Mesenchymal Stem Cell-Derived Exosomes Provide Neuroprotection and Improve Long-Term Neurologic Outcomes in a Swine Model of Traumatic Brain Injury and Hemorrhagic Shock

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Abstract

Combined traumatic brain injury (TBI) and hemorrhagic shock (HS) remains a leading cause of preventable death worldwide. Mesenchymal stem cell-derived exosomes have demonstrated promise in small animal models of neurologic injury. To investigate the effects of exosome treatment in a clinically realistic large animal model, Yorkshire swine underwent TBI and HS. Animals were maintained in shock for 2 h before resuscitation with normal saline (NS). Animals were then resuscitated either with NS (3 × volume of shed blood) or with the same volume of NS with delayed exosome administration (1×10^{13} particles/4 mL) ($n=5$ /cohort). Exosomes were administered 9 h post-injury, and on post-injury days (PID) 1, 5, 9, and 13. Neurologic severity scores (NSS) were assessed for 30 days, and neurocognitive functions were objectively measured. Exosome-treated animals had significantly lower NSS ($p<0.05$) during the first five days of recovery. Exosome-treated animals also had a significantly shorter time to complete neurologic recovery (NSS=0) compared with animals given NS alone (days to recovery: NS = 16.8 ± 10.6 ; NS + exosomes = 5.6 ± 2.8 ; $p=0.03$). Animals treated with exosomes initiated neurocognitive testing earlier (days to initiation: NS = 9.6 ± 0.5 vs. NS + exosomes = 4.2 ± 0.8 ; $p=0.008$); however, no difference was seen in time to mastery of tasks. In conclusion, treatment with exosomes attenuates the severity of neurologic injury and allows for faster neurologic recovery in a clinically realistic large animal model of TBI and HS.

Keywords: exosomes; hemorrhagic shock; mesenchymal stem cells; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) remains a leading cause of death and disability worldwide.^{1,2} In the United States alone, approximately 1.7 million people are affected by TBI each year, and TBI ultimately contributes to approximately 30% of all injury-related deaths.³ In traumatic settings, TBI is often accompanied by other traumatic insults, including vascular injury and hemorrhage. Hypoxia and hypotension secondary to hemorrhagic shock (HS) drastically affect progression of TBI.^{4,5} Each episode of hypotension can double TBI-associated morbidity and death.^{4,6}

Current treatment of these combined injuries has focused on hemorrhage control and maintenance of cerebral perfusion through crystalloid and blood product resuscitation. Advances have been made in research exploring new strategies for treatment of TBI and

HS in recent years, including utilization of valproic acid (VPA).^{7,8} Combined TBI and HS, however, remains a serious concern and further work to develop new treatment modalities is warranted.

Within recent years, mesenchymal stem cell (MSC)-derived exosomes have emerged as a new potential treatment for those with TBI.⁹ Exosomes are small, membrane-bound nanovesicles that participate in intercellular communication and contain molecular cargo including cellular proteins, lipids, microribonucleic acid (miRNA), and messenger RNA (mRNA). The therapeutic effects of exosomes have been attributed widely to their surface markers, molecular contents, low immunogenicity, ability to cross the blood–brain barrier (BBB), and roles in mediating neural regeneration.^{10–15}

In small animal models of TBI, we and others have shown that MSC-derived exosomes promote functional neurologic recovery and

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can improve rescue pattern separation and decrease spatial learning impairments after TBI.^{16–18} Potential mechanisms for this therapeutic benefit are suspected to be secondary to promotion of neurogenesis and angiogenesis, and suppression of neuro-inflammation.⁹ The full effects of exosomes in this setting require further elucidation, however.

