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Drug-like delivery methods of stem cells as biologics for stroke

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Abstract

Introduction—Stem cell therapy is an experimental treatment for brain disorders. Although a cellular product, stem cells can be classified as biologics based on the cells' secretion of therapeutic substances. Treatment with stem cell biologics may appeal to stroke because of the secondary cell death mechanisms, especially neuroinflammation, that are rampant from the onset and remain elevated during the progressive phase of the disease requiring multi-pronged biological targets to effectively abrogate the neurodegenerative pathology. However, the optimal delivery methods, among other logistical approaches (i.e., cell doses and timing of intervention), for stem cell therapy will need to be refined before stem cell biologics can be successfully utilized for stroke in large scale clinical trials.

Areas covered—In this review, we discuss how the innate qualities of stem cells characterize them as biologics, how stem cell transplantation may be an ideal treatment for stroke, and the various routes of stem cell administration that have been employed in various preclinical and clinical investigations.

Expert opinion—There is a need to optimize the delivery of stem cell biologics for stroke in order to guide the safe and effective translation of this therapy from the laboratory to the clinic.

Keywords

stem cells; cerebral ischemia; drug; inflammation; translational research

1. The dirty drug: Stem cells as "biologics"

Stroke is a neurodegenerative and neurovascular disorder with 795,000 cases occurring annually in the United States [1]. The disease is characterized by an occlusion of a blood vessel within the skull or neck that supplies blood to the brain, which consequentially

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reduces cerebral blood flow and induces cell death in the affected areas [2]. Additionally, ischemic stroke is accompanied by an increase in inflammatory events in the brain and periphery that contribute to further apoptosis and subsequent neurological damage [3]. Although tissue plasminogen activator (tPA) is available as a treatment for stroke, its narrow 4.5 hour window of administration following ischemic stroke limits its effectiveness in the clinic [4]. Thus, alternative treatment regimens are warranted. Stem cell therapy may be a viable treatment for stroke given stem cells' neuroprotective properties and ability to mitigate inflammation in the brain, as well as in the periphery via the spleen [5,6]. For instance, stem cells transplanted systemically after stroke demonstrate preferential migration to the spleen [5]. Indeed, stem cells such as mesenchymal stem cells (MSCs) are "biologics" that exhibit the ability to home to sites of inflammation [5–7], which would enable them to effectively render their anti-inflammatory effects and hinder further pathological development of a stroke. As recent evidence points to the systemic inflammatory response as a key pathological component in exacerbating secondary cell death post-stroke [8], stem cell transplantation appears to be a promising therapy to attenuate this system-wide immune response and ameliorate stroke outcomes.

Generating a dependable source of transplantable cells with consistent ethical and quality control will be important to create effective, reproducible, and reliable cell therapies for ischemic stroke. Additionally, further research is necessary to identify the optimal cell types that are both safe and effective for transplantation. However, stem cells remain promising therapeutic biologics as they demonstrate multiple avenues for combating neurodegeneration such as by mitigating neuroinflammation and increasing angiogenesis, neurogenesis, and vasculogenesis [9–14]. Of note, the ischemic site in the stroke brain comprises a toxic microenvironment that is detrimental to stem cell survival and maintains a heightened state of inflammation [15,16]. Thus, mitigating this toxic microenvironment by lowering cytotoxicity and inflammation may prove beneficial for increasing the quantity of surviving grafted cells and enhancing their therapeutic effects.

Initially, the regenerative mechanism exerted by stem cell therapy in the nervous system was believed to be the replacement of dead or dying neurons. However, further stem cell transplantation investigations have demonstrated poor engraftment rates yet robust increases in functional recovery and neuronal survival [17]. One of the main mechanisms to explain these observations is the secretion of neurotrophic factors by the engrafted cells. Several growth factors are involved in cell survival pathways and limiting their decline represents a valid strategy to ameliorate stroke outcomes. Administering BDNF, VEGF, or other growth factors prevents apoptosis [18–20] and improves neurological outcomes in experimental disease models, but is unlikely to be effective in clinical settings. In contrast, stem cells are able to secrete a variety of neuroprotective factors, inducing anti-apoptotic and anti-inflammatory responses. Moreover, stem cells are sensitive and responsive, capable of regulating their growth factor secretion to proper levels and avoiding any dosage issues. This point is crucial because drug-induced overproduction can exert counter-productive neurological activities, such as how BDNF overexpression can induce epileptic seizures [21]. Another main mechanism of action is represented by the mobilization of endogenous stem cells. The discovery of brain regions which harbor endogenous stem cells (neurogenic niches), has challenged the dogma that mammalian adult brains are incapable of generating

