Mesenchymal stem cells regulate activation of microglia cells to improve hippocampal nerve damage of heat stroke rats

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Research

Keywords: Mesenchymal Stem Cells, Heat Stroke, Microglia Cells, Hippocampal, Central Nervous System

Posted Date: July 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38801/v1

Abstract

Background

Heat stroke (HS) is a severe systemic inflammatory response disease caused by high fever, mainly with nervous system damage. Currently, mesenchymal stem cells (MSCs) have inflammation and immunomodulatory effects. Therefore, we aimed to explore the protective effect and mechanism of MSCs on HS-induced excessive inflammation and neurological dysfunction.

Methods

A heat stroke Sprague-Dawley (SD) rat model was established at continuous high temperature (42 °C) and high humidity (70%-80%). After modeling, the rats were randomly divided into a heat stroke model group (HS group), a MSCs treatment group (HS + MSCs group), and a Control group in which the rats were kept at room temperature without any treatment. Survival analysis, neurological deficit scoring, pathological staining of hippocampus and cerebellum, immunofluorescence staining of microglia cells, and tissue level detection of inflammatory cytokines were performed in the three groups on day 1, 3, 7, 14 and 28 separately.

Results

After heat stroke modeling, the rats were severely paralyzed and had a high mortality. MSCs treatment significantly reduced the mortality in both early stage (day 3) and late stage (day 28). MSCs treatment also significantly reduced the neurological impairment of heat stroke rats, and improved hippocampal and cerebellar pathology and neuronal cell damage. In addition, MSCs treatment significantly inhibited the overactivation of microglia in the hippocampus of HS rats as well as the levels of pro-inflammatory factors and chemokines in the hippocampus. In the early stage (day 1) of MSCs treatment, the activation of cerebellar microglia in the heat stroke rats was significantly promoted. Meanwhile, MSCs treatment had no significant inhibitory effect on the levels of pro-inflammatory factors in the cerebellar tissues of heat stroke rats, but can inhibit the levels of chemokines in the early stage.

Conclusions

The application of MSCs for heat stroke treatment in rats can significantly reduce the mortality and neurological defects and improve the hippocampal injury. Meanwhile, MSCs can inhibit the over-activation of microglia cells in the hippocampus of heat stroke rats, which may be a mechanism of MSCs in protecting heat-stroke-caused hippocampal injury.

Background

As global warming is intensified year by year, the incidence of heat stroke increases significantly(1, 2). Thermal radiation can result in systemic inflammatory response syndrome, which leads to multiple organ failure, with a fatality rate as high as 50%-60% (3, 4).

Clinically, almost all heat stroke patients suffer from varying degrees of brain injury, which is also the main cause of death or serious sequelae(5, 6). In addition to high fever, ischemia, hypoxia and other factors, central nervous system (CNS) inflammatory response also plays an important role in the occurrence and development of heat-stroke-caused brain injury, and the level of such response is significantly correlated with nervous system injury, angioedema and neuron death (7). The occurrence and development of CNS inflammatory response are closely related to the microglia, which are widely distributed in the CNS and are involved in the nutrition, support, antigen presentation and tissue repair in the CNS (8–11). Microglia are also the main medium of CNS immune response and play a crucial role in the pathological and physiological processes of the CNS (8–11).

At present, there is still no effective targeted method for treatment of heat stroke, especially for brain injury. Studies have confirmed that mesenchymal stem cells (MSCs) have a strong immune regulatory capacity, and can secret or induce the production of cytokines that have a significant protective effect on various brain injuries (12–14). However, the protective effect of MSCs on brain injury caused by radiation diseases has been seldom studied.

Therefore, this study is aimed to explore the significance of MSCs in the treatment of heat stroke in rats, especially the effect on heat-stroke-caused brain damage, to analyze the possible mechanism, and to underlie the clinical application and research of stem cells.

Methods

Experimental animals

Ninety male adult Sprague-Dawley (SD) rats (8 weeks, 210 ± 12 g) and 10 four-week-old SD male mice (80–100 g) were provided by the Laboratory Animal Center of People's Liberation Army (PLA) General Hospital (laboratory animal license No. SCXK (Beijing) 2016-0002). All animal experiments were reviewed and approved by the Animal Experiment Ethics Committee of PLA General Hospital and conducted in accordance with National Institutes of Health (NIH) Guidelines for Care and Use of Experimental Animals (4th edition, 2008). The animals were kept at room temperature (22–25 °C) and allowed to eat and drink freely. After the experiments, the rats were sacrificed by cervical dislocation.

Isolation, culture and identification of MSCs

According to previous reports, MSCs were isolated from the inguinal fats of juvenile rats (15). Specifically, after the rats were killed by cervical dislocation, groin fat tissues were sterily separated, cut into 1-mm-thick pieces and placed in digestive juices containing 0.05% pancreatic enzymes and 0.1% type I collagen enzyme. Then after shock digestion at 37 °C for 30 min, the filtrates were collected after filtration and

centrifuged. Then the precipitates were collected, washed with PBS 2 times, and suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 80 u/ml penicillin, and 0.2 mg/ml streptomycin, followed by cultivation in a 5% CO_2 incubator at 37 °C. The identification of MSCs was based on previous reports. The phenotypes of the cultured MSCs were identified by flow cytometry, and the adiponectic and chondrogenic differentiation abilities of the stem cells were identified by oil red O staining and alizarin red staining respectively (15). The freshly-cultured 4th generation MSCs were used in subsequent experiments.

Establishment of heat stroke rat model

The rat model was constructed with reference to previous reports (16, 17). The rats were placed constantly at 40 °C and 80% humidity. The anal temperature of each rat was continuously monitored every 10 minutes. After removal, the rats were observed and identified as paralysis. After modeling, the rats were randomly divided into a heat stroke (HS) group and an MSCs treatment group (HS + MSCs group), with 30 rats in each group. Rats in the HS + MSCs group were each given via tail vein with 0.3 ml of normal saline solution containing 2×10^6 MSCs, and rats in the HS group were only given the same volume of normal saline. Rats in the two groups were further divided into subgroups of day 1, 3, 7, 14 and 28. Ten normal rats were kept at room temperature without any treatment.

Rat survival analysis

Survival analysis was conducted in early stage (day 3) and later stage (day 28). Kaplan-Meier method was used for survival analysis of rats in each group.

Neurological deficit scoring in rats

According to a modified scoring system reported before, each rat in each group at each time point was scored for neurological impairment, including motor, sensory and balance tests (18, 19). The score was set as 0–10, and the higher score meant the more serious neurological impairment. Exclusion criteria were: (a) death within a given time; (b) score of 0 at any given time.

Hematoxylin-eosin (HE) staining of brain tissues

Hematoxylin-eosin (HE) staining, Nissl staining, and silver staining were used. Hippocampus and cerebellum tissue specimens were prepared by perfusion fixation on days 1, 3, 7, 14 and 28 following MSCs or saline infusion, and the samples were eluted three times using PBS. Hippocampus and cerebellum tissues were dissected and immersed in 4% paraformaldehyde (Wuhan Servicebio Technology Co., Ltd) for 12 h initially and then immersed in 30% sucrose solution for 24 h at room temperature. The tissues were implanted in a cooled embedding medium (optimal cutting temperature solution; Sakura Finetek USA, Inc.). Following immediate freezing, tissues were cut using a frozen section machine (Leica Microsystems) into 7 μm thick slices for dyeing. The sections were stained with hematoxylin and eosin. Stained sections were visualized and scanned using a Pannoramic MIDI CaseViewer 2.0 System (3DHISTECH Ltd.).