Although MSC-derived exosomes have been confirmed to be a potentially promising therapy for TBI, neurologic functional outcomes and mechanistic studies have only been conducted in small animal studies.^{16,18} Thus, exosome administration in large animal models of TBI, which are more clinically relevant and better reflect human disease, are lacking at this time. If found to show benefit in large animal models, this would support translation of exosome treatment into clinical trials. In this study, we sought to investigate the effects of exosome administration on long-term neurologic outcomes in a large animal model of TBI and HS. We hypothesized that exosome administration would attenuate neurologic impairment after injury and allow for faster neurologic recovery.

Methods

This study protocol was approved by the University of Michigan Institutional Animal Care and Use Committee. Experiments were conducted in compliance with all guidelines and regulations regarding animal welfare and research. Strict aseptic technique was employed for all surgical procedures. Figure 1 provides a schematic representation of the model used in this experiment.

Exosome preparation, harvesting, and isolation

MSCs were isolated from the bone marrow of a single healthy human donor (male, aged 34) and were seeded into flasks and grown into two passages. Three million cells were then seeded into a Quantum incubator (Terumo, Somerset, NJ) and were grown in media circulated through fibronectin-coated hollow fibers. Media were harvested starting on day three, followed by every other day for six days, and then every day for four days, at which time cells were confluent and harvests were ceased.

Exosomes were isolated from the cell culture media via multi-step centrifugation at 4°C, as described previously,^{16,18} with slight modification. Briefly, the cell culture media were centrifuged at $250 \times g$ for 5 min, and then the supernatants removed and centrifuged at $3000 \times g$ for 30 min, followed by filtration through a $0.22 \mu\text{m}$ filter (SLMP025SS; EMD Millipore Corporation, Billerica, MA) to remove small debris. The resulting media were further centrifuged at $100,000 \times g$ for 2 h. The final pellets, consisting mainly of exosomes, were resuspended in phosphate-buffered saline and stored at 4°C.

Exosome concentration was measured using a qNano (Izon, Cambridge, MA) and suspensions diluted to 1×10^{13} particles/4 mL bolus. This dose was chosen based on our previous studies in rat (4×10^{11} particles/kg),^{16,18} divided by the allometric scaling factor for swine.

Animal selection and acclimation

Female Yorkshire swine (35–45 kg; Michigan State University, East Lansing, MI) were used for this study. All animals underwent an acclimation period of at least five days.

Induction and maintenance of anesthesia

Animals were induced with Telazol (0.5 mg/kg intramuscular injection; Pfizer, New York, NY) and inhaled isoflurane (2–4%). After endotracheal intubation (7.0 mm tube), anesthesia was maintained with isoflurane (1–3%) for the duration of the procedure. Before instrumentation, a transdermal fentanyl patch (75 µg/h) was applied and left in place until 72 h post-operatively. Peripheral intravenous (IV) catheters (18–22 G) were placed bilaterally in ear veins. Prophylactic antibiotics (cefazolin, 1 g) were administered before incision and every 8 h until 24 h post-operatively.

Instrumentation and monitoring

Instrumentation and monitoring have been described in depth previously.^{7,19} Briefly, bilateral groin incisions were made and femoral vessels exposed using cut-down technique. Femoral arteries were dissected, isolated, and cannulated with 5F catheters. One arterial catheter was designated for invasive blood pressure monitoring, while the other was designated for blood collection and hemorrhage. Using ultrasound-guided percutaneous technique, a 9F catheter was placed in the right external jugular for pulmonary artery catheterization, and a 5F catheter was placed in the left external jugular for post-operative fluid management, blood collection, and drug administration.