new neurons [22,23]. The endogenous mobilization of stem cells in the brain is only slightly effective in reverting or impeding the activation of cell death pathways. This is a result of the limited ability of endogenous stem cells to commit to the neuronal lineage, migrate, and differentiate from the neurogenic niches to the injured area [24]. To exert therapeutic activities, the endogenous stem cells need to migrate from these distal, neurogenic areas. Of note, transplanted stem cells mediate the mobilization of host stem cells [25]. This mechanism called a “biobridge” helps stem cells provide neuroprotective benefits and can facilitate stem cell migration to the injured area, enhancing the host’s regenerative activity [25]. Moreover, an additional mechanism of action has been reported involving secretome (microvesicles and exosomes) activity, which exerts therapeutic effects in neurovascular diseases [26].

While stem cell transplantation has promise as a potential treatment for ameliorating ischemic stroke, these grafted cells also carry possible risks including a host immune response to reject the transplanted cells and formation of detrimental teratomas. Immunosuppression regimens are an available solution for host rejection of stem cell grafts, though the risk of an undesirable immune response is dependent on the type of host [27]. Of note, stem cells with the capacity to differentiate into multiple cell lineages such as induced-pluripotent stem cells and embryonic-derived stem cells possess a higher chance of developing teratomas than adult stem cells [28]. Additionally, a reduction in the stemness of transplanted stem cells over time is actually favorable, as there is less risk for undesirable, excessive proliferation. This limitation is particularly dependent on specific cells’ ability to differentiate, though all stem cells possess this potential hazard.

To circumvent the host rejection response and teratoma development while preserving the regenerative benefits of stem cells, it has been proposed to use derivatives of stem cells such as vesicles, rather than the cells themselves. It is widely accepted that MSCs exert their action via paracrine effects via secretome or extracellular vesicles, rather than through transdifferentiation to replace injured neurons. Specifically, MSC-secreted extracellular vesicles are able to promote neural repair and to improve the functional outcome in stroke animal model. In this case, cell-derived therapies, such as components derived from secretomes possessing beneficial factors, can be utilized to increase neurogenesis and angiogenesis and decrease inflammatory processes [28–32]. Therefore, extracellular vesicles may be developed as a novel cell-free therapy for neurological disorders. Indeed, intravenous delivery of exosome-enriched vesicles released by bone marrow-derived MSCs significantly improve the neurite remodeling as well as neural plasticity in MCAO rats [33]. Stroke triggers the mobilization of bone marrow MSC-derived secretome in patients with severe stroke, and these vesicles exert restorative activity [34]. Such cell-free therapeutic effect is recently seen in a porcine model of stroke, demonstrating for the first time that hNSC-derived vesicles preserve functional activity and neural tissue integrity post-MCAO, suggesting that it may represent a potential therapy for stroke patients [35]. To date, systematic comparative vis-à-vis studies between transplantable cells and cell-free therapeutic substances are missing, which will be a key step in identifying the optimal regenerative medicine product for stroke therapy.

2. Stroke as a candidate target for stem cell biologics

Stroke can be classified as either ischemic or hemorrhagic. Of recent, ischemic stroke appears to be an ideal candidate for stem cell therapy [36, 37]. As mentioned above, at present, only a few treatment options exist for ischemic stroke and these regimens possess deficiencies that compromise their safety and efficacy in clinical settings. Thus, stem cell therapy emerges as an alluring potential treatment for the chronic symptoms of cerebral ischemia, such as the cell death and detrimental neuroinflammation associated with the subacute and chronic stages of ischemic stroke [38].

Three main phases characterize the ischemic events that occur after a stroke: the acute, subacute, and chronic phases. First, the initial few hours after the occlusion mark the acute phase of stroke and are accompanied by excitotoxicity and oxidative damage at the infarct area due to insufficient blood supply [39–41]. Cell death is exacerbated by the production of reactive oxygen species (ROS) and increasing concentrations of Na^+ and Ca^{2+} ions in the ischemic region. The compromised homeostasis of ions enables water to enter the cells, resulting in the development of vasogenic edema in the infarct zone. Moreover, the neurons in the ischemic penumbra that initially survive the ischemic event begin to secrete signals that promote further cellular damage [39–41]. Next, the subacute phase involves elevated neuroinflammation and the secretion of matrix metalloproteases (MMPs), cellular adhesion molecules (CAMs), chemokines, and cytokines from damaged astrocytes, neurons, and microglia; and occurs during the early days after the onset of ischemia, following the acute phase [5,39–41]. Additional leukocytes are drawn to the infarct site due to elevated amounts of CAMs enabling increased leukocyte adhesion to cerebral vessels. Furthermore, as a result of rising MMP levels, blood-brain barrier (BBB) permeability increases, facilitating the infiltration of peripheral leukocytes into damaged regions and further escalating inflammation [39–41]. Finally, astrocytes and activated microglia perpetuate the state of heightened inflammation into the chronic stages of stroke that follow the subacute phase [39–41]. Additional macrophages and neutrophils from the periphery are recruited via the BBB due to the release of CAMs, chemokines, and cytokines from diseased brain cells. Consequently, the brain infrastructure is at risk to damage from the cell death and cerebral edema generated by this chronic inflammation [39–41].

