Administration of Umbilical Cord Blood Cells Transiently Decreased Hypoxic-Ischemic Brain Injury in Neonatal Rats


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Key Words
Neuroprotection · Oxidative stress · Cell therapy · Asphyxia

Abstract
This study aimed to investigate whether the administration of mononuclear cells derived from human umbilical cord blood cells (UCBCs) could ameliorate hypoxic-ischemic brain injury in a neonatal rat model. The left carotid arteries of 7-day-old rats were ligated, and the rats were then exposed to 8% oxygen for 60 min. Mononuclear cells derived from UCBCs using the Ficoll-Hypaque technique were injected intraperitoneally 6 h after the insult (1.0 × 10^7 cells). Twenty-four hours after the insult, the number of cells positive for the oxidative stress markers 4-hydroxy-2-nonenal and nitrotyrosine, in the dentate gyrus of the hippocampus in the UCBC-treated group, decreased by 36 and 42%, respectively, compared with those in the control group. In addition, the number of cells positive for the apoptosis markers active caspase-3 and apoptosis-inducing factor decreased by 53 and 58%, respectively. The number of activated microglia (ED1-positive cells) was 51% lower in the UCBC group compared with the control group. In a gait analysis performed 2 weeks after the insult, there were no significant differences among the sham-operated, control and UCBC groups. An active avoidance test using a shuttle box the following week also revealed no significant differences among the groups. Neither the volumes of the hippocampi, corpus callosum and cortices nor the numbers of neurons in the hippocampus were different between the UCBC and control groups. In summary, a single intraperitoneal injection of UCBC-derived mononuclear cells 6 h after an ischemic insult was associated with a transient reduction in numbers of apoptosis and oxidative stress marker-positive cells, but it did not induce long-term morphological or functional protection. Repeated administration or a combination treatment may be required to achieve sustained protection.

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Introduction

Perinatal hypoxia-ischemia (HI) remains an important cause of neonatal death and permanent neurological deficits [1]. Notwithstanding the developments made in perinatal medicine, perinatal HI occurs in 1.3–1.7/1,000 live births; its incidence is high even in developed countries [2]. Many survivors of perinatal HI experience long-term neurological disabilities and impairments resulting in major socioeconomic burdens. At present, there are no effective treatments for HI-induced brain damage, except for brain hypothermia [3], which is not effective in severe cases [4, 5]. Therefore, it is of the utmost importance to develop a novel and effective therapy against perinatal HI-induced brain injury.

Stem cell therapy is expected to be used in the treatment of many central nervous system diseases in the future. Various kinds of stem cells are possible sources of cell therapy for future clinical applications [6, 7]. We recently demonstrated that intracerebroventricular injection of neural stem/progenitor cells together with chondroitinase ABC – which digests glycosaminoglycan chains on chondroitin sulfate proteoglycans – significantly decreased the degree of cerebral infarction after perinatal HI injury in a rat model [8]. However, ethical concerns hinder the use of postmortem human brains as a source of neural stem/progenitor cells in future clinical applications. Furthermore, intracerebral administration is an invasive procedure, and the injected cells themselves may lead to gliotic changes in the host brain [9], thereby necessitating more detailed examinations to ensure the safety of the procedure.

Umbilical cord blood cells (UCBCs) are a promising source of stem cell therapy. They are readily available and can be used for autologous transplantations. Thus, many ethical considerations can be avoided. Furthermore, UCBCs can be administered intravenously [10] and cross the blood-brain barrier [11]. Meier et al. [12] first reported the treatment effects of UCBCs in the amelioration of HI-induced brain damage in a neonatal rat model; moreover, several recent studies reported favorable effects of UCBCs [13–18]. However, the mechanisms underlying the favorable effects remain to be fully elucidated. In the present study, we administered UCBCs to HI rats to investigate their effects and the underlying mechanisms.

Materials and Methods

Animals

All animal experimental protocols in the present study were approved by the Institutional Review Board of Nagoya University School of Medicine (Nagoya, Aichi Prefecture, Japan; permit No.: 23181-2011 and 24337-2012). Wistar/ST rat pups were obtained from Japan SLC Inc. (Shizuoka, Japan) and maintained under a 12-hour light/dark cycle (8.00 a.m. to 8.00 p.m.) with ad libitum access to food and water. The animal room and experimental space were always maintained at 23°C.

UCBC Preparation

Human UCBCs were donated by women who delivered at Nagoya University Hospital. Written, informed consent was obtained from the donors and their spouses, and this experimental protocol using human cells was reviewed and approved by the local ethics committee of our hospital (permit No.: 794). The donors underwent normal delivery or elective cesarean section because of previous cesarean section, breech position or cephalopelvic disproportion. The donors and infants had no major perinatal complications; all were singleton pregnancies of more than 36 weeks of gestational age.