A suprapubic cystostomy tube was placed into the bladder via mini-laparotomy and secured temporarily with a purse-string suture. The animal was then placed in the prone position and the head fixed in a custom-made stereotactic frame with a mouthpiece. Two skull pegs were affixed to the zygoma to prevent movement during injury. A U-shaped scalp incision was made to expose the skull. A 20-mm burr hole was made anterolateral to the coronal and sagittal sutures on the right side of the skull, while a 6 mm burr hole was made 10 mm anterolateral to the bregma on the left side. The 20 mm burr hole was used for brain injury, and the 6 mm burr hole was used for intracranial pressure (ICP) and oxygenation monitoring. Physiological parameters were measured continuously as described previously.^{7,19}

FIG. 1. Injury protocol. Injury consisted of 40% total blood volume hemorrhage with concurrent traumatic brain injury (TBI) followed by 2 h period of shock. Resuscitation was with normal saline ($3 \times$ volume of shed blood) over 1 h. Packed red blood cells (pRBCs) were administered 5 h into the critical care phase over 1 h. Exosomes were administered after pRBC infusion and at four intervals post-operatively including post-injury days (PID) 1, 5, 9, and 13. Total survival time was 30 days.

Intraoperative laboratory monitoring

Arterial blood gases (ABGs; Nova Biochemical, Waltham, MA) and whole blood samples were obtained at baseline, post-shock, post-resuscitation, and at the end of the observation phase. Additional ABGs were performed as needed throughout the experiment. Whole blood samples were withdrawn into vacuum-sealed tubes, centrifuged for separation into serum and plasma, and flash frozen for future analysis. All laboratory samples were included in the calculation of hemorrhage volume.

TBI and hemorrhage

A computer-controlled cortical impact (CCI) device developed by the University of Michigan Innovation Center was used to induce TBI with a 20 mm cylindrical impactor (4 m/sec velocity, 100 millisecond dwell time, 8 mm cortical penetration depth). This insult was designed to produce a severe, but survivable injury. Concurrently, 40% estimated total blood volume (ETBV [mL] = weight [g] $\times$ 0.06 + 0.77) was hemorrhaged over 12.5 min using a Masterflex pump (Cole-Palmer, Vernon Hills, IL). Blood was collected in standard blood collection bags (Terumo, Ann Arbor, MI; containing CPDA, AS-5) and separated by plasma centrifugation for autologous transfusion of packed red blood cells (pRBCs) in the observation phase. During the hemorrhage period, if mean arterial pressure (MAP) decreased below 30 mm Hg, hemorrhage was held, isoflurane was turned off, and a 50–100 mL bolus of normal saline (NS) was administered. Hemorrhage was restarted when MAP returned to above 30 mm Hg. The scalp incision was closed temporarily after TBI using silk suture.

Hemorrhagic shock, resuscitation, and treatment

The resuscitation schedule was designed to represent a pre-hospital environment, with a prolonged period of shock before access to definitive care (i.e., “worse case” scenario). Therefore, after completion of hemorrhage, animals were maintained in shock (MAP 30–35 mm Hg) for 2 h via titration of isoflurane. Animals were then randomized to one of two groups: NS alone (control) or NS + exosomes (treatment) ($n=5$ /cohort). At the end of the shock period, all animals were resuscitated with NS ($3 \times$ hemorrhage volume, over 1 h).

Observation and recovery

Animals were observed for 6 h after resuscitation with continuous monitoring of physiological and laboratory parameters. They were treated for any electrolyte disturbances, and physiologic temperature was maintained using a Bair Hugger (Arizant Healthcare, Inc., Eden Prairie, MN). At 5 h post-resuscitation, all the animals received transfusion of autologous pRBCs over 60 min.

Animals randomized to the treatment group received a single IV injection of exosomes (1×10^{13} particles/4 mL NS, administered over 5 min) at 6 h post-resuscitation. Control animals received the same volume (4 mL) of NS alone at the same time point. This time point was chosen to represent evacuation to a higher-level center from an austere setting. After exosome injection, invasive monitoring was discontinued, and all catheters removed except for the left central venous line, as mentioned previously. All incisions were closed in multiple layers using absorbable suture and covered with surgical adhesive (3 M Vetbond, 3 M Animal Care Products, St. Paul, MN) after infiltrating with local anesthetic (1% bupivacaine, 2 mg/kg). Animals were weaned from mechanical ventilation and extubated. Animals were then returned to housing for observation for a 30-day period.