Stem cell transplantation is capable of mitigating the heightened inflammation present in both the subacute and chronic phases of stroke. As the ischemic injury is aggravated by ongoing inflammatory processes during the subacute stage, it is critical to promote neuroprotection and preclude further damage during this period [36]. Additionally, facilitating anti-inflammatory and neuroregenerative events in the subacute and chronic phases are paramount for successful therapeutic outcomes [5,39–41]. Indeed, administering stem cells in the chronic phase of stroke can revitalize the brain, restore normal blood flow, repair the disruptions in the BBB, and reduce inflammation via regenerative processes such as angiogenesis, neurogenesis, synaptogenesis, and vasculogenesis. Moreover, the apoptosis, inflammation, mitochondrial dysfunction, and oxidative stress that lead to ischemia-mediated early secondary cell death can be inhibited by stem cell injections during the subacute stage [4,5,42,43]. Thus, stem cell therapy can fulfill the unmet need for an effective treatment targeting the subacute and chronic phases of ischemic stroke through its ability to

promote recovery after stroke-induced damage by increasing reinnervation and diminishing inflammation.

The disease pathology of ischemic stroke is now thought to involve a mounting peripheral immune response in addition to the central neuroinflammation [44], and this connection between the peripheral systems and the brain is likely mediated through inflammatory signals in the circulatory system. Indeed, neuroinflammation is facilitated by both central and peripheral inflammation in which the initial ischemic insult creates an inflammatory response that is further enhanced by systemic inflammation [45]. Furthermore, lymphocytes, Tcells, macrophages, and monocytes pass through the BBB which has been compromised by the ischemic injury, and further elevate inflammation levels [5,45].

The systemic inflammation that accompanies ischemia in the brain may be mediated by the spleen. In fact, following ischemic stroke, spleen sizes decrease and splenocytes are released into the circulatory system, leading to increased neurodegeneration [46]. Additionally, the spleen has demonstrated a role in exacerbating other neurodegenerative diseases such as traumatic brain injury, as cognitive function and injury sizes improve when the spleen is removed post-injury [47].

The spleen appears to play a crucial role as a “seducer” in the body’s physiological response to transplanted stem cells [5,25]. Human bone marrow MSCs injected into the circulatory system preferentially migrate to the spleen after stroke [5]. Hence, stem cells may mitigate the spleen’s inflammatory processes post-stroke, given that these cells possess innate anti-inflammatory qualities. With the knowledge that the spleen contributes to inflammation and neurodegeneration during the chronic stage of stroke, anti-inflammatory treatments for ischemia may also benefit from approaches that target and abate the peripheral immune response, such as stem cell therapy.

The stem cells’ ability to modulate splenic activity in the chronic phase of stroke may prove to be beneficial for developing effective stroke treatments. These cells may not need to enter the brain and may possibly only need to interact with the spleen to exert their neuroprotective effects [5]. Indeed, if stem cells can reduce neuroinflammation via indirect avenues, their ability to ameliorate ischemic stroke will not be compromised by their limited potential to cross the BBB. This solves the dilemma in which systemic administration of stem cells is rendered less effective following repair of the damaged BBB. Furthermore, stem cell treatments that focus on the spleen in the chronic phase of stroke will remain potent even after the BBB is restored and impedes the bioavailability of therapeutics in the brain. Stem cell therapies at later post-stroke time points will benefit from additional studies that further investigate how stem cells interact with the spleen to reduce inflammation.

While several types of cells such as induced pluripotent stem cells, embryonic stem cells, amnion, umbilical cord blood, adipose, CTX0E3 cells, and NT2N cells have been employed in bench and clinical cell transplantation investigations for ischemic stroke [4,48–55], recent studies have focused on cells harvested from bone marrow. Multi-potent adult progenitor cells (MAPCs), SB623 cells, endothelial progenitor cells (EPCs), multilineage-differentiating stress enduring (Muse) cells, MSCs, and other stem cells derived from bone

marrow demonstrate a favorable safety profile in several diseases [56,57] and have been comprehensively examined in various animal models [5,58], making them attractive for stem cell transplantation in stroke.

