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SI: CHALLENGES AND CONTROVERSIES IN TRANSLATIONAL STROKE RESEARCH

Stem Cell Therapy and Administration Routes After Stroke

Berta Rodríguez-Frutos[1] · **Laura Otero-Ortega**[1] · **María Gutiérrez-Fernández**[1] · **Blanca Fuentes**[1] · **Jaime Ramos-Cejudo**[1] · **Exuperio Díez-Tejedor**[1]

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Abstract Cell-based therapy has demonstrated safety and efficacy in experimental animal models of stroke, as well as safety in stroke patients. However, various questions remain regarding the therapeutic window, dosage, route of administration, and the most appropriate cell type and source, as well as mechanisms of action and immune-modulation to optimize treatment based on stem cell therapy. Various delivery routes have been used in experimental stroke models, including intracerebral, intraventricular, subarachnoid, intra-arterial, intraperitoneal, intravenous, and intranasal routes. From a clinical point of view, it is necessary to demonstrate which is the most feasible, safest, and most effective for use with stroke patients. Therefore, further experimental studies concerning the safety, efficacy, and mechanisms of action involved in these therapeutic effects are required to determine their optimal clinical use.

Keywords Administration routes · Stem cell therapy · Stroke · Translational research

Berta Rodríguez-Frutos, Laura Otero-Ortega and María Gutiérrez-Fernández contributed equally to this work.

✉ María Gutiérrez-Fernández
mgutierrezfernandez@salud.madrid.org

✉ Exuperio Díez-Tejedor
exuperio.diez@salud.madrid.org

[1] Department of Neurology and Stroke Center, Neuroscience and Cerebrovascular Research Laboratory, Neuroscience Area of IdiPAZ (Health Research Institute), Autonomous University of Madrid, La Paz University Hospital, Paseo de la Castellana 261, 28046 Madrid, Spain

Introduction

Stroke is the leading cause of death and disability worldwide. Pathophysiological responses after stroke are complex, and there is currently no therapy to repair the damage triggered after the insult. Only i.v. thrombolysis (tPA), endovascular treatment, and their management in stroke unit are effective therapies to treat the acute phase [1], as well as early diagnosis and hemostasis. Other therapies include open surgery or minimally invasive surgical techniques to remove clots and intraventricular blood and management of intracranial pressure in intracerebral hemorrhages [2]. Along these lines, cell-based therapy has emerged as a novel strategy for stroke treatment. Based on the results of preclinical studies, clinical trials have been conducted on stroke patients (Table 1). Most studies have evaluated the safety of stem cell-based therapy; however, details need to be refined before establishing stem cell as an effective and common stroke treatment.

An important aspect of stem cell therapy that is still to be determined is the most appropriate route of administration. Several delivery routes have been used in experimental stroke models, including intracerebral, intraventricular, subarachnoid, intra-arterial, intraperitoneal, intravenous, and intranasal.

We review here the status of the routes used in the treatment of stroke, their therapeutic effects, and their advantages and disadvantages in terms of clinical translation.

Routes of Administration in Stroke

Stem cell treatment after stroke has led to improvements in functional outcome in preclinical studies. Intracerebral, intraventricular, subarachnoid, intra-arterial, intraperitoneal, intravenous, and intranasal administration were the optimal routes for treatment. The most appropriate route, however, is still

Table 1 Ongoing and completed clinical trials in stroke. All listed trials are small safety and feasibility studies

