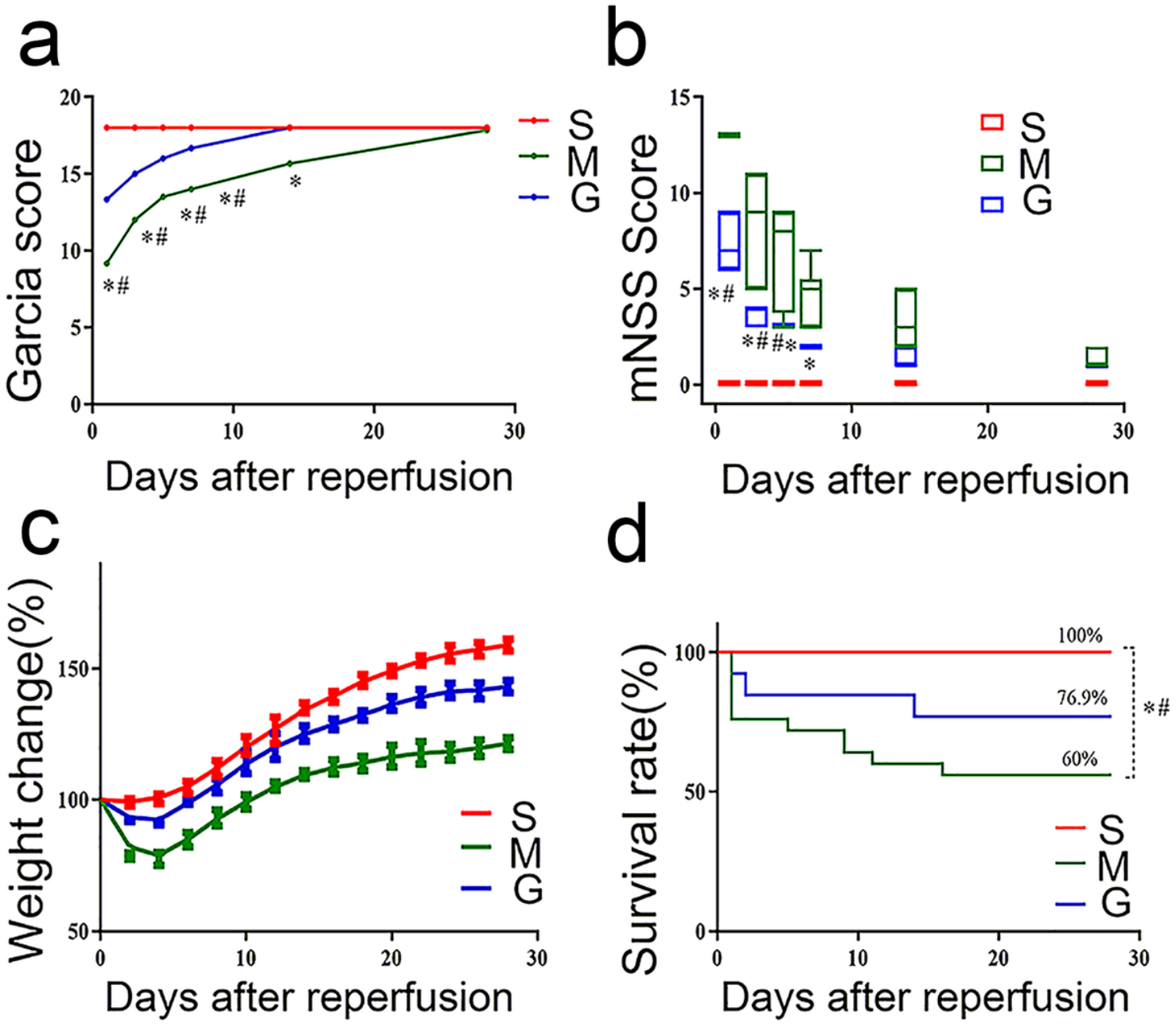

Figure 2

The long-term functional benefit of SGB. A, Results of the Garcia test. B, Results of the mNSS test. C, Comparison of weight change. D, SGB increased the 28-day survival rate. (n=6). The data represent the mean ± SD. Kaplan-Meier survival curves were compared using log-rank testing. *p<0.05 vs. group S. #p<0.05 vs. group G.