Nissl staining of brain tissues

The freezing process was performed as described previously, and frozen sections of 35 mm thickness were rinsed in distilled water for 2 minutes. The sections were then sectioned and stained with Nissl staining solution (Beyotime Institute of Biotechnology, China) for 10 minutes at 37 °C, replaced with fresh distilled water, and washed for 2 minutes. Next, they were soaked in 95% ethanol for 5 s and then washed twice with 70% alcohol every 5 seconds. Stained sections were visualized and scanned using a Pannoramic MIDI CaseViewer 2.0 System (3DHISTECH Ltd.).

Bielschowsky Silver staining of cerebellum tissues

Cerebellar tissue sections were stained using the modified Bielschowsky method(20). Briefly, coronal brain sections (40 μm) were cut with a slicer, and rehydrated sections were covered with 20% $AgNO_3$ in the dark 20 minutes, then rinsed with distilled water and incubated in silver ammonia solution in the dark for 10 minutes. After incubation, the slides were immersed in ammonia to develop the slides. Finally, the sections were rinsed, dehydrated, washed and covered. Counting and photographing positive silver-stained neurons under a Pannoramic MIDI CaseViewer 2.0 System (3DHISTECH Ltd.).

Immunofluorescence detection of microglia in brain tissues of different sizes

Frozen sections (5 μm thick) of the hippocampus and cerebellum of the brain were prepared. After fixation with cold acetone, the sections were rinsed with PBS for 3 times (each 5 min), drilled with PBS containing 0.5% TritonX-100 for 15 min, and washed with PBS for 3 times (5 min each). Then the sections were closed by serum with the same source of secondary antibody (normal sheep serum) at 37 °C for 30 min, and washed with PBS three times (5 min each time). The slides in different species were added at the same time with the source of a target protein (IBA, 1:100), incubated at 4 °C overnight, and restored for 20 min at room temperature. After washing with PBS three times (each time 5 min), FITC-labeled anti-rabbit IgG (1:100) was added for incubation at 37 °C and in the dark for 1 h. After washing with PBS three times (each time 5 min), the immunofluorescence film sealing pieces after dyed DAPI nucleus were directly photographed under a fluorescence microscope.

Detection of cytokines and chemokines in brain tissues

The levels of inflammatory cytokines (interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α) and chemokines (growth-related oncogene (GRO)-α, monocyte chemotactic protein (MCP)-1, and Rantes) in brain tissues of all sizes were detected using a multi-cytokine detection platform by referring to the technical methods provided by the kit.

Statistical method

SPSS software (version 23.0; IBM Corp.) was used to the analyze data. All experiments were conducted independently in triplicate. Data is expressed as mean ± standard deviation. A Kolmogorov Smirnov test was used to assess whether the data was normally distributed, and a Levene test was used to analyze the homogeneity of variance. ANOVA and Tukey’s test was used for continuous variables subject following data tests of normality and equivariance. Otherwise, nonparametric statistical analysis (Man

Whitney-U test) was conducted for neurological deficit scoring of brain tissue. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effect of MSCs on survival rate of heat stroke rats

The rats were placed constantly at 40 °C and 80% humidity. After the core temperature (anal temperature) reached 42 °C, the rats were taken out. After modeling, rats in the HS + MSCs group were each given 0.5 ml of normal saline solution containing 1×10^6 MSCs, while in the HS group, only the same volume of normal saline was given. The mortality rate of heat stroke rats was higher after the modeling, and the death rate of rats was mainly distributed in the early stage (within day 3). The mortality of rats in the HS + MSCs group was significantly lower than that in the HS group both in the early stage (Fig. 1A) and later stage (Fig. 1B). The rats were observed in a paralysis state and especially, the hind limbs were completely relaxed and basically had no response to pain or other stimuli (Fig. 1C). Compared with the HS group, the neurological deficit score of rats in the HS + MSCs group was significantly reduced on day 1, day 3, day 7 and day 14 (Fig. 1D). It was suggested that MSCs infusion can significantly reduce the death rate and neurological deficit of heat stroke rats and can significantly improve the survival rate and of heat stroke rats at early stage.

Figure 1

MSCs improve pathological damage of hippocampal neurons in heat stroke rats

Then we conducted HE staining and Nissl staining in the vulnerable hippocampus of rats, and intuitively analyzed the protective effect of MSCs on central nerve injury through pathology. HE staining showed that neurons and vertebral cells in the vulnerable hippocampus (CA1 area) were found with edema, vacuoles, nuclear condensation and loose cell arrangement after day 1 of the thermal radiation injury (Fig. 2A). Similar pathological damage remained in this area after day 28, and no significant changes were observed among different time points. Compared with the HS group, the nerve cells in the vulnerable hippocampus of the HS + MSCs group were less damaged at day 1, and were significantly better at the same subsequent time point. The neurons in the vulnerable hippocampal area were further observed by Nissl staining (Fig. 2B). Similar to the changes of pathological damage, neurons in the hippocampal area of the HS group were sparsely arranged from day 1, and the Nissl bodies were significantly reduced in count or even disappeared. The neurons of rats in the HS + MSCs group showed mild damage at day 1, and were significantly better at the same subsequent time point than in the HS group. These results suggest that the infusion of MSCs plays a protective role on neurons in the vulnerable hippocampal area of heat stroke rats, and the protective effect occurs in the early stage of injury.

Figure 2

MSCs improve pathological damage of cerebellar nerve cells in heat stroke rats

The protective effect of MSCs on cerebellum injury was further analyzed. HE staining showed that cerebellar neurons and vertebral cells presented edema, vacuoles, nuclear condensation and loose cell arrangement after day 1 of radiation injury; the damage was aggravated at day 14, and similar pathological damage was maintained in this area at day 28, but was alleviated (Fig. 3A). Compared with the HS group, the neuron damages in the cerebrum of the HS + MSCs group at day 1 and day 3 were both less, and were aggravated at day 7, and significantly reduced at both day 14 and 28. Similarly, Nissl staining (Fig. 3B) and silver staining (Fig. 3C) also showed that significant neuronal injury occurred in the cerebellum of the rats one day after the thermal radiation injury. The cerebellum had fewer Purkinje cells and nerve fibers, lighter staining and sparse arrangement. The damages were the most severe at day 3 and 7, and were still severe at day 14 and 28, despite recovery somewhat. The damages of cerebellar neurons (Purkinje cells) and nerve fibers in the HS + MSCs group were mild at 1 d, and were aggravated at day 3 and 7, but still lighter than in the HS group, and the damages were significantly reduced at day 14 and 28 and were significantly better than in the HS group. These results indicate that MSCs infusion can protect cerebellar neurons (Purkinje cells) in the early stage of injury.

Figure 3

MSC therapy inhibits excessive activation of hippocampal microglia in heat stroke rats

Microglia play an important role in CNS injury. Considering that the CNS damage induced by heat stroke may involve the immune inflammatory response of the brain, we observed the microglia activation in the vulnerable hippocampus at different time points. Microglial cell activation occurred in the hippocampal vulnerable area of heat stroke rats at day 1, decreased at day 3, increased at day 7 and 14, and declined at day 28, all significantly (Fig. 4). The activation of glial cells fluctuated throughout the disease course, and overactivation happened on day 1, 3 and 7. Although microglia cells in the vulnerable hippocampal area in the HS + MSCs group were activated at day 1, the activation level was low, and a similar stable state was maintained at all subsequent time points. It is suggested that the overactivation of microglia cells is involved in hippocampal injury of heat stroke rats. MSCs therapy can inhibit the microglia overactivation at the above time points and stabilize the activation state in the whole process, which may be an important mechanism for MSCs to protect the central nerve injury of heat stroke rats.