Umbilical cord blood was collected immediately after placental delivery in bags containing citrate phosphate dextrose (CPC-20; Nipro Corporation, Osaka, Japan). Mononuclear cells were isolated using the Ficoll-Hypaque technique, suspended in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies, Carlsbad, Calif., USA) at a concentration of 1 × 10⁷ cells/ml, and cryopreserved in liquid nitrogen with an equal amount of a cryoprotectant (CP-1; Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan). CP-1 is a mixture of dimethylsulfoxide and hydroxethyl starch, which makes it possible to preserve stem cells in a frozen state. Immediately before administration, the cells were thawed to 37°C, washed 3 times with phosphate-buffered saline (PBS) and resuspended in 0.3 ml of RPMI 1640 medium.

HI Insult and UCBC Administration

HI brain damage was produced using postnatal day 7 (P7) rats according to the method of Rice et al. [19] with slight modifications. Each pup was anesthetized using isoflurane inhalation and the left carotid artery was subsequently doubly ligated and incised between the ligatures. After a 1-hour rest with a dam, the pups were exposed to a hypoxic environment of 8% O₂ at 37°C for 60 min, after which they were returned to the dam in the animal room maintained at 23°C. Six hours later, the pups in the treatment group (UCBC group) were injected intraperitoneally with mononuclear cells derived from UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml). A control group underwent ligation of the left carotid artery and hypoxia in the same manner, but received UCBCs (1 × 10⁷ cells/0.3 ml).

Histological and Immunohistochemical Procedures

Histological and immunohistochemical procedures were performed as previously described [20] with minor modifications. Briefly, rats were deeply anesthetized and intracardially perfusion-fixed with 0.9% NaCl followed by 4% paraformaldehyde in PBS. The brains were removed and immersion-fixed in the same solution at 4°C for 24 h, dehydrated with a graded series of ethanol and xylene, embedded in paraffin and cut into 5-μm-thick coronal sections. After deparaffinization and rehydration, antigen retrieval was performed by heating the sections for 10 min in 10 mM citrate buffer (pH 6.0). Nonspecific binding was blocked with 3% donkey serum in PBS. Then, sections were incubated overnight at 4°C with rabbit anti-active caspase-3 (product No. 559565; dilution 1:200; BD Pharmingen, Franklin Lakes, N.J.), 559565; dilution 1:200; BD Pharmingen, Franklin Lakes, N.J., followed by 0.01% 559565; dilution 1:200; BD Pharmingen, Franklin Lakes, N.J., followed by 0.01% 559565; dilution 1:200; BD Pharmingen, Franklin Lakes, N.J., followed by 0.01% 559565; dilution 1:200; BD Pharmingen, Franklin Lakes, N.J., followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%, followed by 0.01%.
USA), goat anti-apoptosis-inducing factor (AIF; product No. SC-9416; dilution 1:100; Santa Cruz Biotechnology, Dallas, Tex., USA), rabbit anti-4-hydroxy-2-nonenal (4-HNE; product No. HNE11-S; dilution 1:400; Alpha Diagnostic International, San Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit anti-4-hydroxy-2-nonenal (4-HNE; product No. HNE11-S; dilution 1:400; Alpha Diagnostic International, San Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit anti-4-hydroxy-2-nonenal (4-HNE; product No. HNE11-S; dilution 1:400; Alpha Diagnostic International, San Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA), rabbit antinitrotyrosine (product No. A-21285; dilution 1:200; Life Technologies; control samples are Antonio, Tex., USA).
stress markers (4-HNE and nitrotyrosine) and an activated microglia marker (ED1). Photomicrographs of representative hippocampi are shown in figure 1. The number of the apoptosis marker-positive cells in the ipsilateral GCL significantly decreased in the UCBC group compared with those in the control group (activated caspase-3, 53%, and AIF, 58%; fig. 2a, b; p < 0.05 and p < 0.01, respectively). The numbers of oxidative stress marker-positive cells also decreased in the UCBC group compared with the control group (4-HNE, 36%, and nitrotyrosine, 42%; fig. 2c, d; p < 0.05). Moreover, the number of ED1-positive cells was 51% lower in the UCBC group compared with the control group (fig. 2e; p < 0.05).
Impact of UCBCs on Behavior after HI

Gait Analysis
To evaluate the effect of HI on motor function and of UCBC administration on HI-induced motor deficits, gait analysis was performed 14 days after the insult (P21) using the CatWalk system. There were no significant differences in run duration, right front paw (RF) print area, RF swing speed, RF/left front paw (LF) ratio of mean intensity, RF duty cycle or RF stride length among the three groups (sham-operated, control and UCBC groups; fig. 3).