Indeed, stem cell transplantation will likely be beneficial for ischemic stroke given the stem cells' ability to bolster neuroprotection and neuroregeneration in diseased animal models [58–63]. As the onset of stroke is uncertain, stem cell regimens will likely be used as a preventative or regenerative treatment in the clinic. The idea of stem cells as prophylactic biologics will be reinforced by advancements in diagnostic methods that incorporate genetics, co-morbidity factors, and family history to indicate patients that are prone to stroke and may benefit from cell transplantation. Clinical utilization of stem cells will benefit from future studies that probe these cells' capabilities to impede the occurrence of an ischemic stroke and promote post-stroke recovery. Particular interest is provided in optimizing the route of stem cell delivery because of the need to distribute the biologics relased by the stem cells to the stroke brain. The rescue of the injured brain and resulting functional outcomes are likely to be dictated by the appropriate bioavailabity of the stem cells and their secereted factors within or close to the site of injury.

3. Delivery routes of stem cell biologics in stroke

Stem cell therapy after stroke improves functional outcomes in preclinical studies. There are different routes for treatments, including intracerebral, intra-arterial, intraperitoneal, intraventricular, intravenous, and intranasal (Figure 1), but the most suitable route is still uncertain. After stroke, the original concept of transplanting exogenous stem cells is to reestablish the cytoarchitecture of injured tissue. This treatment involves the grafted cells' survival in an inhospitable environment with inflammation, oxidative stress, glial scars, and cell death [15,16]. Initially, it was believed that intracerebral administration was the best route to enable exogenous neural stem cells (NSCs) to reach brain tissues. These cells are multipotent, able to self-renew and generate neural cells, and can replace lost neurons [64,65]. Subsequently, the mechanistic view of stem cell therapy has evolved into a by-stander effects, with stem cells such as NSCs exerting their functional effects through paracrine pathways, secreting different growth factors, and expressing mRNA. Besides NSCs, numerous other cell types can perform these paracrine functions and differentiate into multiple lineages, which is necessary for replacing lost neurons in the injured brain [66,67]. In addition to NSCs displaying cell replacement and by-stander effects in animal models [68–70], embryonic stem cells [71,72], induced pluripotent stem cells (iPSCs) [73,74], and MSCs have also been demonstrated to achieve such mechanisms of neurovascular repair.

Intracerebral delivery indicates that more implanted cells exist in the injured regions compared to other administration routes, in which millions of stem cells are delivered into the brain and approximately one third are able to migrate to the injured areas [75–77], as well as to the intact hemisphere [78,79]. Intra-striatal injection of human iPSCs induces neural recovery as these cells are able to differentiate into immature and mature neurons, indicating that this administration route is feasible in subcortical stroke [80]. However, less invasive methods than stereotaxic injections have been used and the intracerebral method is not the only route capable of replacing lost neurons and neuronal connections. Using

subarachnoid [81,82] and intraventricular [16] routes for therapeutic delivery in stroke rats improves cell survival and enriches the microenvironment. Additionally, exogenous stem cells administered via intraventricular techniques can reach ischemic areas after stroke.

Clinical trials have reported the feasibility and safety of NSCs. Although some patients show improvement, the trial results demonstrate no significant benefits in motor function [83]. The use of NSCs in patients, particularly the CTX0E03 (CTX) line, has been evaluated in the PISCES trial in order to test their safety after injection (NCT01151124), since few in vitro and in vivo studies have been performed and are not enough to render significant conclusions. Interestingly, a single intracerebral dose of CTX (up to 20 million cells) induces no adverse events and is associated with improved neurological function [84].

Bone marrow stem cells have demonstrated effectiveness concomitant with some improvements in the neurological condition of patients [85,86]. Clinical trials performing intracerebroventricular administration of autologous bone marrow stem cells [87,88] and fetal cells [89] report ameliorations in functional activity compared with the control groups. However, numerous issues hinder intraventricular and intracerebral routes of stem cell administration for brain repair, such as poor cell availability, invasiveness, immune rejection, and uncertain cell “fate” within the brain, which represent important limits to translational applications [90]. To overcome these obstacles, less invasive routes of administration represent promising candidates for stem cell therapy after stroke.