Figure 4

MSCs therapy can promote microglia activation in cerebellum at early stage

We further examined the effect of MSCs on microglia activation in the cerebellum of thermally infected rats. Microglia activation was obvious in the cerebellar area of heat stroke rats at day 1, but not at day 3 or 7, and increased at day 14, and transited to a resting state without obvious activation at day 28

(Fig. 5). The cerebellar microglia cells of the HS + MSCs rats were under a stronger activation state at day 1 compared with the HS group, and remained inactive at all subsequent time points, with no significant difference from the HS group. It is suggested that microglia may promote microglia activation in the cerebellar region of HS rats at early stage (day 1) and there is no obvious regulatory effect on microglia at the subsequent time points. This phenomenon is completely different from that in the hippocampus and needs further study.

Figure 5

MSCs therapy inhibits hippocampal levels of proinflammatory factors and chemokines in heat stroke rats

To investigate the effect of MSCs on the activation and inflammatory response of brain microglia in the HS rats, we further examined the levels of pro-inflammatory factors (IL-1, IL-6, TNF-α) in the brain of rats. Compared with the normal control group, with the extended time, the above factors in the HS group had the potential volatility change, were significantly increased at day 1, day 3 were significantly lower than the levels at day 1, but were still significantly higher than that of the normal control group. Moreover, the levels at day 3 all showed up, then reduced, dropped to the lowest level at day 28, but were still significantly higher than in normal control group (Fig. 6A, B, C). The levels of the above factors in the brain tissues of the HS + MSCs group compared with the normal control group were also significantly higher at day 1, and the IL-1 level was still significantly higher at day 3, although the IL-6 and TNF-α levels decreased. The Il-1 level increased significantly at day 7 and then decreased compared with the normal control group. No significant difference in the IL-1 level was found from the normal control group at day 14 or 28 (Fig. 6A). The IL-6 level increased at day 7 and 14, with significant difference between the normal control group and the control group, and then decreased, without significant difference between the two groups at day 28 (Fig. 6B). However, the TNF-α level remained relatively stable after day 3, which was not significantly different from the normal control group (Fig. 6C). Although the brain levels of the above proinflammatory factors also fluctuated in the HS + MSCs group, the overall levels were low, and the levels at each time point were significantly lower compared with the HS group. Furthermore, the brain tissue levels of chemokines (GRO-α, MCP-1 and Rantes) also remarkably changed. Compared with the normal control group, the three chemokines in the HS group at day 1 significantly increased, and subsequently fluctuated and were still significantly higher, but an overall significant downtrend existed compared with the levels at day 1. In the HS + MSCs group, the chemokine levels in rat brain tissues also rose first and then declined, and the levels were still significantly higher than in the normal control group at most of the time points (MCP-1 level in day 7 and Rantes level in day 28 were not significantly different compared with the normal control group). Nevertheless, the levels at 1–7 day were significantly lower than in the model group rats at the same time points, and no significant difference between the HS + MSCs group and the HS group was found in the GRO-α or MCP-1 level at day 14 or 28. At day 28, the Rantes level in the HS + MSCs group was significantly lower than in the HS group at the same time point (Fig. 6D, E, F). Although the levels of these chemokines in the brain tissues of the HS + MSCs group also

increased to certain extent compared with the normal control group, they were still lower than those in the HS group on the whole.

Figure 6

Effects of MSCs therapy on cerebellar levels of proinflammatory factors and chemokines in heat stroke rats

We further examined and analyzed the levels of related inflammatory cytokines and chemokines in cerebellar tissues. Although the levels of IL-1, IL-6 and TNF-α in cerebellar tissues of HS rats all similarly fluctuated, the mean levels of IL-1 from dau 1 to day 14 were significantly higher than in the normal control group, and no significant difference was found between the two groups at day 28 (Fig. 7A, B and C). The mean levels of IL-6 and TNF-α at 1–7 day were significantly higher than the normal control group, but decreased and were not significantly different at day 14 or 28 (Fig. 7B, C). The change trends of the above inflammatory factors in the cerebellar tissues of the HS + MSCs group were basically consistent with those of the HS group, except that the IL-6 level was significantly lower than that of the HS group at day 1 (Fig. 7B), and no significant differences were found at all other time points from the HS group. Moreover, the levels of GRO-α, MCP-1 and Rantes in cerebellar tissues were more complex. The GRO-α level increased significantly at day 1, but then significantly dropped at day 3 and 7, and increased significantly at day 28, and the levels at each time point were significantly higher than those in the normal control group. Compared with the normal control group, the GRO-α level in the HS + MSCs group increased significantly at day 1 and decreased significantly at day 3, without significant difference, and then increased significantly, and was significantly higher at each time point. The GRO-α level in cerebellar tissues of the HS + MSCs group was significantly lower than in the HS group at day 3, and significantly rose at day 7 and 14, with no significant difference at each time point (Fig. 7d). The MCP-1 level was significantly higher at day 1, significantly lower at day 3, but significantly higher compared with the normal control group at day 3, and then increased. The MCP-1 level compared with the normal control group was also significantly higher at day 7, but not significantly different at day 14 or 28. The level change trends were consistent between the HS + MSCs group and the HS group, but the levels of the HS + MSCs group were significantly higher on day 1, 3 and 7 compared with the normal control group, while no significant difference was found between the HS + MSCs group and the normal control group on day 14 or 28. The MCP-1 level in the HS + MSCs group was significantly lower than in the HS group at day 1 and 7 at the same time point, and no significant difference existed between the two groups at each time point (Fig. 7E). The variation trend of Rantes level was similar to that of MCP-1 level. Compared with the control group, the Rantes level increased on day 1, dropped on day 3, and rose on day 7 than on day 3, all significantly. However, the average level at each time was significantly higher than that of the control group. The Rantes level in the HS + MSCs group also changed in the same trend, and the levels on day 1, 3 and 7 were significantly higher than that of the normal control group, while the level on day 3 was significantly lower than that of the model group, and there was no significant difference among the other groups (Fig. 7F). It is suggested that MSCs may not have a significant regulatory effect on the pro-inflammatory factors in the cerebellum of heat stroke rats, but may have a certain regulatory effect on

chemokines in the early stage, which may also be a potential mechanism for MSCs to protect heat stroke rats from cerebellar injury.

Figure 7

Discussion

Clinically, heat stroke is characterized by CNS dysfunction, multi-organ failure and sharp elevation of body temperature (usually > 40.5 °C) (1–4). At present, there is no uniform and objective diagnostic criterion for diagnosis of heat stroke. Moreover, there is no unified standard for the establishment of heat emission disease animal models. In most experimental studies, the occurrence of animal heat emission diseases is defined as core temperature > 40 °C and low mean arterial pressure (< 50 mmHg) (17, 21). Therefore, based on previous studies, we adopted the experimental condition of exposing rats to 40 °C and 80% humidity until the anal temperature reached 42 °C, as the standard condition for construction of the heart stroke rat model. In the modeling, the anal temperature of the rats increased rapidly at first, which was characterized by restlessness, irritability and more salivation, then the temperature rose slowly and increased again in the later stage. Meanwhile, the activity of the rats decreased significantly. In this experiment, the heat stroke model was established when the rats were awake, and therefore, it failed to monitor the change of mean arterial pressure in rats. Reportedly, after late thermal exposure of critical point (40 °C), the changes in temperature and mean arterial pressure were very similar (22). Hence, the heat stroke rat model was established successfully, which laid a foundation for subsequent heat stroke-induced damage experiments, especially for further research on the effects of the CNS damage.