Active Avoidance Test
Further, to evaluate the effects of UCBCs on HI-induced learning impairments, an active avoidance test was performed 21–24 days after the insult (P28–31). The mean avoidance proportion of each group was calculated for each consecutive day (fig. 4). The avoidance rates increased with time in all groups; however, there were no significant differences among the three groups for each day.

Impact of UCBCs on Histological Changes after HI
Finally, to assess the absolute tissue loss after HI, the volumes of the cortex, corpus callosum and hippocampus of both hemispheres were evaluated. After the behavioral tests, sections throughout the whole cerebrum were evaluated.

Representative photomicrographs stained for NeuN are shown in figure 5a and b. In both groups, there was apparent unilateral atrophy with partial collapse. We evaluated the volumes of the cortex (fig. 5c), corpus callosum (fig. 5d) or hippocampus (fig. 5e), but found no significant differences in the volumes between the groups.
Therefore, we examined whether there was a difference in the number of neurons between the groups, even though the volume reductions were equivalent. The numbers of NeuN-positive neurons in the hippocampus were counted using stereological principles (Stereo Investigator, MicroBrightField) but they were not significantly different between the groups in the hippocampus of either the ipsilateral or contralateral hemisphere (fig. 5f).

**Discussion**

In the present study, we demonstrated that intraperitoneal UCBC administration caused antiapoptotic and antioxidative effects 24 h after the insults; however, we failed to demonstrate a prolonged reduction of neurological damage. We administered UCBCs in the early phase (6 h after HI). The optimal timing of administration is one of the most critical points to establish a new cell therapy. In regenerative medicine using stem cells, grafting in the early postinjury phase is generally not recommended. In neural stem/progenitor cell transplantation, early grafting in the acute phase (i.e. 24 h after insult), during which inflammatory chemical mediator and
cytokine concentrations are increased [24], can induce greater differentiation of grafted cells to astrocytes, decrease the survival rates and/or decrease the beneficial treatment effects in various models such as spinal cord injury [25] and stroke [26] in adult rodents, and irradiation-induced brain injury in young mice [9]. In addition, early administration of mononuclear cells from bone marrow showed fewer treatment effects in a model of adult stroke [27]. However, in neonatal HI, there are two phases of pathological events: primary and secondary energy failure [28, 29]. Primary energy failure occurs within minutes after initial cerebral ischemia; in this phase, acidosis and depletion of oxygen, glucose and adenosine triphosphate lead to acute derangement of intracellular metabolism, resulting in necrosis and cell death. The subsequent secondary energy failure occurs after a variable period following the initial insult. Inflammation, excitatory amino acid release, intracellular calcium inflow, and production of nitric oxide and reactive oxidative species occur in this stage. Therefore, it is reasonable that most therapies, including hypothermia therapy, should be commenced at least before the second phase. The treatment effect induced by UCBC administration for brain injury is considered to be involved in secretion of neurotrophic factors that promote endogenous neurogenesis, prevent loss of neuronal cells and regulate immunity [28], which was shown also in the effect of bone marrow-derived mesenchymal stem cells [30]. Expression levels of various pro-inflammatory cytokines, including interleukin-1 and tumor necrosis factor, are elevated in the early phase of perinatal HI; UCBC administration can decrease these levels, which are also accompanied by decreased expression of cluster of differentiation 68, a biomarker of activated microglia/macrophages in the brain.

Fig. 5. Impact of the UCBCs on the histological change after HI. The volumes of whole cortex, corpus callosum and hippocampus, and the number of NeuN-positive neurons in whole hippocampus were evaluated using Stereo Investigator version 10 (stereology software) after behavioral tests. a, b Representative photomicrographs of the brain stained for NeuN from vehicle (a) and UCBC-treated rats (b) 2 months after HI. Bar = 1,000 μm. There was no significant difference in the volumes of the cortex (c), corpus callosum (d) or hippocampus (e) between the groups. The numbers of NeuN-positive neurons in the GCL were not significantly different between the vehicle and UCBC groups in the hippocampus of ipsilateral hemispheres (f). Contra. = Contralateral; Ipsi. = ipsilateral.
Oxidative stress plays an important role in HI brain damage [32]. Here, we demonstrated a decrease in 4-HNE- and nitrotyrosine-positive cells in the dentate gyrus following UCBC administration (fig. 1c, d, 2c, d). To our knowledge, this study is the first to report antioxidative effects of UCBCs in a neonatal HI rat model. Arieri-Zakay et al. [33] reported antioxidative effects of UCBC-derived neural progenitor cells on insulted PC12 cell lines. Furthermore, suppression of oxidative stress after adult transient focal ischemia was observed in an interleukin-1 knockout murine model [34]. As described above, UCBC administration can decrease the elevated expression of proinflammatory cytokines including interleukin-1 [31]. Moreover, the decreased expression of ED1 in the present study (fig. 1e, 2e) indicates that UCBC administration decreased HI-induced inflammation. Thus, the antioxidative effect of UCBC administration might be exerted directly and/or via suppression of inflammation.