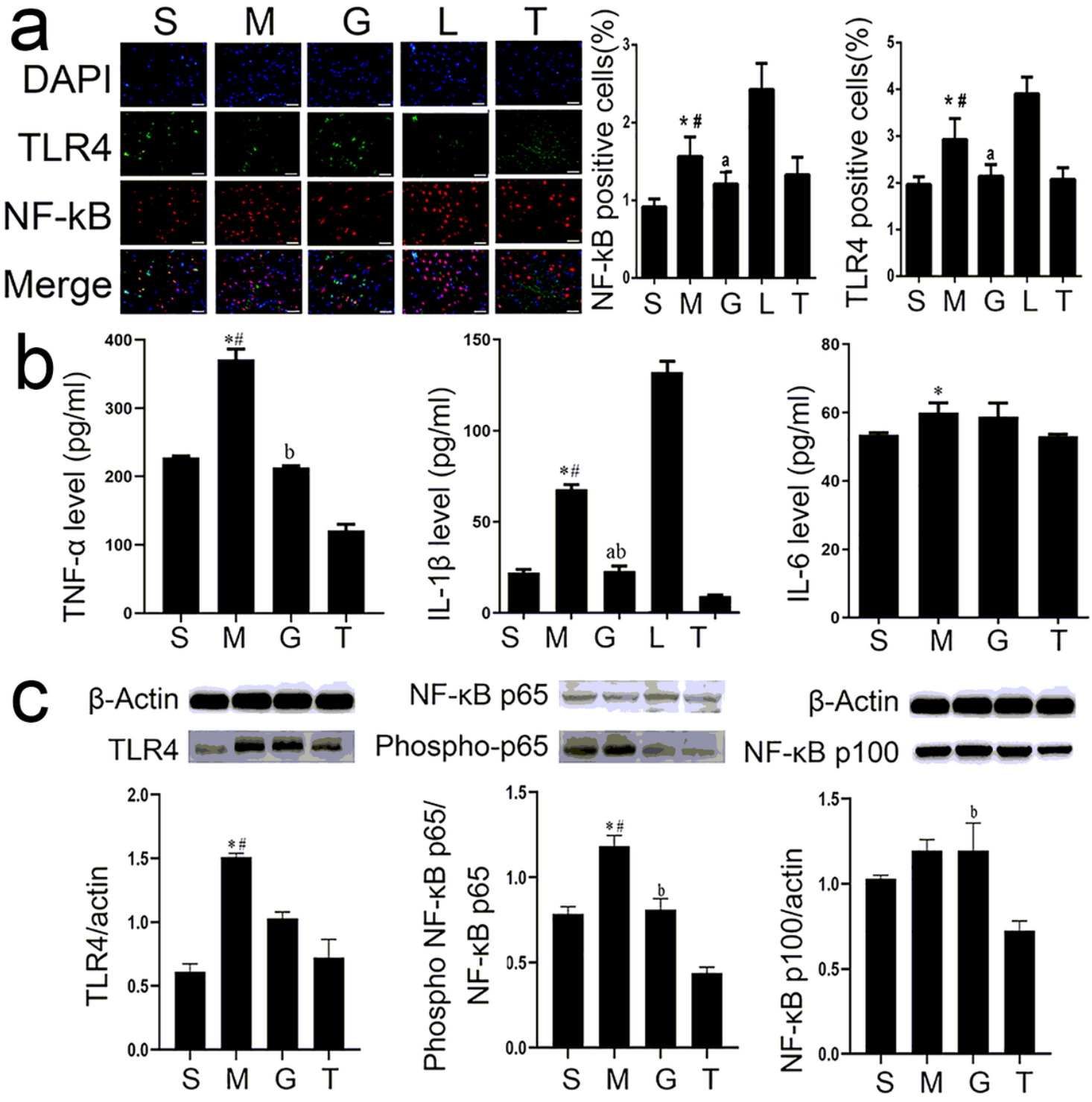

Figure 3

Results of dual immunofluorescence, SGB on inflammation and western-blot. A, Representative double immunofluorescence, scale bar = 50μm. Index of TLR4 positive cells and NF-κ B positive cells. B, IL-6, IL-1β, and TNF-α. C, Results of western-blot. (n=6). The data represent the mean ± SD. Difference among groups were analyzed by using one-way ANOVA followed by the Student–Newman–Keuls post hoc test. *p<0.05 vs. group S. #p<0.05 vs. group G. ap<0.05 vs. group L.