Heat stroke is one of the most dangerous stages in the course of thermal injury. As the core body temperature continues to rise, the cytotoxic effect and inflammatory response are intensified, leading to a vicious circle as well as extensive tissue and organ damages and eventually resulting in multiorgan failure. If no effective treatment is taken in the early stage of heat stroke, it will raise the mortality (3, 4). At present, there is still no effective and targeted method for treatment of heat stroke. Our study confirmed that the mortality rate was higher after the modeling of heat stroke rats, and was mainly distributed in the early stage (day 3). MSCs therapy, whether in the early stage (day 3) or later stage (day 28), can significantly reduce the mortality rate of heat stroke rats. As reported, the earlier stem cell treatment given led to a lower mortality rate of heat stroke rats (23). MSCs have a good therapeutic effect on various disease models, and the underlying mechanism mainly includes: the secretion of various growth factors and cytokines to regulate the overactivated inflammatory response (24, 25); to promote damaged tissues to regenerate and secret microRNA and mitochondrial microvesicles with regulatory functions (26). The pathogenesis of heat stroke is similar to that of systemic sepsis, a systemic inflammatory response syndrome. Factors such as acute inflammatory response, increased vascular permeability, abnormal coagulation mechanism, and the outbreak of oxygen free radicals all directly affect the survival rate and severity of heat stroke rats (3, 4). MSCs from a variety of sources play an important role in anti-inflammation, immune regulation and organ function improvement, thereby reducing the mortality rate in sepsis animal models (27–29). These studies all suggest that exogenous

MSCs therapy can significantly reduce the mortality of heat stroke rats and play a significant role in the early stage.

CNS injury is a common complication of heat stroke. Our previous analysis of clinical data found that almost all patients will suffer central damage to different degrees, which is also the main cause of death or serious sequelae (5). The mechanism of central nerve injury in thermal radiation disease is relatively complex and may be related to two factors. Firstly, the high temperature in the occurrence of thermal radiation diseases directly damages the CNS, causing a series of pathological reactions in brain cells and leading to cerebral edema and metabolic disorders, and further development results in degeneration and necrosis of brain cells. Secondly, cerebral ischemia and hypoxia are the main pathophysiological basis of brain injury during heat stroke. Heat stress caused by high temperature can lead to decreased cerebral blood flow and rapid death of central nerve cells under the stimulation of high temperature and hypoxia (7). In our study, HE staining, neuron Nissl staining, and cerebellar silver staining showed that after the heat stroke injury, the hippocampal vulnerable area (CA1 area), cerebellum, neurons, cones, cerebellar Purkinje cells were found with edema, cavitation, nucleus pycnosis and cell loose arrangement, significant reduction in Nissl bodies, and other damages. Especially, the damages were severe at early stage, but at late stage (day 28), although the above areas still suffered damages, the damages were significantly restored. MSCs therapy significantly reduced the damage of nerve cells in the vulnerable areas of hippocampus and cerebellum in heat stroke rats compared with the HS group at the same time point. It is suggested that MSCs infusion can protect nerve cells (neurons and Purkinje cells) in vulnerable hippocampal and cerebellar areas of heat stroke rats, and the protective effect can happen at the early stage of injury. Extensive studies confirm that MSCs have significant protective effects various CNS damages. MSCs have significant protective effects on cerebral ischemic injuries, external injury caused by brain damage, Alzheimer's disease, dementia, Parkinson's disease and other neurodegenerative diseases. The specific mechanisms include: MSCs can secrete various neurotrophic factors, regulate immune response, improve cerebral vascular permeability, and adjust nerve cell communication (12–14). Therefore, the treatment of MSCs has an important protective effect on the common central system damage of heat stroke, and is of great significance for the survival rate and prognosis of heat stroke rats.

In addition to high fever, ischemia, hypoxia and other factors, inflammatory response also plays an important role in the brain injury caused by heat stroke. A clinical case report shows that in thermal radiation patients, abnormal high signals in the bilateral cortex and sub hippocampal white matter can be observed by craniocerebral magnetic resonance, indicating obvious inflammatory response in brain tissues (30). As reported, the increase of inflammatory cytokines IL-1 and IL-6 in brain tissues after heat stroke is significantly correlated with the nervous system damage, vascular edema and neuron death, while the cortex and hippocampus are the most common damage sites (31). However, the main mechanisms about the occurrence, development and outcome of CNS inflammation during heat stroke are still not fully understood, which leads to the insufficient understanding about the importance of CNS inflammation in clinical treatment of heat stroke. The occurrence and development of inflammation in CNS are closely related to microglia. Microglia cells are widely distributed in the CNS and play roles of nutrition, support, antigen presentation and tissue repair in CNS. They are also the main mediators of

CNS immune response and play a crucial role in the pathological and physiological processes of the CNS (8–11). We found that microglial cell activation in the hippocampal vulnerable areas of heat stroke rats was significant in early stage (day 1) and middle stage (day 7 and 14), and significantly decreased in late stage (day 28). Although the microglia cells in the vulnerable hippocampal area of MSCs-treated rats were activated in early stage (day 1), the activation level was low, and the cells maintained a similar stable state at all subsequent time points. It is suggested that the overactivation of microglia cells is involved in the hippocampal injury of heat stroke rats. MSCs therapy can inhibit the overactivation of microglia at the above time points and stabilize the activation state in the whole process. However, the activation of microglia in the cerebellar region of the rats was not obvious during the whole observation period. However, the microglia cells in the cerebellar region of MSCs- treated rats showed a slightly stronger activation state in early period (day 1), and the activation state was not obvious in the subsequent time points. However, microglia cells in the cerebellum did not obviously respond to heat damage, and MSCs may promote the activation of cerebellar microglia cells in heat stroke rats only in early stage (day 1), which is a completely different phenomenon from the hippocampus and needs further study. The mechanism of central inflammatory response involves the activation of microglia, infiltration of inflammatory cells, and release of cytokines and inflammatory chemokines (32). On the one hand, heat stroke can cause central neuron apoptosis or necrosis, such as secondary injury, release of various chemical factors by damaged nerve cells. Moreover, the dead cell fragments can activate the brain neural immune cells-microglia, which then are activated to produce numerous inflammatory factors, resulting in severe inflammation of CNS. On the other hand, a variety of inflammatory factors in the inflammatory response can directly stimulate vascular endothelial cells, and destroy the interconnections between endothelial cells, causing edema in perivascular tissues and aggravating brain damage (33). Studies have confirmed that MSCs have an important regulatory effect on microglia cells after CNS injury, and the main mechanism is that MSCs can regulate the inflammatory response mediated by microglia cells and reduce the release of pro-inflammatory factors (34–36). Our study found that the levels of inflammatory cytokines (IL-1β, IL-6 and TNF-α) and chemokines (GRO-α, MCP-1 and Rantes) in the hippocampal brain tissues of thermophilic rats fluctuated. On the whole, MSCs therapy can significantly inhibit the increase of these cytokines. However, MSCs had no overall significant regulatory effect on the levels of IL-1β, IL-6 and TNF-α in cerebellar tissues of thermally injected rats, but can inhibit the levels of GRO-α, MCP-1 and Rantes at early stage. It is suggested that CNS inflammation plays an important role in the pathogenesis of heat stroke, and the regulation of inflammation may become an important target of MSCs in the treatment of heat stroke.