Post-operative care

After the surgical procedure, animals were monitored for oral intake. All animals received 1 L of 5% dextrose solution in Lactated

Ringer's fluid on post-injury day (PID) 1. Oral intake of water and regular diet were encouraged immediately after the procedure. The IV fluids were continued on an as-needed basis until adequate oral intake was established. Antibiotic coverage and post-operative analgesia were given as mentioned previously.

Post-operative exosome administration and blood sampling

Exosomes were administered further on PID 1, PID 5, PID 9, and PID 13. Whole blood samples were obtained from all animals at PID 2, PID 7, and PID 11 and processed as mentioned previously.

Neurological Severity Score (NSS)

A validated scoring system for neurological function was utilized to evaluate both severity of injury and recovery trajectory.^{7,19,20} The NSS is a 32-point scale (0 = no deficit, 32 = severe injury) based on nine domains: level of consciousness, utterance, behavior, head position, standing position, gait, and motor function (hind limbs/forelimbs). Two observers who were blinded to the treatment administered independently assessed the animals twice daily until the animal returned to baseline level, or until the end of the observation period.

Neurocognitive function testing

Once the blinded behavioral observers determined that the animals were able to stand and move freely without assistance, training was initiated to test neurocognitive functions. A modified operant conditioning model, involving prioritization, integration of spatial memory, long-term memory processing and recall, and identification of color, was utilized. As described previously,^{7,19,20} animals were presented with three bins containing food, only one of which could be opened. The bins were identical, except for color (white, yellow, blue). Animals were assessed on how quickly they learned to identify the bin that could be opened (blue), while ignoring other bins. A daily performance score was generated to assess progress and to identify task mastery. Mastery was considered when animals correctly identified the bin that could be opened without giving attention to the other bins on three consecutive days.

Statistical analyses

All statistical analyses were performed using GraphPad Prism v6.00 (GraphPad Software, San Diego, CA). Hemodynamic and physiological parameters were compared at individual time points using unpaired *t* tests with Holm-Sidak correction. The Mann-Whitney test was used to detect differences in NSS between groups. Data are represented as mean $\pm$ standard deviation (SD), unless specified otherwise. Statistical significance was defined as $p < 0.05$.

Results

Survival

All animals survived until the end of the 30-day observation period. There were no adverse effects observed related to the exosome treatment during this period.

Hemodynamic and physiological parameters

Animals underwent predictable changes in physiological parameters. After injury, animals experienced tachycardia, which recovered toward baseline after resuscitation (Fig. 2A). As expected, MAP decreased after injury and improved after resuscitation (Fig. 2B). The ICP increased after resuscitation and remained elevated throughout the remainder of the experiment (Fig. 2C). As expected, cerebral

FIG. 2. Hemodynamic and physiologic response to injury. Intraoperative measurements included (A) heart rate (HR), (B) mean arterial pressure (MAP), (C) intracranial pressure (ICP), and (D), cerebral perfusion pressure (CPP). BL, baseline; S, shock period (2 h); R, resuscitation (1 h); PR, post-resuscitation period; pRBC, transfusion 5 h after resuscitation; EXO, exosome injection; E, end of injury phase (7 h after shock period completion).

perfusion pressure (CPP) decreased after injury, but recovered after resuscitation (Fig. 2D). No statistically significant differences were noted in hemodynamic and physiological parameters between the two groups.

Laboratory parameters

Overall, the severity of shock and the response to resuscitation were similar between the groups (Table 1). No significant differences were seen in pH, partial pressure of oxygen, partial pressure of CO₂, lactate, and glucose between the groups. After NS resuscitation, hemoglobin levels showed a predictable decrease in both NS and NS + exosomes animals (g/dL: NS = 5.7 ± 0.6; NS + exosomes = 5.9 ± 0.7; $p = 0.46$);

however, this resolved after pRBC transfusion at the conclusion of the 6-h observation phase (g/dL: NS = 9.9 ± 0.6; NS + exosomes = 9.7 ± 0.3; $p = 0.52$).