Calculations of the immunohistochemically stained cells were focused on the dentate gyrus of the hippocampus, which is one of the most vulnerable areas to hypoxic ischemic insult. Although we calculated the immunohistochemically stained cells with density counts, which is less sensitive than stereological counts, the results revealed that UCBC administration suppressed apoptosis, as indicated by the decrease in the number of cells positive for active caspase-3 and AIF (fig. 1a, b, 2a, b). In the present study, we performed high-resolution analyses of walking patterns using the CatWalk system; however, they were not sensitive enough to detect motor impairment after HI injury. In contrast to human neonates, rat pups after HI injury did not show obvious locomotor abnormalities, as in other studies using the same model [35]; this may have been because of the higher degree of plasticity of the immature rat brain [36]. We also evaluated the learning memory after HI with the shuttle avoidance test and found only a mild tendency to improve the learning memory in the UCBC group; the difference was not significant. This may be a type 2 error, and further studies are required to clarify the potential effects of UCBC therapy on motor impairment and cognitive deficits after HI. In addition, we found no differences between the vehicle and UCBC groups in absolute tissue loss or the number of neurons in the cortex, corpus callosum or hippocampus (fig. 5). Similarly, some former studies failed to show histological improvement following UCBC therapy [12, 16], whereas others did [14, 15]. We have summarized the experimental protocols and results [37]. In many previous reports, $1 \times 10^7$ mononuclear cells were administered intraperitoneally 24 h after the insult. We administered the same dose of cells at an earlier time point. Pimentel-Coelho et al. [13] administered UCBCs even earlier (3 h after the insult) using a lower dose ($2 \times 10^6$) of cells, and showed improvement in morphology and behavior. It is still unclear how the differences between protocols can affect the results. Other possible reasons for the different outcomes can be the severity of the insult and other experimental settings. Considering the fact that the present study failed to show any effect on morphological changes in the chronic phase or improvement of behavioral impairments, despite the fact that several acute injury markers were suppressed, a modified protocol (e.g. repeated administration, combination with some other treatments) should be tried with an aim to achieve sustained neuroprotection.

In the present study, cryopreserved mononuclear cells were used. Even frozen-thawed UCBCs are known to produce various cytokines and chemokines [38], and exert a neuroprotective effect in various animal models [13, 37, 39]. Moreover, from the viewpoint of clinical applications, cryopreservation is essential in the case of allogeneic transplantation, which may be applicable for patients without access to their own cord blood cells.

We used human UCBCs in a rodent injury model. It might have been more suitable to use rat UCBCs. However, it was very difficult to get sufficient numbers of cells from the umbilical cord of rats without expansion in culture. Because the purpose of the present study was to evaluate the treatment effect of mononuclear cells from the umbilical cord without using culture procedures, we used human cells, as in previous publications [13–18].

Another possible limitation in the present study is that we could not monitor/control body temperature in each pup. We placed the pups on/into the temperature-controlled plate/chamber during the HI insult, and returned them to the dam in a temperature-controlled room after the insult. There might be some variation in brain temperature, leading to variation in the degree of brain damage [40].
The choice of injection site is an important issue when using cell infusion for the treatment of brain injury. We administered UCBCs intraperitoneally, as in most previous studies. According to our recent unpublished results, only a small number of cells either injected intraperitoneally or intravenously could be found in the brain, and cells injected intraperitoneally were less seen in the liver, lung or spleen than cells injected intravenously, indicating that many intraperitoneally injected cells might have stayed in the peritoneal cavity. The treatment effect might be through trophic factors secreted by the cells [41]. The extent of brain damage can be influenced by the peripheral inflammatory response [42]. Modulating peripheral inflammation can be a therapeutic target. In a traumatic brain injury model, multipotent adult progenitor cells exerted a neuroprotective effect through interaction with resident splenocytes [43].

In summary, these results indicate that a single intraperitoneal injection of UCBC-derived mononuclear cells 6 h after HI reduced caspase-3, AIF, microglial activations and oxidative stress, but it did not induce morphological or functional protection. Repeated administration or a combination treatment may be required to achieve sustained protection.

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Disclosure Statement

None of the authors have any conflicts of interest associated with this study.

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