Stem Cells as a Potential Adjunctive Therapy in Aneurysmal Subarachnoid Hemorrhage

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Abstract

Background—Despite advances in the management of subarachnoid hemorrhage, a considerable proportion of patients are still left with severe and disabling long-term consequences. Unfortunately, there are limited therapeutic options to counteract the sequelae following the initial insult. The role of stem cells has been studied in the treatment of various diseases. The goal of this study was to provide a literature review regarding the potential advantages of stem-cell therapy to counteract or minimize the sequelae of aneurysmal subarachnoid hemorrhage.

Methods—PubMed, Google Scholar, and ClinicalTrials.gov searches were conducted to incorporate pertinent studies that discussed stem cell use in the management of subarachnoid hemorrhage. Included articles were subjected to data extraction for the synthesis of the efficacy of stem-cell therapy.

Results—Four preclinical studies with 181 animal model subjects (44 mice, 137 rats) were incorporated in our review. Endovascular punctures (65%) and blood injections in subarachnoid spaces (17%) were used to induce hemorrhage models. Stem cells were administered intravenously (3.0 × 10⁶ cells) or intranasally (1.5 × 10⁶ cells). According to literature, mesenchymal cell therapy significantly (p<0.05) induces stem-cell migration to lesion sites, decreases associated neural apoptosis and inflammation, improves ultrastructural integrity of cerebral tissue, and aids in improving sensorimotor function post subarachnoid hemorrhage.

Conclusion—Stem cells, particularly mesenchymal stem cells, have shown promising cellular, morphological, and functional benefits in animal models suffering from induced subarachnoid hemorrhages. However, further studies are warranted to elucidate the full effects of stem-cell therapy for aneurysmal subarachnoid hemorrhage.

Keywords

Intracranial aneurysms; mesenchymal stem cells; stem cells; subarachnoid hemorrhage

Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) remains a devastating disease with high morbidity and morality, affecting roughly 30,000 people annually in the United States [1,3–5,21]. Even if patients survive the initial insult, vasospasm with secondary delayed ischemic neurological insult can further exacerbate brain damage [2,5,11,13,14,17,19,20]. Currently, management of aSAH focuses on preventing rebleeding from the ruptured aneurysm and averting the comorbidities that arise due to vasospasm [12]. Clinically, vasospasm is managed with triple-H therapy (hypervolemia, induction of hypertension, and hemodilution) and calcium-channel blockers (e.g., nimodipine), or endovascularly with intra-arterial vasodilators and/or balloon angioplasty [1]. Nonetheless, the optimal treatment necessary to circumvent the late-effects of aSAH remains controversial, and hence, there has been greater investigation into providing efficacious adjunct treatments to patients to mitigate delayed consequences.
One such experimental method beginning to emerge is the use of stem-cell therapy. The self-renewal capacity and repopulating properties that stem cells possess may make them a prime therapeutic candidate. They have previously shown tremendous benefits in improving functional outcomes in patients with various endovascular conditions, such as stroke, so potential use in patients with aSAH may offer similar outcomes [6,7,18,22]. However, the role of stem-cell therapy in the setting of aSAH remains to be adequately established. Therefore, the primary focus of this paper is to provide a systematic review of recent literature regarding the use of stem cells in the management of SAH and provide future perspectives regarding treatment.

Materials and Methods

Using the medical subject headings (MeSH) database system through PubMed, a literature search was completed between the years 1995 and 2015 for all articles that included the terms stem cells, mesenchymal stem cells, or neural stem cells, and subarachnoid hemorrhage, with related phrases, such as ruptured aneurysm (e.g., “subarachnoid hemorrhage” [MeSH] and “stem cells” [MeSH], “subarachnoid hemorrhage” [MeSH] and “neural stem cells” [MeSH], “aneurysm, ruptured” [MeSH] and “stem cells” [MeSH] or “neural stem cells” [MeSH], and “subarachnoid hemorrhage,” [MeSH] and “mesenchymal stem cells” [MeSH]). The articles were limited to English with either human or animal subjects whose SAH was treated using stem-cell therapy or if there was reference of stem-cell incorporation near the injury site. Additionally, the article types were limited to case reports, clinical trials, randomized-controlled trials, and case studies, while editorials, meta-analyses, reviews, and commentaries were excluded. The initial inclusion criteria focused on stem-cell utilization in SAH or papers exhibiting evidence of stem-cell migration and proliferation at hemorrhage site. All articles that met these requirements underwent a title and abstract review to establish the validity of each paper. Papers that failed to meet our study standards or any duplicates were discarded. The full text of each remaining paper was screened, and the final papers were utilized for our analysis.