Stem cell therapy through intra-arterial administration in stroke animal models has demonstrated positive results. The most common intra-arterial method requires the use of catheterization as a guide for the cells through the carotid artery, enabling the delivery of a large number of cells to the brain injury [91]. However, when using this delivery method, around 10% of exogenous cells reach the injured region [92]. Transplanting stem cells intra-arterially leads to the replacement of lost neural connections and induces the release of trophic factors that enhance brain repair [91]. Moreover, intra-arterial administration of NSCs confers successful recovery after stroke, implying that grafted cells do not need to be administered near the injured area to be effective. For stroke treatments in animal models, several different types of stem cells apart from NSCs have been delivered intra-arterially, including umbilical cord mesenchymal stromal cells, human umbilical cord blood mononuclear cells, and MSCs [91–93]. Intra-arterial delivery has the advantage of being less invasive compared to the intralesional routes and is a promising route for stem cell therapy in stroke patients. Exogenous stem cells administered intra-arterially can reach the brain, showing efficacy via improved recovery. However, the risk of vessel blockage has been noted due to the large size of the cells [94] or microemboli [95]. In contrast, no adverse effects from microemboli after intra-arterial administration have also been observed [91]. Systemic delivery of stem cells could also lead to vasculature blockage. Increasing extra-vascular activity from the lumen to parenchymal brain [96] or targeting cells through overexpression of molecules [97] represent strategies to increase engraftment of these cells to the brain and minimize microemboli formation. The current therapies for stroke, such as thrombectomy, include an intra-arterial intervention that is effective until 8 h after stroke [98]. Treatments featuring an intra-arterial thrombectomy procedure may be well worth

combining with intra-arterial administration of stem cells, possibly offering an advantage in clinical translation.

Despite several studies exploring the utility of intra-arterial transplantation, its safety and efficacy remain inconclusive requiring further investigations. The mortality of stroke rodents after intra-arterial administration of NSCs is reduced using a microneedle instead of a catheter [99]. Moreover, microemboli occur in some cases, while other studies report no adverse effects from microemboli [91]. The infusion time, dose, and cell size can all contribute to an increase in the complications after intra-arterial graft transplantation. In fact, low infusion is linked to increased complications, while transplanting a high dose of cells triggers augmented embolic events [100]. Intriguingly, fast infusion has also induced an increase in embolic events [101]. In light of this, infusion velocity requires further investigations as the results remain contradictory. Additionally, reports regarding efficacy are equally as conflicting. While the intra-arterial route of administration in stroke animal models has demonstrated positive outcomes [91,92,99], intra-arterial delivered exogenous bone marrow MSCs exhibit limited ability to improve middle cerebral artery occlusion recovery in rats, even after indicating effective homing to the infarcted hemisphere [97–102]. These preclinical results indicate that further studies are necessary to identify clinically effective therapies for stroke [103]. Of note, clinical trials of cell therapies for stroke have also evaluated intra-arterial administration, showing that intra-arterial delivery of bone marrow cell grafts [104] or autologous bone marrow mononuclear cells [105] is safe and feasible. Moreover, 30% of patients with moderate to severe strokes show clinical improvement, and 40% of patients have a positive clinical outcome at 90 days [106].

Compared with intra-arterial administration, the intravenous route of cell delivery is more attractive because it is less invasive for stroke patients and equally effective compared to other delivery methods. In fact, most ongoing clinical trials use this route of administration [107]. Using intravenous delivery, the administered cells are confined to the peripheral organs, leading to low cell concentrations in the infarct zone [108]. Additionally, no cases of tumor formation or adverse effects have been reported. While intravenous and intra-arterial routes of administration show comparable protective properties and feasibility, intravenous delivery is considered preferable [91]. Different preclinical studies have reported promising results after intravenous cell therapy administration in stroke using several types of cells. Exogenous bone marrow stromal cells administered intravenously are able to migrate into the brain, survive, and improve recovery [109]. In addition, bone marrow mononuclear cells reduce lesion sizes and ameliorate functional outcomes in rats [110]. Moreover, adipose-derived MSCs improve brain plasticity and attenuate inflammation and apoptosis [111,112]. However, stem cell administration post-stroke may not be sufficient to enhance recovery in an aged brain environment [113]. iPSCs transplanted into the stroke-afflicted cortex are able to survive, differentiate into neurons, and improve functional recovery [114]. After intravenous NSC administration in rodents with intracerebral hemorrhage, these cells migrate and differentiate into astrocytes and neurons, and enhance post-stroke functionality in rodents [115]. Moreover, a small percentage of injected NSCs accumulate in the stroke injury area and most of these exogenous cells remain undifferentiated [116]. Interestingly, it has been demonstrated that NSCs migrate preferentially to the spleen compared to the brain and reduce apoptosis, inflammation, and edema formation after an ischemic insult. These

effects are not observed in splenectomized rats, implicating that the stem cells provided this neuroprotection by interrupting splenic inflammatory responses [117]. Additionally, human bone marrow stem cells administered intravenously also migrate more to the spleen than to the brain, attenuating inflammation and reducing the infarct area in the striatum. Indeed, these results demonstrate that stem cells injected intravenously represent a potential therapy for post-acute stroke capable of abrogating the inflammation-plagued secondary cell death [5].