NSS and rate of recovery

The NSS were noted to be highest on PID 1 (NSS on PID1: NS = 24.6 ± 2.9; NS + exosomes = 9.6 ± 3.6). Animals treated with exosomes had significantly lower NSS ($p < 0.05$) during the first five days post-injury (Fig. 3A) and demonstrated significantly faster full neurological recovery (NSS = 0) (days to recovery: NS = 16.8 ± 10.6; NS + exosomes = 5.6 ± 2.8; $p = 0.03$). All animals returned to baseline neurological functions by the end of the observation period.

TABLE 1. SELECTED BLOOD GAS VALUES BEFORE AND AFTER INJURY

Parameters	Group	BL	PS	PR	6 h PR/E
pH	NS	7.46 ± 0.02	7.48 ± 0.01	7.39 ± 0.02	7.42 ± 0.02
	NS + Exo	7.45 ± 0.02	7.46 ± 0.03	7.37 ± 0.02	7.41 ± 0.02
pO ₂ , mm Hg	NS	141.5 ± 14.8	145.2 ± 17.0	141.7 ± 22.4	110.5 ± 10.3
	NS + Exo	138 ± 14.9	136 ± 13.1	132 ± 7.42	102.4 ± 16.4
PCO ₂ , mm Hg	NS	39.4 ± 2.6	34.4 ± 0.9	41.5 ± 2.0	38.8 ± 1.2
	NS + Exo	40.0 ± 2.0	36.9 ± 1.5	43.0 ± 2.0	40.5 ± 1.9
Lactate, mmol/L	NS	1.28 ± 0.2	1.96 ± 0.4	1.82 ± 0.5	1.64 ± 0.4
	NS + Exo	1.34 ± 0.4	2.60 ± 0.7	1.86 ± 0.5	1.60 ± 0.5
Glucose, mmol/L	NS	54.3 ± 8.3	80.7 ± 3.7	77.7 ± 12	90.7 ± 27.4
	NS + Exo	59.6 ± 15	86.8 ± 15	91 ± 9.5	103 ± 7
Hemoglobin, g/dL	NS	9.9 ± 0.3	9.7 ± 0.2	5.7 ± 0.6	9.9 ± 0.6
	NS + Exo	9.8 ± 0.3	10.2 ± 0.4	5.9 ± 0.7	9.7 ± 0.3

BL, baseline; PS, post-shock; PR, post-resuscitation; E, end; NS, normal saline. Data presented as group mean ± standard deviation values.

FIG. 3. Long-term Neurologic Severity Scores and neurocognitive function testing. Neurologic impairment was significantly lower in exosome-treated animals (post-injury days [PIDs] 1 through 5) (A). Exosome-treated animals also had a faster neurological recovery with return to baseline (B). Time to initiation was significantly shorter for animals treated with exosomes. No difference was noted in days to mastery of neurocognitive testing. Data are presented as group mean $\pm$ standard deviation. Asterisks indicate time points that were significantly different ($p < 0.05$). NS, normal saline; EXO, exosomes.

Neurocognitive function

Exosome-treated animals were able to initiate neurocognitive testing earlier (days to initiation: NS = 9.6 ± 0.5 ; NS + exosomes = 4.2 ± 0.8 , $p = 0.008$), however, no difference was seen in time to mastery of tasks (days to mastery: NS = 6.6 ± 0.5 ; NS + exosomes = 6 ± 0.7 , $p = 0.37$; Fig. 3B).

Discussion

This study tested the efficacy of MSC-derived exosomes in a large animal model of combined TBI and HS. We found that exosome treatment attenuates the severity of neurological injury and improves functional neurological recovery. While these findings build on previous work in small animal models, they are the first to demonstrate that post-injury administration of exosomes induces robust and sustained functional recovery in a large animal model of TBI.