Searches were also conducted on alternate Internet databases, including PubMed excluding the MeSH function, Google Scholar, and ClinicalTrials.gov. Key search terms included SAH and ruptured aneurysms with mesenchymal stem cell, neural stem cell, or simply stem cell. This process was identical to the process employed in the PubMed MeSH database. The included studies were screened based on the characteristics of the participants, the number of study participants, the origin and site of induced SAH, the administration method of stem cells for treatment, the volume of stem cells administered, and statistical significance of each outcome reported by the authors.

Articles that focused on intracranial hemorrhage, including intraventricular bleeding, were excluded, as our study aims were limited to SAH. The last search was conducted on March 31, 2015.

Results

Study Selection

The initial PubMed literature search resulted in 27 papers, with three of these studies being duplicates. The additional database searches resulted in five more studies. After the title and abstract review, these papers were assessed using the previously mentioned inclusion criteria. Articles were included in the analysis if there was relevant information regarding stem-cell therapy for treatment of SAH in humans or in animal models or if there was stem-cell proliferation near the SAH site. From this screening, only three papers were included from the 27 articles recognized by the PubMed search, while only one of the five papers found through the other databases was included. A total of four papers were ultimately included in our paper. The study selection process is outlined through the PRISMA flowchart in Figure 1. A summary of these articles is presented in Table 1.

Study Demographics

No clinical trials were present at the time of the review. A total of 181 animal test subjects were included. All four papers included data regarding the type of animal model and the genders of the study subjects [8,9,10,15]. One paper utilized 44 adult male CD-1 mice, whose weight ranged from 35 g to 40 g for their research [15]. The other three utilized Wistar rats (105 males; 32 females), whose weight ranged from 275 g to 350 g [8–10]. Core temperature for all animal subjects was maintained at 37°C to 37.5°C. All had open access to food and water. Two of the four papers included data regarding mortality rates of animals that underwent induced SAH, and the incidence of death reported was 32.9% for the sample cohort with SAH [10] and [15].

Experimental SAH Model

All four papers included information regarding the induced SAH model [8–10,15]. The endovascular punc-
Figure 1. The PRISMA diagram summarizes the systematic process used to identify, screen, and include the case reports, case series, clinical trials, and randomized controlled trials that we analyzed for this review.
ture model was utilized in animals from two studies \((n = 127)\) where a suture was advanced through the vasculature to perforate an artery in the circle of Willis \([10] \text{ and } [15]\). The specific arteries to be punctured were different throughout both of the studies. The middle cerebral artery (MCA) was punctured in 83 animals \([10]\). In 34 animals, the anterior cerebral artery (ACA) was perforated \([15]\). The model utilized in the remaining two studies was through the injection of 0.3 mL of blood directly in the subarachnoid space \((n = 32)\) \([8] \text{ and } [9]\). A total of 32 sham operations were used as controls in all the studies. A summary of the experimental SAH models is shown in Figure 2.

### Stem Cell Administration Sites and Doses

Three studies utilized mesenchymal stem cells (MSCs). In two studies by Khalili et al., MSCs were administered intravenously through the tail vein of study rats 24 hours post-SAH procedure. A total of \(3.0 \times 10^6\) cells were given in a 1.0 mL injection \([8] \text{ and } [9]\). In the third study by Kooijman et al., MSCs were administered intranasally in Wistar rats six days postinduced SAH. A total of \(1.5 \times 10^6\) cells were delivered in 24 μL; however, this dose was broken in two rounds with 12 μL \([10]\).

### Treatment Effects

#### Cellular Outcomes

In the three studies that involved administration of MSCs post-SAH, significant improvements in cellular outcomes were observed, such as stem-cell migration, decreased apoptotic response, and decreased macrophage infiltration, when compared to animal controls not treated with MSCs. Stem cells labeled with 5′-bromo-deoxyuridine (BrdU) were identified in localized patterns of neural tissue surrounding the SAH lesion sites; however, no localization of BrdU-positive MSCs was noted in control animals, suggesting MSC migration to areas damaged by SAH \([8]\). MSC treatment for SAH also significantly reduced the number of apoptotic cells around the injury site when tested using TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling) method. Percentages of TUNEL-positive cells (those undergoing apoptosis) were significantly lower \((p<0.05)\) in SAH + MSCs \((9.30 \pm 1.7\%)\) rats than in animal controls \((17.42 \pm 3.3\%)\) \([8]\). Finally,

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**Table 1. Studies included in this review that analyzed the use of MSCs as a therapy against SAH.**