Many clinical studies have employed cell-based therapies with intravenous administration. For instance, MSCs injected intravenously have improved neurological deficits in five patients with severe stroke [118]. A clinical evaluation of the efficacy and safety of intravenously injected autologous MSCs in a larger cohort has deemed this treatment as safe for 5-years follow-up [119]. Additionally, autologous MSCs expanded using autologous human serum are safe and capable of reducing the ischemic lesion by more than 20% after one week of treatment [120]. Other types of cells such as bone marrow mononuclear cells have been administered intravenously and have demonstrated feasibility and safety in patients with stroke [121,122]. After autologous cell transplants in the chronic phase of stroke, patients have shown improvements in the Barthel Index and augmented brain plasticity without adverse effects [123]. Recently, intravenous transplantations of autologous bone marrow mononuclear cells have also demonstrated enhanced neurological recovery and cerebral blood flow in stroke patients [124]. Overall, autologous mononuclear stem cell treatment via intravenous administration has been demonstrated to be feasible and safe for stroke patients, and there are several ongoing clinical trials testing the feasibility, safety, and efficacy of intravenous administration of other cell types in acute stroke.

In tandem with intra-arterial and intraperitoneal routes, the intraperitoneal route of administration stands as another potent delivery method but it remains underexplored that necessitates the need for using *in vivo* stroke models to assess safety, efficacy, and feasibility as a prior step to clinical studies. As noted above, the most appealing feature of intraperitoneal route is its minimally invasive procedure thus lessening the trauma associated with stem cell delivery associated with the other cell delivery approaches. However, the minimal migration of the intraperitoneally transplanted cells may limit the successful deposition of the cells and their biologics into the ischemic brain and inflammatory sites, which would necessitate increasing the cell dose to achieve an efficacious outcome. In the end, laboratory studies are needed to enhance cell migration with the intraperineal route. When contemplating with the intraperitoneal route of stem cell injections for stroke, it has been demonstrated that the grafted cell distribution may affect the therapeutic outcomes. For instance, higher quantities of MSCs reach the spleen, lungs, and brain when injected intravenously, relative to the intraperitoneal route [125]. That relatively few stem cells reach the ischemic brain or inflammatory peripheral organs (i.e., spleen) may warrant an ample amount of cell dose for intraperitoneal route of delivery. Such logistical requirements may limit the use of intraperitoneal route, despite its minimally invasive approach that appears practical in the clinical setting of stroke.

Another minimally invasive procedure but allows robust cell migration potential is the intranasal administration which has gained traction for stem cell delivery primarily because

of its safe, effective, and feasible route of delivery. Intranasal delivery is the most recent route used for cell-based treatments for stroke and currently, only preclinical studies utilizing this method have been performed. Of interest, intranasally administered cells are able to bypass the BBB and reach the brain [126]. These intranasally delivered cells migrate from the nose through the olfactory bulb or cerebrospinal fluid [127]. Additional experimental investigations probing proper dosages and techniques to reduce cell clumping or other adverse effects are necessary to advance this route of delivery. Compared with the other peripheral routes of delivery, the intranasal route appears to circumvent the problem of directing cells to the ischemic brain. Mouse models of ischemia treated with intranasally administered bone marrow MSCs have shown enhanced cell homing to the ischemic area and optimized therapeutic efficacy [128]. Additionally, intranasal delivery of bone marrow MSCs in neonatal stroke rats reduces infarct sizes and BBB disruption. Moreover, these animals show improved brain plasticity, enhanced cerebral blood flow, and increased functional recovery [129]. In a comparison between an intranasal delivery of MSCs and BDNF-secreting MSCs in neonatal hypoxic-ischemic brain injury rats, it has been demonstrated that both treatments reduce brain injury, ameliorate behavioral performance, and promote cell proliferation after stroke [130]. To reduce possible tumorigenic effects and increase the survival rate of grafted cells after intranasal administration, conditional medium can be used. Indeed, intranasal administration of conditional medium from human umbilical cord MSCs ameliorates functional outcomes, reduces BBB damage, and improves the vasculature post-stroke [131]. Additional experimental investigations probing proper dosages and techniques to reduce cell clumping or other adverse effects are necessary to advance this route of delivery. Compared with intraperitoneal route, the intranasal route appears to circumvent the problem of directing cells to the ischemic brain.

Finally, an invasive route of delivery has also been explored for stem cell administration. Intralesional (intracerebral, intraventricular, or subarachnoid) route of administration is that the transplanted cells participate in reestablishing and reconstructing the cytoarchitecture of damaged tissue after stroke. In fact, it has been demonstrated that these cells can replace most of the lost neurons after stroke [76,132]. However, this type of delivery has the disadvantage of a limited survival rate in an inhospitable milieu [15,16]. To overcome this obstacle and improve grafted cell survival, migration, differentiation, and proliferation, different hydrogels have been used. For instance, encouraging results for stroke therapies has been reported using Matrigel or hyaluronic acid (HA), which are promising material for delivering cells to neural tissues [133].