Conclusion

In summary, the changes of CNS inflammatory factors were analyzed in combination with brain tissue pathology and microglial cell activation. The change trends of the three were basically the same, suggesting that CNS inflammation was closely related to brain tissue pathological damage and microglial cell activation after heat stroke, and the three may be related pathological processes. MSCs can inhibit the overactivation of microglia in the hippocampus of heat stroke rats, stabilize the activation

of microglia, and inhibit the overexpression of cytokines, which may be an important mechanism for MSCs to protect the central nerve injury of heat stroke rats.

Abbreviations

MSCs: mesenchymal stem cells; HS:heat stroke; IL-1β:interleukin-1beta; IL-6:interleukin-6; IL-10:interleukin-10; TNF-α:tumor necrosis factor alpha; GRO-α:growth-related oncogene alpha; MCP-1:monocyte chemotactic protein-1; CNS:central nervous system.

Declarations

Ethics approval and consent to participate

The current research complied with the statement of relevant ethical standards (the Animal Research: Reporting of In Vivo Experiments reporting guidelines) and was approved by the Ethics Committee of the Chinese PLA General Hospital (approval no. 2017-X13-10).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or 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, and all authors should confirm its accuracy.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81671966), Natural Science Foundation of Beijing Municipality (No. 7182155) and Application Research and Achievement Extension of Clinical Characteristics in Chinese Capital Foundation (No. Z171100001017160).

Authors' contributions

HK and ZD: study design; JH: develop the study; MY: assist some of the experiments; YL: isolate and culture of mouse MSCs; RY: analyze the histopathologic change; YZ: assist the establishment of HS rats;

HK and FZ: supervised research and manuscript writing; LW and YZ: study design, perform the research, analyze the data, and write the paper.

Acknowledgements

Not applicable.

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Figures

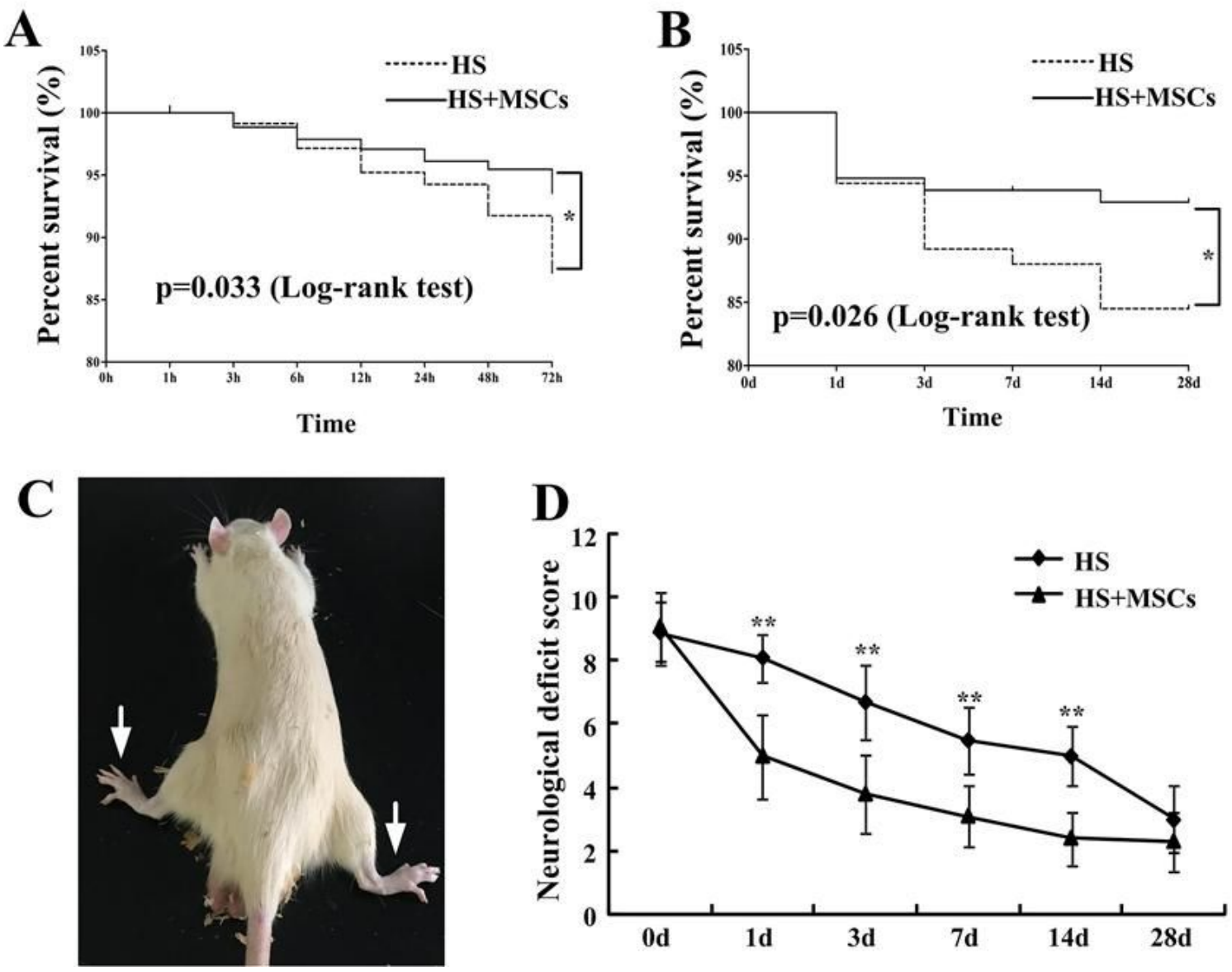

Figure 1

MSCs infusion can improve the survival rate of HS rats. When the rectal temperature reached 42 ° C, the rats were placed at room temperature, and 5 ml of saline was intraperitoneally injected. The survival rate in the early (3 days) (A) and long-lasting stages (28 days) (B) was assessed. The HS rats were observed and validated to have acute hindlimb paralysis (C). After administration of MSCs, the neurological deficit scoring of HS rats was evaluated and recorded (D). n=40 in each group. * P<0.05 vs. HS group, ** P<0.01 vs. HS group.