TBI remains a leading cause of death and disability worldwide.^{1,2} In the United States alone, TBI affects 1.7 million people each year, resulting in more than \$76 billion in healthcare costs annually.²¹ Currently, treatment of those with TBI targets two main areas: neuroprotection and neurorestoration.⁹ Neuroprotective strategies involve the attenuation of brain injury progression, while neurorestorative strategies focus on regulation of

angiogenesis, neurogenesis, and gliosis as part of neurovascular remodeling.

Despite recent improvements in supportive and rehabilitative care for TBI, no pharmacological agents exist that are able to reduce the deaths associated with TBI and improve neurological outcomes after injury.⁹ Over the last few decades, many studies have focused on neuroprotective treatment strategies, which have culminated in more than 30 clinical trials.⁹ Unfortunately, the majority of these have failed in Phase II/III clinical testing.^{22,23} VPA, among other agents, has shown promise in the management of TBI and HS.^{7,19} Further exploration of treatment options for TBI is warranted, however.

In recent years, MSC-derived exosomes have demonstrated promise in being a new and potentially valuable therapeutic strategy for TBI. Exosomes are the smallest endocytic membrane-bound vesicle (30–100 nanometers in size) found in eukaryotic cells and play an integral role in complex intercellular communication. These vesicles contain a wide array of cellular cargo including cell-specific proteins, lipids, and coding and noncoding RNA.²⁴ Proteins found in exosomes include membrane-associated proteins, proteins involved in multi-vesicular biogenesis, heat shock proteins, and proteins required for membrane transport and fusion.^{25,26} Cytoskeletal proteins, metabolic enzymes, and signal transduction proteins, including kinases, are also found within the exosomes.²⁷ The specific contents of exosomes vary according to cellular origin and the physiological or pathological condition of the cell.

Although exosomes can be released by various cell types, those derived from MSCs have become one of the most popular stem cell-derived exosomes in neurological disease research, including stroke, neurodegenerative diseases, and TBI. MSCs are adult multipotent stem cells with the potential for self-renewal and differentiation and have been demonstrated to induce cerebrovascular plasticity post-stroke and TBI.^{28–30} The paracrine mechanisms by which MSCs induce neurorestorative effects after neural injury, however, have been attributed recently to exosomes secreted by the MSCs.¹³ Compared with other cell types, MSCs are among the most prolific producers of exosomes.^{15,16,31,32}

Although numerous MSC sources exist, the most common source includes bone marrow,^{16,33} which we utilized in this study. We chose to use human bone marrow MSCs as the source of exosomes, because we wanted to ensure this study was as translational as possible. We and others have used human MSC products in several rodent studies of TBI, and they are efficacious and do not cause interspecies immune complications.^{34–36}

The MSC-derived exosomes are promising therapeutic agents in the treatment of those with TBI. We were the first to demonstrate that MSC-derived exosomes attenuated neurological injury and improved functional recovery when given 24 h after injury in a rat model of TBI.¹⁶ Other studies have validated such findings. Kim and associates¹⁷ demonstrated further that exosomes reduce cognitive impairment in a mouse model of TBI, aiding in recovery of pattern separation and spatial learning.

We demonstrated further that exosomes derived from MSCs cultured in three dimensional (3D) scaffolds, which have been shown to stimulate higher levels of trophic factor secretion compared with monolayer cell culture, provide significantly improved outcomes in spatial learning in rat models when compared with two dimensional conditions.¹⁸ Overall, this study is a logical extension of our previous work involving MSC-derived exosomes administered in small animal studies. The present study, however, is the first to demonstrate that these results can be translated into a clinically relevant large animal model.