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Study species</th>
<th>Sample size, (n)</th>
<th>Weight (grams)</th>
<th>Experimental SAH model</th>
<th>MSCs administration site</th>
<th>Volume of MSCs</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mino et al 2003 ([15])</td>
<td>Adult male CD-1 mice</td>
<td>(n = 14)</td>
<td>35–40 g</td>
<td>Endovascular perforation (ACA)</td>
<td>N/A</td>
<td>N/A</td>
<td>Neurogenesis in the hippocampus may affect functional outcome after SAH. The induction of neurogenesis can provide therapeutic value against SAH.</td>
</tr>
<tr>
<td>Khalili et al 2012 ([8])</td>
<td>Female Wistar rats</td>
<td>(n = 16)</td>
<td>275–300 g</td>
<td>0.35 mL blood injected in SAS</td>
<td>Intravenous (tail vein)</td>
<td>(3 \times 10^6) cells (1 mL)</td>
<td>Intravenous MSCs provided significant ((p&lt;0.05)) functional recovery in animals with SAH. Differentiation of stem cells to glia, neurons, and endothelial cells was also noted along with fewer apoptotic cells in SAH + MSC group.</td>
</tr>
<tr>
<td>Khalili et al 2014 ([9])</td>
<td>Female Wistar rats</td>
<td>(n = 16)</td>
<td>275–300 g</td>
<td>0.35 mL blood injected in SAS</td>
<td>Intravenous (tail vein)</td>
<td>(3 \times 10^6) cells (1 mL)</td>
<td>Electron microscopy analysis revealed that MSCs were capable of improving the damaged areas within cerebral tissues post-SAH. Compared to control cohort, blood vessel of the treatment group exhibited less neuronal degeneration, pre-neural edema, and mitochondrial abnormalities. Sensorimotor and mechanical function was significantly improved after MSC administration. A decrease in gray and white matter loss also noted when comparing control versus treatment groups</td>
</tr>
<tr>
<td>Kooijman et al 2014 ([10])</td>
<td>Male Wistar rats</td>
<td>(n = 105)</td>
<td>300–350 g</td>
<td>Endovascular perforation</td>
<td>Intranasal</td>
<td>(1.5 \times 10^6) (24 μL)</td>
<td>Sensorimotor and mechanical function was significantly improved after MSC administration. A decrease in gray and white matter loss also noted when comparing control versus treatment groups</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACA, anterior cerebral artery; MSC, mesenchymal stem cell; N/A, Not Available; SAH, subarachnoid hemorrhage; SAS, subarachnoid space.
MSC treatment for SAH was tested in regards to long-term inflammatory responses by macrophage and microglia activation (21 days post-SAH). Rats who underwent induced SAH indicated high levels of macrophage and microglia activation by Iba-1 probing, while rats treated with MSCs showed significant Iba-1 downregulation, indicating a decreased inflammatory response [10].

**Morphological Outcomes**

Morphological studies were conducted using transmission electron microscopy (TEM) to study cerebral vasculature integrity post SAH with and without MSC treatment. Rats with induced SAH without MSCs administration exhibited arterial abnormalities around examined blood vessels with smooth-muscle necrosis noted. Mitochondrial degeneration was also visualized by TEM. However, SAH rats with MSC therapy revealed uninterrupted arterial walls, with no areas devoid of endothelial cells. Moreover, no muscle necrosis was visualized in MSC treated rats [9].

Additionally, gray and white matter volumes 21 days post-treatment were determined using microtubule-associated protein 2 (MAP2) and myelin basic protein (MBP) staining. MAP2, which is involved in microtubule formation, measured the degree of gray matter damage, whereas MBP, which is vital in the myelination of neurons, assessed the degree of white matter damage. Damage to both gray matter and white matter was sig-
nificantly lower in MSC-treated rats compared to rats without treatment (p<0.001 and p<0.05, respectively) as determined by increased MAP2 and MBP expression.

**Functional Outcomes**

Sensorimotor and neurological function was monitored in animal models to examine possible improvements as a response to MSC treatment. Testing was completed by way of the neurological severity score (NSS), the adhesive removal task (ART), and by use of von Frey hairs (to test mechanosensory) [8] and [10]. These assessments collectively measured sensorimotor function, mechanosensory function, balance, and reflexes.

NSS is an index used to score neurological function on a scale of 0 to 18, where a larger number corresponds to a greater deficit. NSS was utilized at 1, 7, and 14 days post-SAH. While NSS scores for rats treated with MSCs at all time points were consistently lower than nontreated animals, the most significant difference in treatment versus nontreatment animals came at 14 days post-SAH (3.5 vs. 7.75, p<0.05), indicating that neurological deficit was reduced in SAH + MSC rats [8]. Kooijman et al. (2014) observed neurological functions 21 days post-SAH and found that intranasal administration of MSCs attenuated long-term sensorimotor and mechanosensory deficits after induced SAH. ART times were approximately 50% shorter in MSC treated rats than in control rats. The animals recognized the foreign object sooner and attempted to remove the adhesive faster than rats with SAH without MSC therapy [10]. Lastly, mechanosensory function assessment demonstrated that animals treated with MSC showed a significantly lower threshold of contact required to elicit a hind paw withdrawal as compared to nontreated animals, suggesting an enhanced response to touch after MSC therapy [10].