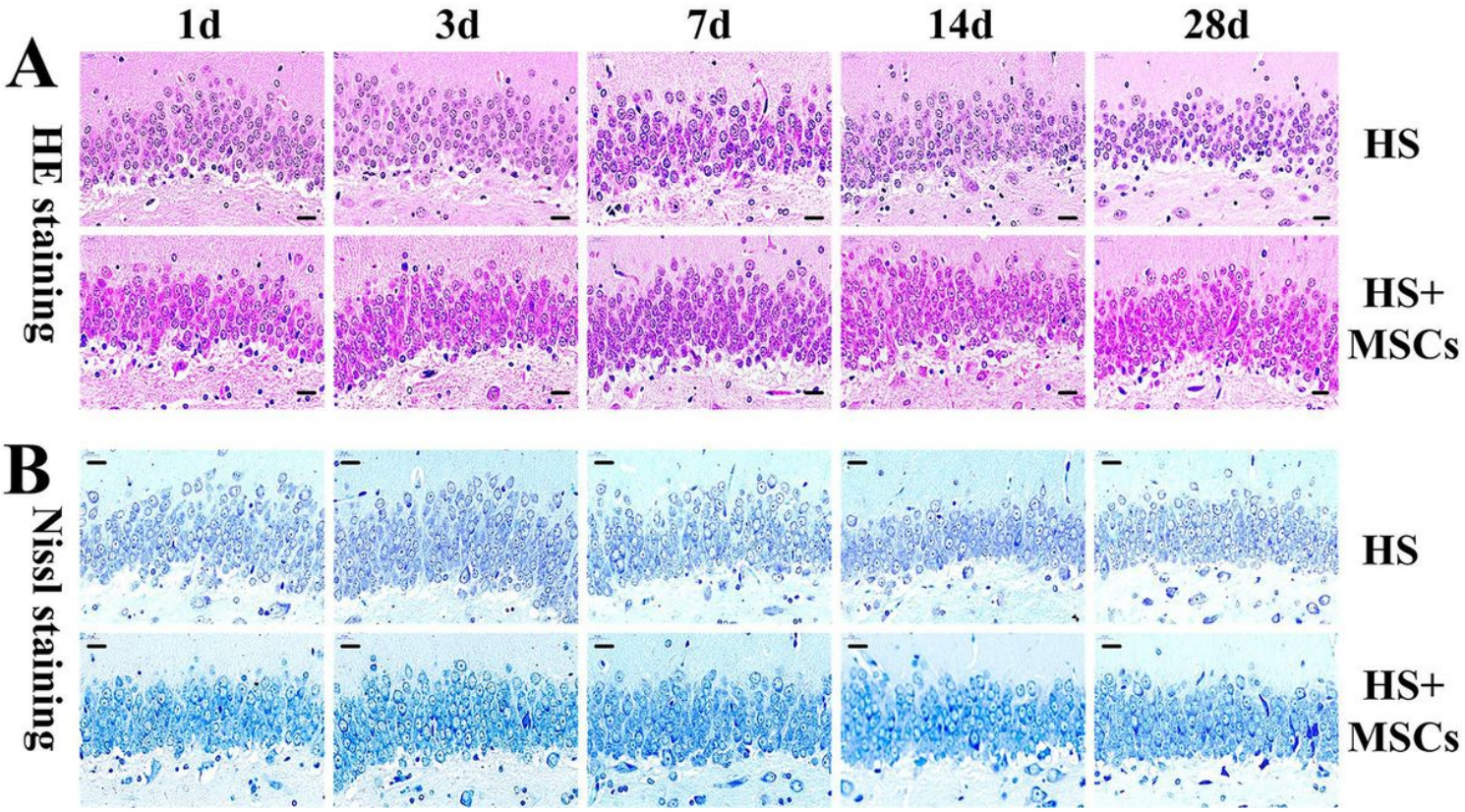

Figure 2

MSCs improve pathological damage of hippocampal neurons in HS rats. At different time points after MSCs or saline infusion, the hippocampal histopathological properties were observed by HE staining (A) and Nissl staining (B), respectively. The representative images were shown, Scale bar, 20 μm.

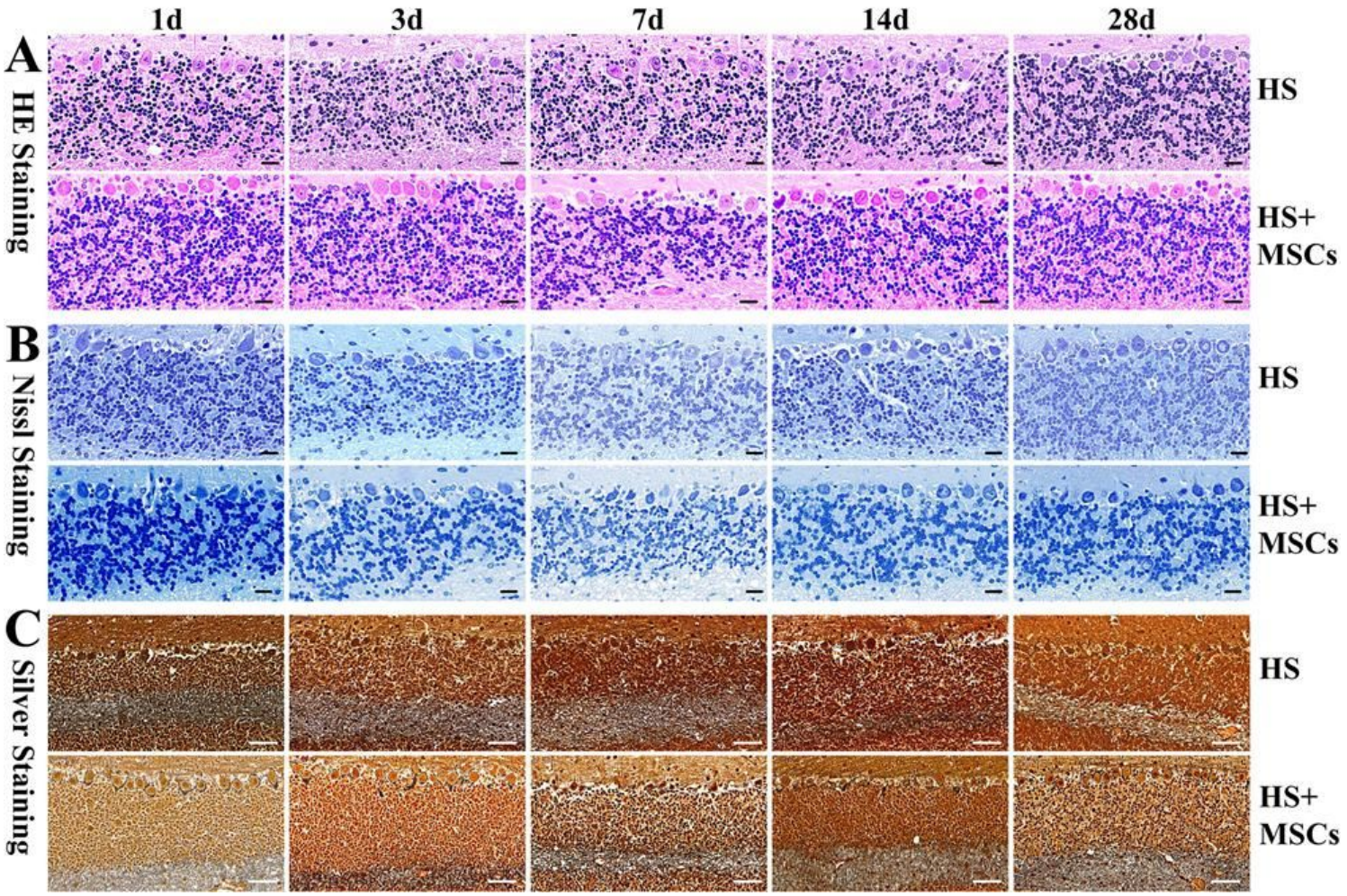

Figure 3

MSCs improve pathological damage of cerebellar nerve cells in HS rats. At different time points after MSCs or saline infusion, the cerebellar nerve cells histopathological properties were observed by HE staining (A, Scale bar, 20μm), Nissl staining (B, Scale bar, 20μm) and silver staining (C, Scale bar, 50 μm), respectively. The representative images were shown.