Importantly, the majority of small animal brain injury models that have shown a significant improvement in functional recovery after exosome therapy have focused on neurorestoration, typically treating at subacute to chronic time points after injury. As this study demonstrates, the timing of exosome administration may play a significant role in providing neuroprotection. Previous studies demonstrating the therapeutic effects of MSC-derived exosomes on TBI involved single-dose administration of exosomes at 24 h after TBI.^{16,18} In this setting, significant improvements in spatial learning and sensorimotor functional recovery after TBI were observed at 33–35 and 14–35 days, respectively.^{16,18} In the present study, exosomes were administered 9 h post-injury, as well as repetitive dosing in the 30-day period following. In contrast to previous reports, our study showed significant neurological improvement as early as PID 1.

These data show that exosomes may play a multi-faceted role in neuroprotection and neurorestoration. We speculate that differences in our large animal model, which includes combined TBI and HS, may contribute to the neuroprotective effects that have not been reported previously in small animal TBI models. It is unclear to what extent the differences are species dependent, or whether exosomes may act to mitigate the harmful effects of combined HS and TBI. Exosome therapy after combined HS and TBI has not been thoroughly investigated in rodent models. We are planning to perform additional experiments to identify the optimal dosing schedule, and to perform more mechanistic studies in the swine model.

Our previous studies and other reports demonstrate that MSC-derived exosomes provide their therapeutic effects by decreasing neuroinflammation and promoting neurovascular remodeling, including neurogenesis and angiogenesis.⁹ In facilitating decreased neuroinflammation, numerous studies have suggested that MSC-derived exosomes can increase levels of anti-inflammatory cytokines, while reducing levels of pro-inflammatory cytokines.³⁷ Further, inhibition of glial fibrillary acidic protein + astrocytes and CD68 + microglia and macrophages by suppression of Toll-like receptor signaling and inhibition of hypoxic inflammation by suppression of proliferative pathways involving STAT3 phosphorylation have also been demonstrated to be involved.^{16,18,38,39} In addition, MSC-derived exosomes have also been shown to promote neurogenesis and angiogenesis through the transfer of miRNA, which can modify recipient neural cellular phenotype and physiology and promote cellular proliferation and differentiation.

We have not yet investigated the mechanisms of action in our pig model, but we speculate that the neuroprotective effects we observed may derive from modulation of the central nervous system and peripheral immune response, because brain remodeling is a late phase recovery process that occurs days, weeks, and even months after injury, and thus is unlikely to contribute to observable changes 24–48 h after injury. Further investigations to this end are ongoing.

There are several limitations to this study. First, sample size in this study was limited by ethical considerations and costs and may be prone to a type I error. Second, although swine are commonly utilized for human translation, they serve as an imperfect surrogate for human subjects. Third, the injury model utilized, while severe, is only an approximation of actual traumatic injuries and hemorrhage. Numerous models of TBI induction exist in the literature, all of which can provide unique brain injury processes. Our model, however, is a validated and highly reproducible model of CCI to induce TBI. Fourth, the assessment of neurological injury severity and recovery in swine is an imperfect method. Although this has been a well-validated tool to evaluate neurocognitive recovery, its translation to humans is unclear.

Last, 35–45 kg swine (4–6 months of age) were used for this study. Young animals are known to recover faster, as well as to have a greater neuroplastic potential, than aged animals. Therefore, the enhanced neurological recovery we observed in this study may not reflect accurately the ability of an adult swine or human to recover after TBI. We have demonstrated, however, that treatment of aged ischemic animals with MSCs improves neurological function^{40,41}; thus, exosomes may also be effective at managing TBI in older swine and humans. Our future studies will focus on moving this proof-of-concept work to older animals.

Conclusion

This study demonstrates that mesenchymal stem cell-derived exosomes reduce the severity of neurological injury and improve neurocognitive recovery in a large animal model of TBI and HS. The findings of this study support continued evaluation of exosomes as a treatment for those with TBI. Future work is required to elucidate the mechanisms of the protective and restorative effects of exosomes and to optimize the dosage and treatment schedule.

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Author Disclosure Statement

No competing financial interests exist.

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