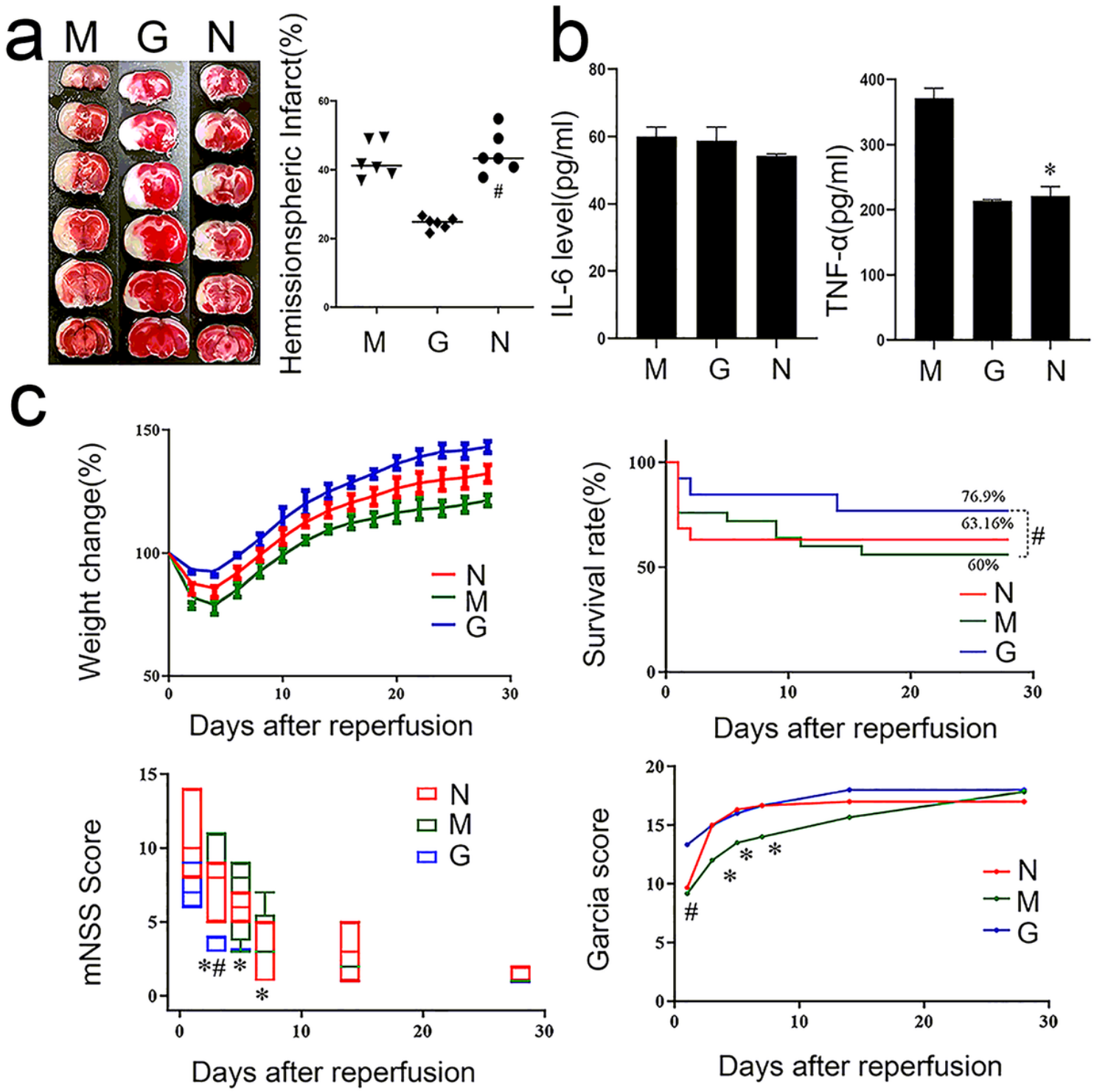

Figure 4

Effect of SGB at night. A, Representative TTC staining in comparable sections. B, Effect of SGB at night on inflammation. C, Comparison of weight change, 28-day survival rate, mNSS test and Garcia test. (n=6). The data represent the mean ± SD. Difference among groups were analyzed by using one-way ANOVA followed by the Student–Newman–Keuls post hoc test, and Kaplan-Meier survival curves were compared using log-rank testing. *p<0.05 vs. group M. #p<0.05 vs. group G.

Supplementary Files

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