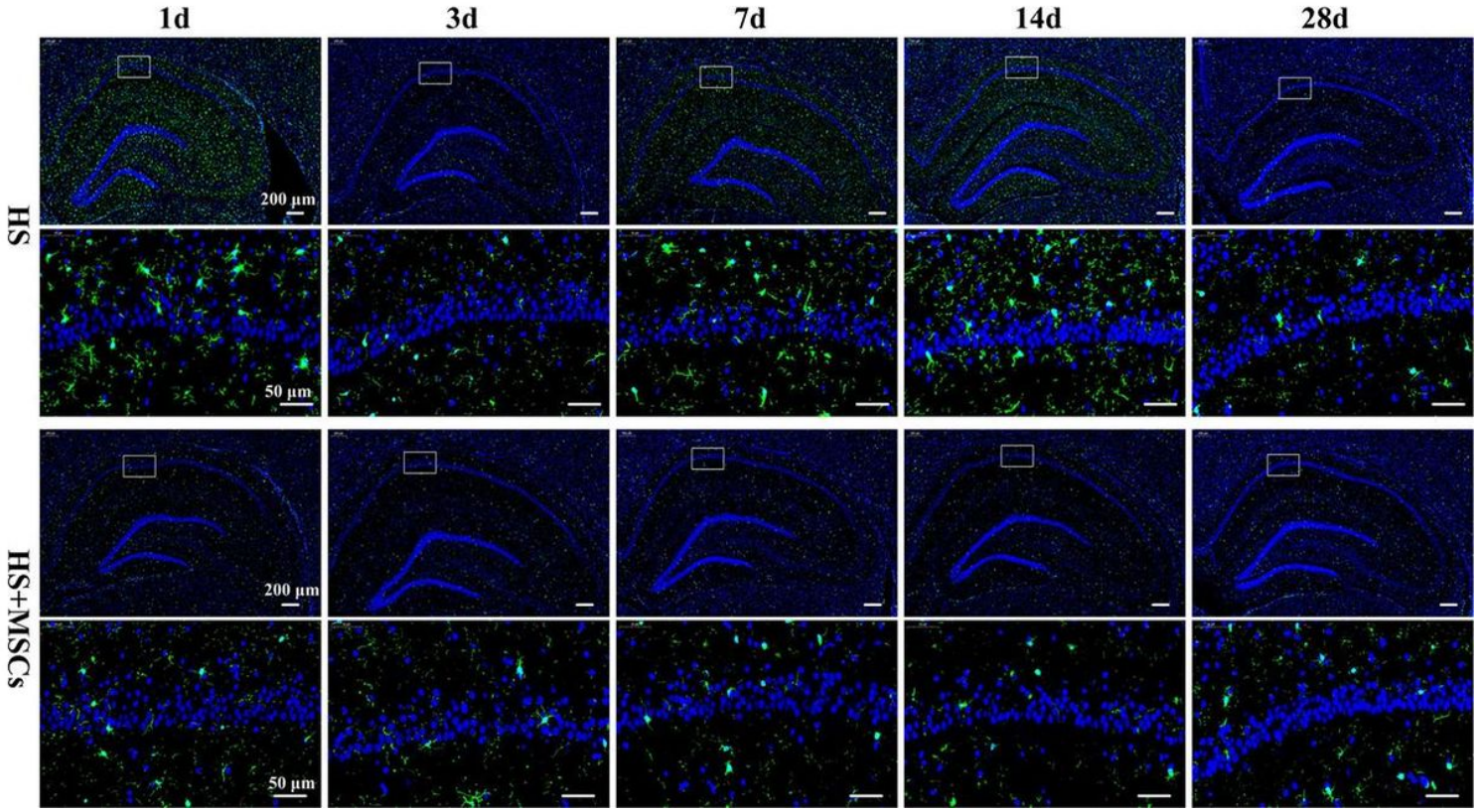

Figure 4

MSCs inhibit excessive activation of hippocampal microglia in HS rats. At different time points after MSCs or saline infusion, the microglia in hippocampus of rats were observed by immunofluorescence staining. The representative images were shown, Scale bar, 50 or 200 μm.

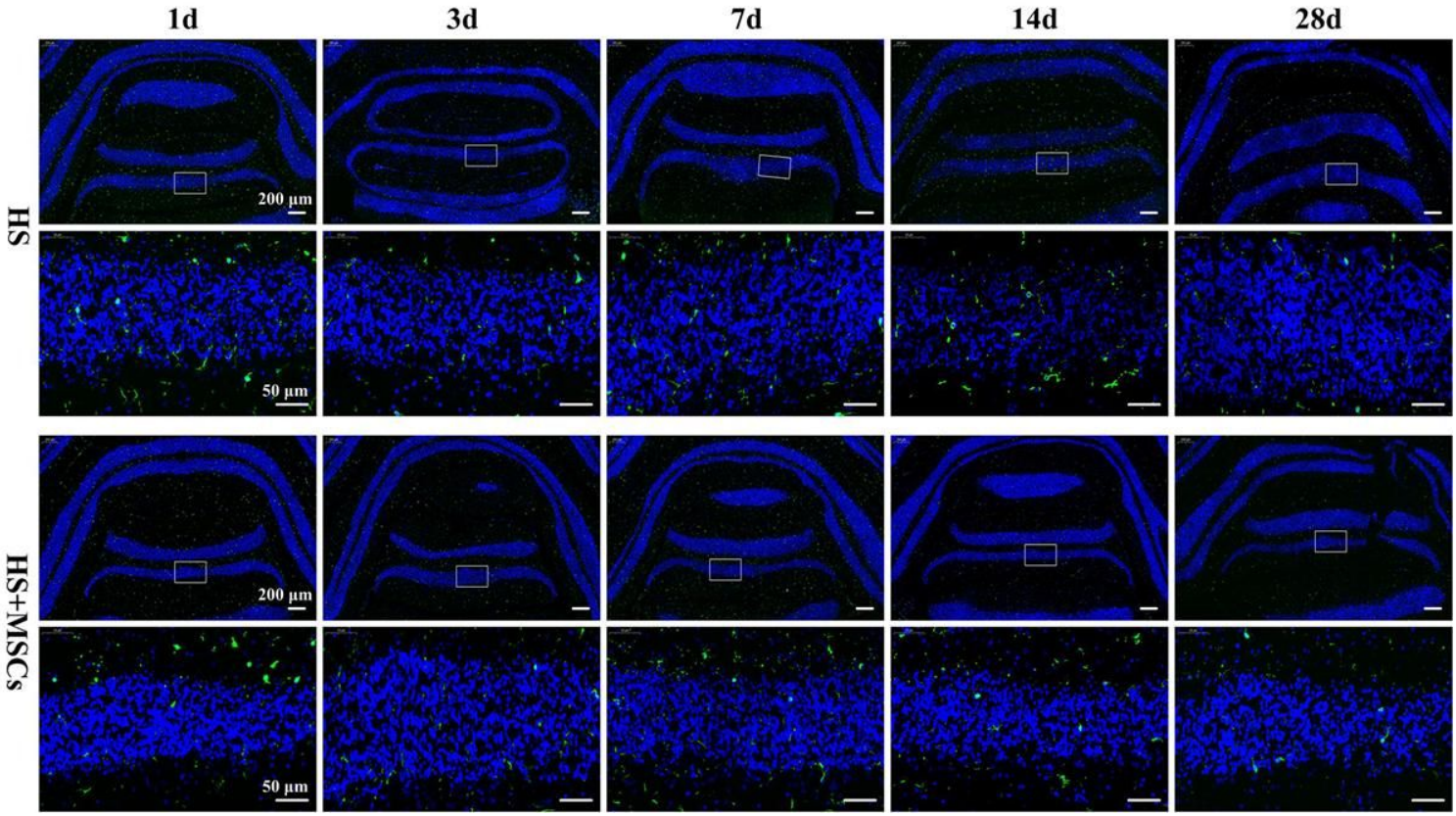

Figure 5

MSCs can promote microglia activation in cerebellum in HS rats at early stage. At different time points after MSCs or saline infusion, the microglia in cerebellum of rats were observed by immunofluorescence staining. The representative images were shown, Scale bar, 50 or 200 μm.