In summary, several delivery methods for stem cells exist in the laboratory which warrant closer examination to reveal each particular route’s safety and efficacy. Clinical studies have tested different routes of administration as well, but since the transplant protocols vary between the clinical trials, assessment of superiority of a particular route of cell delivery compared to others remains elusive.

4. Expert opinion

Stem cell therapy is an experimental treatment for neurological disorders, including stroke. Although stem cells are a cellular product, they may be considered as “biologics” mainly

because of their robust secretion of therapeutic substances, such as neurotrophic, anti-inflammatory, anti-oxidative, and anti-apoptotic factors among others, which have been shown to promote neurovascular recovery after cerebral ischemia. Such stem cell-based biologics approach has targeted stroke primarily due to the secondary cell death pathways accompanying the disease, characterized by downregulated levels of therapeutic substances which the stem cells are known to secrete as noted above, specifically neuroinflammation. That stem cells may represent the dirty drug designation appeals to stroke with its progressive phase associated with multiple cell death pathways requiring a multi-pronged approach to effectively abrogate the neurodegenerative pathology. Preclinical studies have shown the safety and efficacy of stem cell therapy in many stroke models. Limited clinical trials have been underway, and largely show the safety of transplanted stem cells in stroke patients. Efficacy of stem cells in the clinic remains elusive. Clearly, there is a need to improve the transplant regimen in order to realize not just safety, but also efficacy outcomes in the clinic. A key translational research gap may be related to finding the optimal route of delivery. Recognizing the stem cells stand as potent biologics may facilitate finding this optimal stem cell administration approach. To this end, determining the different therapeutic substances, such as anti-inflammatory factors, secreted by stem cells and amplifying their levels, as well as their biological function, may enhance the clinical outcomes of stem cell therapy. Moreover, equating stem cells as biologics with pharmacological properties may allow modification in their delivery method, such that solid and stable functional benefits are achieved. We summarize the different routes of stem delivery in Table 1, which show that each stem cell delivery route necessitates the need for adjusting the dose and timing of stem cells [118, 119, 125–131]. Increasing evidence shows that several types of stem cells reside in the CNS and non-CNS. As mentioned, some of them are not yet transplanted into post-stroke animals or translated in clinic, but they showed the potential to contribute to the brain repair process following stroke [134]. Since the research in this field is of high interest, this potential therapeutic activity will exploit to treat stroke patients in the near future. Because stroke is associated with distinct phases, namely acute, subacute and chronic, as discussed above, the optimal route will need to cater to the timing of intervention based on the stroke stage. Similarly, the cell dose associated with the route and timing of stroke will need to be modified accordingly. In the end, there is likely not a universal route and dose across the phases of stroke, but a range of routes and doses that will be stroke timing-dependent. As a rule of thumb, acute and subacute phases of stroke associated with elevated inflammatory signals acting as cell migratory “help me” signals will likely allow effective outcomes with minimally invasive procedures such as intravenous, intra-arterial, intranasal, and intraperitoneal routes with higher doses. On the other hand, the chronic state of stroke characterized by waning inflammatory signals will necessitate intracerebral or intraventricular administration to deliver the lower doses of cells within or near the ischemic brain region. Integrating our basic science knowledge on the onset and progressive nature of stroke pathology with the translational optimization of stem cell routes and doses will improve the successful clinical application of stem cell biologics for stroke.

In summary, stem cells are cellular products that can be considered as biologics based on their secretion of therapeutic substances, which have been shown to harness regenerative mechanisms in stroke models. Recognizing stem cells as biologics allows optimization of

stem cell delivery routes that take advantage of their drug-like properties, such as absorption and metabolism of stem cell-secreted factors. Such optimized stem cell delivery route, together with appropriate dose and timing of administration, will improve functional outcomes of cell therapy in stroke.