**Discussion**

Despite the continuous advancements in the management of aSAH, there still remains a significant portion of patients who die or are left with devastating long-term sequelae. Novel treatments are necessary to mitigate the associated consequences of this condition.

**Role of MSCs in Improving aSAH Outcomes**

MSCs provide a potentially effective strategy for ameliorating the sequelae of aSAH. Mino et al. investigated the temporal relationship between SAH and neurogenesis in the rat brain [15]. They observed an inhibition of neurogenesis for up to three days following SAH until returning to baseline levels at seven days. They also observed an increase in neural progenitor cell (NPC) activity on the inner surface of the subgranular zone, which then migrated into the granular cell layer of the hippocampus and ultimately differentiated into neurons. Mino et al hypothesized that therapeutically inducing neurogenesis by promoting the activation of NPCs can be a beneficial strategy in improving the outcomes of SAH patients. Activation of NPCs may also be important in humans with aSAH, as was described by Sgubin et al [16]. In their study, the authors analyzed NPC markers from cerebral tissue taken from patients treated for aSAH. The study demonstrated the presence of various stem-cell markers (SOX-2, Musashi, vimentin, nestin, and Ki67) in a majority of the aSAH cases [16].

In the studies reported in this review, animal models that underwent MSC administration, either intravenously or intranasally, showed significant functional, cellular, and ultrastructural outcomes [8–10]. With stem-cell transplantation at both 24 hours post-SAH and at 6 days post-SAH, there was a noticeable improvement in the neurological deficit of these animal models, implying that while rapid intervention with patients suffering from SAH is preferred, MSC treatment may prove effective even if administered later after the initial hemorrhagic event [10]. At the cellular level, animals that had been treated with MSC administration exhibited reduced cell apoptosis and decreased inflammatory responses. Additionally, stem cells seemed to preferentially migrate to areas of damage caused by aSAH. At the morphological level, TEM analysis determined that animal models treated with MSCs displayed less tissue abnormalities such as necrosis and brain matter loss. Finally, functional testing of treated animals showed greater improvements in mechanosensory, balance, and reflex testing than animals in the non-treatment category, showing MSC treatment provides valuable therapeutic value in mitigating the effects of aSAH.

**Clinical Application and Future Perspective**

Though the studies analyzed within our review presented pre-clinical data, the conclusions drawn from these articles indicate promise for clinical application. Administration of MSCs post aSAH was performed intravenously or intranasally depending on the study. No major morbidities were reported in the test animals, suggesting that either method of stem-cell transfer can be safe. Additionally, while the mechanism is not fully characterized, MSCs were able to migrate towards sites of neural.
damage and relieve some commonly seen morphological and cellular responses induced by SAH [8,9]. Furthermore, stem-cell-mediated neurogenesis was noted in regions of damage potentially indicating that functionality can be positively affected with stem-cell therapy.

Based on the results, the most promising clinical application to using MSCs post-SAHA lies in its use as soon as possible after surgical and neuroendovascular intervention. Benefits were seen in animal models when MSCs were administered 24 hours after initial insult; however, results were seen as far as six days out. Future investigation should be geared toward optimizing treatment protocol and examining whether or not the effects of MSC therapy are long lasting, so that transitions to human trials would offer complete results. Moreover, the goals of eventual human trials should be to assess stem-cell therapy in the context of the full-treatment procedures offered to patients with aSAH. Surgical and medical intervention is gold standard, however, offering intravenous or intranasal stem-cell therapy as adjunct treatment may reduce the chance of developing the late sequelae that plagues patient outcomes.

Limitations

A number of factors may have contributed to the observed results of the studies included in this systematic review, and as such, the results of this analysis should be interpreted within the context of several limitations. The participants in these studies were all animal models and only four studies were incorporated due to the fairly recent attention directed toward stem-cell therapy in the context of SAH. Further studies are warranted to ensure the validity of the work currently being conducted. Additionally, some of the papers included in this review presented a sample size in the treatment cohorts that may not hold adequate statistical weight.

Regardless of any limitations present, there are many important clinical implications for this report. aSAH remains a grave condition and there is a need for more efficient treatment options for patients. This paper included information pertaining to the administration of MSCs and the efficacy of their use in animals with positive results. Not only do stem cells promote neurogenesis in damaged areas of the brain, but they can also help mitigate the late effects of aSAH, including strengthening of vasculature and regaining of motor, sensory, and cognitive functions.

Conclusion

Early findings in SAH-induced animal models suggest beneficial effects of MSCs on outcomes if given within 24 hours and up to 6 days following hemorrhage. Mesenchymal stem cells appear to exert their effect at the cellular, morphological, and functional levels. These findings highlight the potential of future utilization of MSCs in the clinical setting to act as a therapeutic option to ameliorate the drastic outcomes of aSAH.

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