Clinical trials

NCT	Country	Design	Cell type	Doses	Route	Time from stroke onset	Simple size	Current status[a]
NCT01151124	UK	Phase 1 Single group assignment Open label	Allogeneic Human neural stem cells	Ascending doses −2, 5, 10, 20 millions	IC	>6 months and <5 years	12	Ongoing, not recruiting
NCT01714167	China	Phase 1 Nonrandomized Open label	Autologous BM-MSC	2–4 millions	IC	>3 months and <60 months	30	Recruiting
NCT01327768	China	Phase 1 Randomized Single blind	Autologous OECs	2–8 millions	IC	>6 months and <60 months	6	Unknown
NCT01438593	China	Phase 1 Single group assignment Open label	Allogeneic Purified umbilical cord blood CD-34	5 millions	IC	>6 months and <60 months	6	Unknown
NCT00950521	China	Phase 2 Randomized Open label	Autologous peripheral blood CD-34	2–8 millions	IC	>6 months and <60 months	30	Completed Results not published yet
NCT02117635	UK	Phase 2 Single group assignment Open label	Allogeneic Human neural stem cells	20 millions	IC	>28 days	41	Recruiting
NCT02448641	USA	Phase 2 Randomized Double blind	SB623	2 groups: −2.5 millions −5 millions	IC	>6 months and <60 months	156	Ongoing, recruiting not started
NCT02245698		Phase 1 Nonrandomized Open label	Autologous BM-MNC	ND	Intrathecal	ND	200	Recruiting
NCT01832428	India	Phases 1 and 2 Single group assignment Open label	Autologous BM-MNC	100 million/week for 3 weeks	Intrathecal	ND	50	Recruiting
NCT01518231	China	Phase 1 Randomized Open label	Autologous Hematopoietic SC	4 millions	IA	<1 year	40	Unknown
NCT00535197	UK	Phases 1 and 2 Single group assignment Open label	Autologous CD34+ BM-MSC	ND	IA	<7 days	10	Unknown
NCT01273337	USA	Phase 2 Randomized Double blind	ALD-401 BM-derived	ND	Intra-carotid	13–19 days	100	Ongoing, not recruiting
NCT00473057	Brazil	Phase 1 Single group assignment Open label	Autologous BM-MSC	500 millions	2 groups: -IA (10 patients) - IV (5 patients)	>3 h and <90 days	15	Completed Results not published yet
NCT01453829	Mexico	Phases 1 and 2 Single group assignment Open label	Autologous AD-stromal cells	ND	IA and IV	ND	10	Unknown
NCT02564328	China	Phase 1 Randomized Single blind	Autologous BM-MSC	ND	IV	>6 months and <60 months	40	Recruiting
NCT01468064	China	Phases 1 and 2 Randomized Single blind	Autologous BM-MSC EPCs	2.5 million per kg (2 doses in 2 weeks)	IV	Recruitmen t <7 days treatment: 4 weeks after recruitment	20	Recruiting
NCT02605707	China	Phases 1 and 2		ND	IV		30	Recruiting

Table 1 (continued)

Clinical trials

NCT	Country	Design	Cell type	Doses	Route	Time from stroke onset	Simple size	Current status[a]
		Randomized Single blind	Autologous EPCs			>6 months and <60 months		
NCT01091701	Malaysia	Phases 1 and 2 Randomized Double blind	Allogeneic MSC	2 millions per kg	IV	<10 days	78	Unknown
NCT01297413	USA	Phases 1 and 2 Single group assignment Open label	Allogeneic BM-MSC	0.5–1.5 millions per kg	IV	>6 months	38	Recruiting
NCT01922908	USA	Phases 1 and 2 Randomized Double blind	Allogeneic BM-MSC	Dose escalation	IV	3–10 days	48	Ongoing, recruiting not started
NCT02378974	Republic of Korea	Phases 1 and 2 Randomized Double blind	Allogeneic UC-MSC	2.0×10^8	IV	<7 days	18	Recruiting
NCT01461720	Malaysia	Phase 2 Nonrandomized Single blind	Autologous BM-MSC	ND	IV	2 weeks–2 months	50	Recruiting
NCT01436487	USA UK	Phase 2 Randomized Double blind	MultiStem	Dose escalation 400 millions 1200 millions	IV	1–2 days	140	Ongoing, not recruiting
NCT00875654	France	Phase 2 Randomized Open label	Autologous BM-MSC	ND	IV	<6 weeks	30	Recruitment complete Ongoing follow-up
NCT01678534	Spain	Phase 2 Randomized Double blind	Allogeneic AD-MSC	1 million per kg	IV	<2 weeks	20	Recruiting
NCT01436487	USA UK	Phase 2 Randomized Double blind	MultiStem	Dose escalation 400 millions 1200 millions	IV	1–2 days	140	Ongoing, not recruiting
NCT02580019	China	Phase 2 Randomized Open label	Allogeneic UC-MSC	2×10^7 /week for 4 weeks	IV	<3 months	2	Not yet recruiting
NCT01716481	Republic of Korea	Phase 3 randomized Open label	Autologous Autoserum cultured MSC		IV	<90 days	60	Recruiting

AD-MSC adipose-derived mesenchymal stem cells, *BM-MNC* bone marrow mononuclear cells, *BM-MSC* bone marrow mesenchymal stem cells, *EPCs* endothelial progenitor cells, *IA* intra-arterial, *IV* intravenous, *MSC* mesenchymal stem cells, *NCT* ClinicalTriasl.gov identifier, *ND* no data, *OECs* olfactory ensheathing cells, *UC-MSC* umbilical cord mesenchymal stem cells

[a] According to information available in ClinicalTrials.gov.org and PubMed. Updated by 24th, February 2016

unknown. Below, we describe studies that have used a variety of routes or pathways to administer stem cell-based treatment.