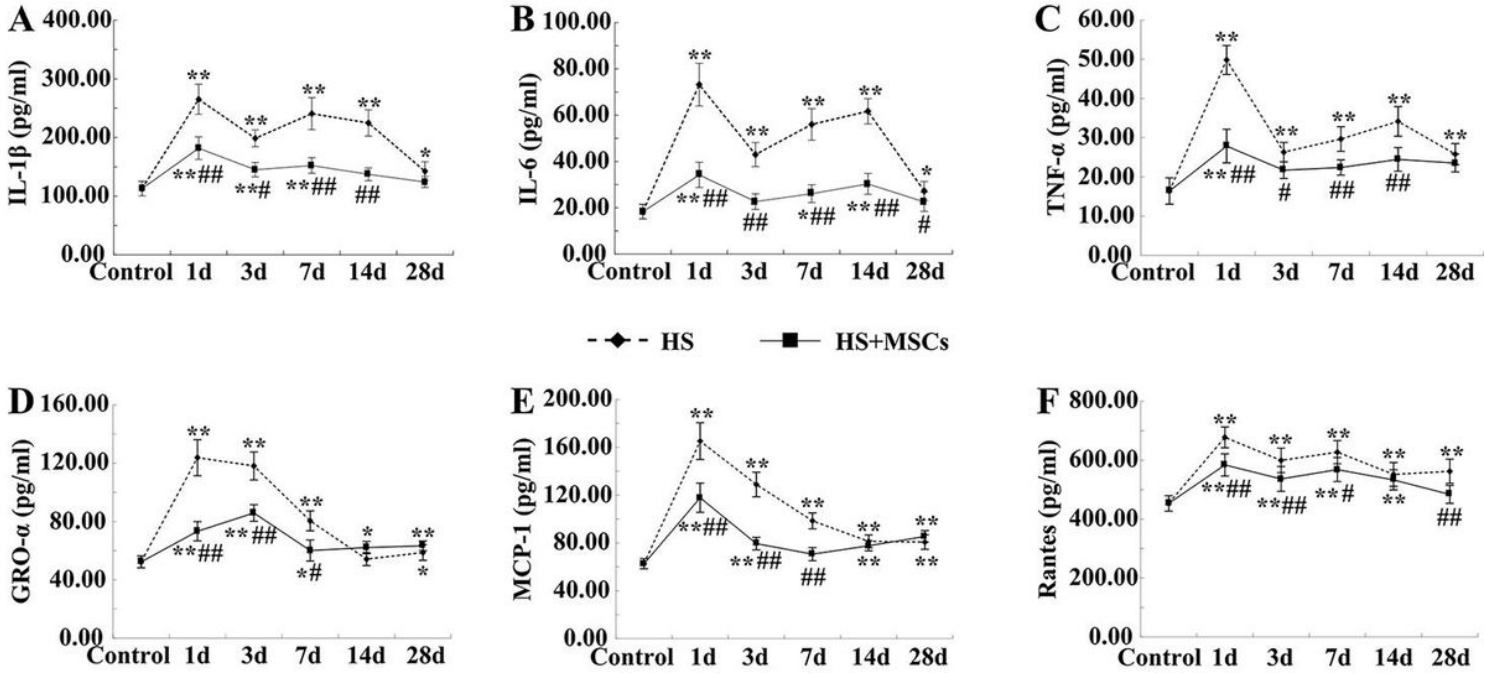

Figure 6

Changes in levels of proinflammatory factors and chemokines in hippocampal brain tissues of rats. At different time points after MSCs or saline infusion, the hippocampal brain tissue of rats in each group were collected and homogenized in 10 volumes of ice-cold PBS. The IL-1β (A), IL-6 (B), TNF-α (C), GRO-α (D), MCP-1 (E) and Rantes (F) levels in the rat's hippocampal brain tissue lysates were assayed. n=10 rats per group. * P<0.05 vs. normal control group, ** P<0.01 vs. normal control group, # P<0.05 vs. HS group, ## P<0.01 vs. HS group.

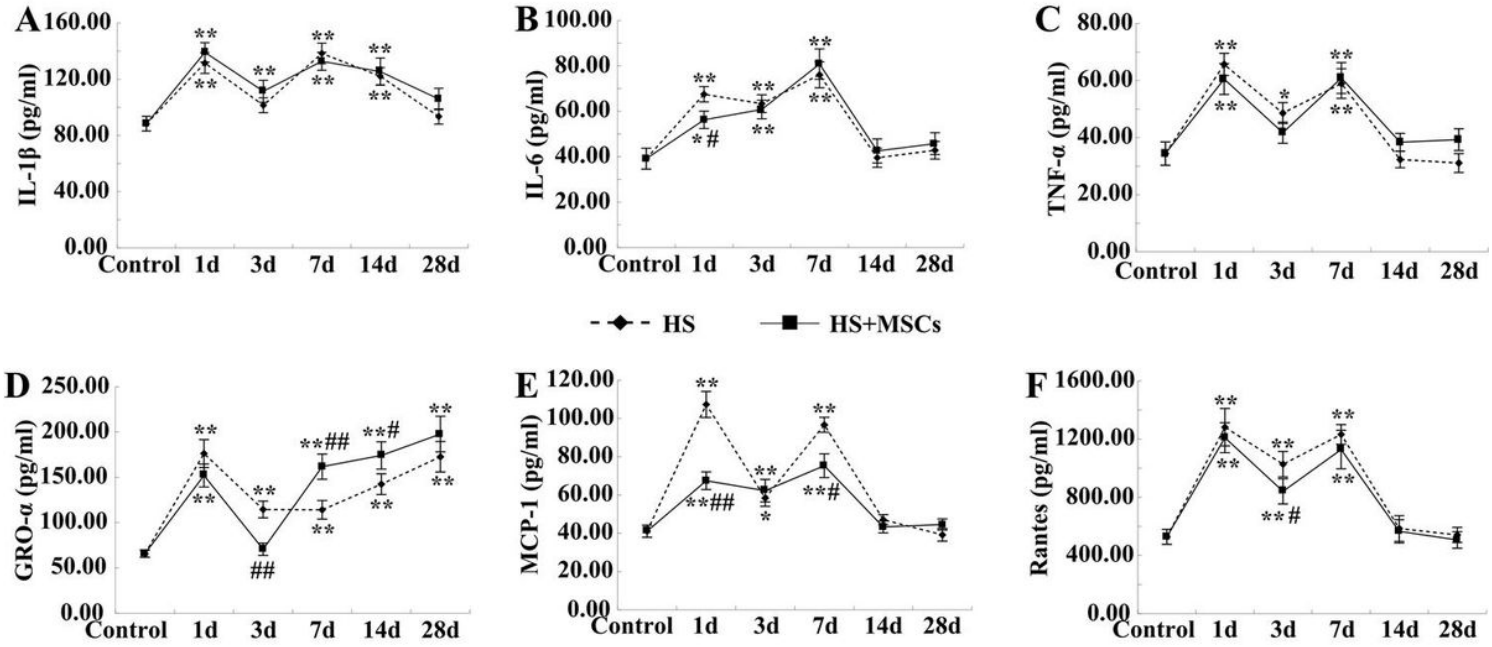

Figure 7

Changes in levels of proinflammatory factors and chemokines in cerebellar tissues of rats. At different time points after MSCs or saline infusion, the hippocampal brain tissue of rats in each group were collected and homogenized in 10 volumes of ice-cold PBS. The IL-1β (A), IL-6 (B), TNF-α (C), GRO-α (D), MCP-1 (E) and Rantes (F) levels in the rat's cerebellar tissue lysates were assayed. n=10 rats per group. * P<0.05 vs. normal control group, ** P<0.01 vs. normal control group, # P<0.05 vs. HS group, ## P<0.01 vs. HS group.