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Article Highlights

- Although long considered as a cellular product, stem cells can be classified as biologics based on the cells' secretion of therapeutic substances
- Stem cell biologics stand as potent stroke therapeutics for sequestering secondary cell death processes
- Cell death cascades, especially aberrant neuroinflammation, plague stroke during the neurodegenerative progression of the disease
- The multifactorial progressive phase of the disease requires multi-pronged biological targets to effectively abrogate the neurodegenerative pathology
- Optimizing the transplant regimen, in particular the route of delivery, will advance the use of stem cell biologics for stroke therapy

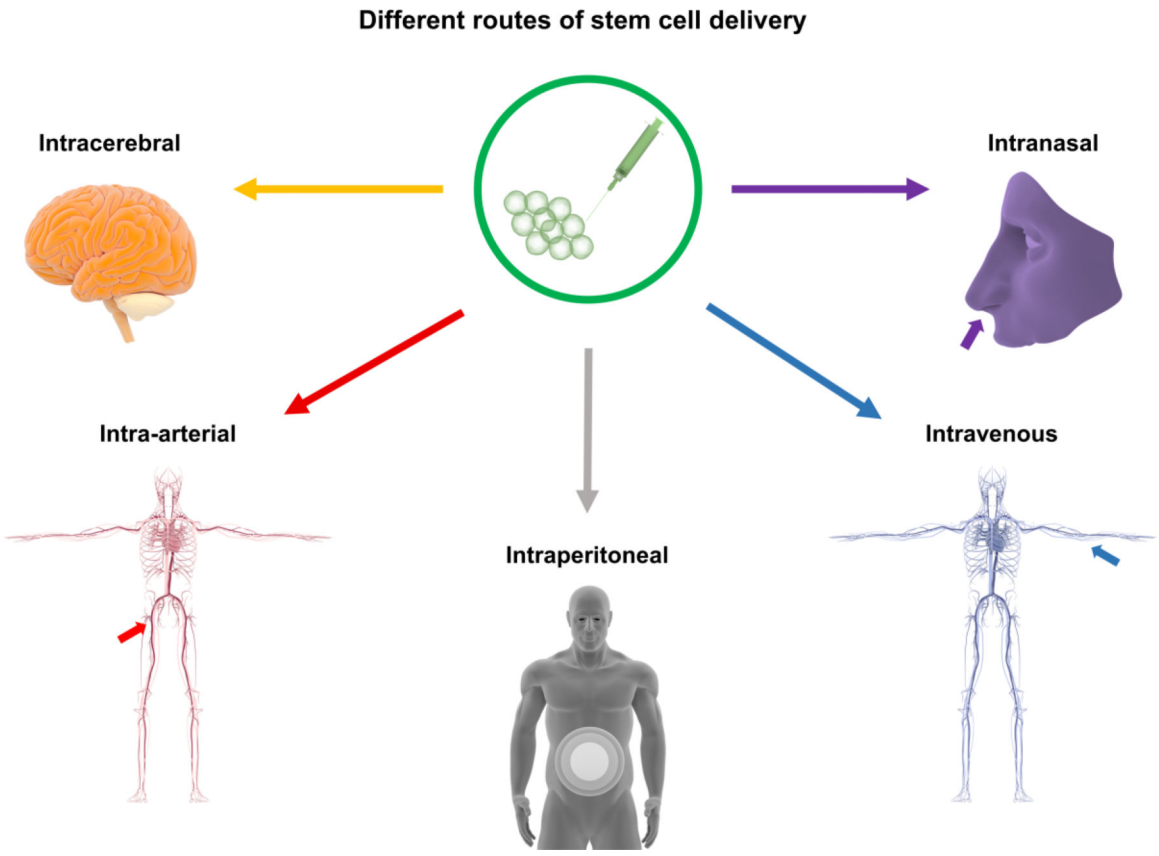

Figure 1.
Different routes of stem cell delivery. Stem cell transplantation for stroke can be administered via several routes, including intracerebral, intra-arterial, intraperitoneal, intraventricular, intravenous, and intranasal treatment.

Table 1.

Routes, doses, and timing of stem cell administration in stroke.

Type of Study	Route	Type of Stem Cells	Physiological Effect	Dose	Timing	Ref.
Preclinical	IP	MSCs MNCs	Improved peripheral distribution compared to IV	1 million	1 day	[118]
Preclinical	IC	CTX0E03	Promotion of behavioural recovery and endogenous neurogenesis	4500, 45000 or 450000	4 weeks	[133]
Clinical	IC	SB623	Improvement in clinical outcome end points	2.5, 5, 10 million	6 – 60 months	[134]
Preclinical	IA	hESCs	Decreased brain injury	1 million	1 day	[135]
Clinical	IA	Autologous CD34+ selected stem/ progenitor	Improvement in functional scores and reduction in lesion volume	100 million	7 days	[136]
Preclinical	IV	hNSCs	Improvement in functional recovery	5 million	1 – 3 days	[137]
Clinical	IV	MAPC	Safety and efficacy No significant improvement	400, 1200 million	1 – 2 days	[138]
Preclinical	IN	BMSCs	Reduction in brain infarct volume and cell death	1 million	1 day	[119]
Clinical	IN	BMSCs	Improvement in clinical outcome	50 million	7 days	[139]