Intracerebral, Intraventricular, and Subarachnoid

During the emergence of cell therapy, one of the goals of transplanted exogenous stem cells was to reconstruct the cytoarchitecture of the damaged tissue after stroke. This therapy requires the survival of grafted cells in an inhospitable milieu including inflammation, cell death, and glial scar [3, 4].

It was initially thought that intracerebral administration was the best way for exogenous neural stem cells to reach the brain. These cells have the capacity to self-renew and to generate neural cells [5], which have been shown to have the ability to replace the neurons lost to stroke [6]. In addition to the potential of neural stem cells to replace the lost neurons, several studies have found therapeutic effects of this kind of cell via paracrine mechanisms, given exogenous transplanted neural stem cells express messenger RNA (mRNA) and secrete several growth factors in vitro. However, many types of

stem cells can perform this paracrine function, and they can also differentiate into multiple lineages, which could be useful for replacing the cells in damaged brain areas [7, 8]. Along these lines, not only neural stem cells [9–11] but also embryonic stem cells [3, 12] induced pluripotent stem cells (iPSC) [13, 14], and mesenchymal stem cells [15–17] are being used for the treatment in animal models via administering them by the intracerebral route. Thus far, the success rate in attaining a mature neuronal phenotype appears to be approximately 2–20 % of exogenous mesenchymal stem cells [4, 18]. Thus, intracerebral administration showed implanted cells in the lesion size in comparison with other delivery routes because several million cells are transplanted into the brain and approximately 1/3 of the stem cells migrate toward the damaged regions [19–21] as well as to the intact hemisphere [22, 23].

Regarding subcortical cerebral infarct, endogenous neural precursor cells from subventricular zone (SVZ) migrate to the peri-infarct striatum. However, whether endogenous neurogenesis is involved in spontaneous recovery is still unknown. Thus, even with endogenous neurogenesis, intrastriatal injection of human iPSC contributes to recovery because these cells have the potential to survive differentiation into immature and mature neurons, demonstrating that this route of administration is feasible in subcortical stroke [24].

There are, however, less invasive methods than direct stereotaxic implantation into the parenchyma. Routes such as the intraventricular [4] and subarachnoid [25, 26] have been used for the treatment of stroke in rats, obtaining an enriched environment, cell survival, and recovery. Not only intracerebrally transplanted cells have the potential to replace the lost neural connections but also intraventricularly administered exogenous stem cells can also reach the lesion area after stroke. Thus, direct brain grafting of transplanted cells has yet to prove safety as an alternative route for intracerebral delivery.

Clinical trials have demonstrated the safety and feasibility of the use of neural stem cells. An improvement was reported in some patients; however, the trial results did not show a significant benefit related to motor function [27]. Although no tumor formations have been observed, along these lines, a clinical trial using intraparenchymal cell implantation showed that three patients had no adverse cell, procedure, or imaging-defined effects. This was the first report on ischemic stroke patients regarding the transplantation of nontumor cells [28]. On the other hand, the use of a neural stem cell line, in particular CTX0E03, is being evaluated in the PISCES trial to test their safety after injection (NCT01151124) in patients [29]. Other authors have proven the effectiveness of bone marrow stem cells, finding that any important adverse events resulted from surgery and observing some improvements in terms of neurological patients' conditions [30, 31]. Some clinical trials that used the intracerebroventricular paradigm have been performed using fetal cells [32] and autologous bone marrow stem cells [33]. In these trials, functional activity significantly increased compared with a clinically compatible control group.

Several problems are associated with using the intracerebral or intraventricular route for stem cell administration for brain repair: invasiveness, poor cell availability, immune rejection, and an uncertain fate in the brain, which present hurdles to the translational application of cell therapy [34]. To solve these matters, less invasive routes are promising candidates for cell-based therapy after stroke.

Intra-arterial

Many studies have reported good stem cell therapy results using the intra-arterial route in experimental animal models of stroke. The more common method of intra-arterial administration is to use catheterization to guide cells into the carotid artery, which prevents initial uptake by systemic organs to enable the delivery of large numbers of cells directly to the brain lesion [35].

Using intra-arterial delivery, however, even via the carotid artery, fewer exogenous cells (1–10 %) arrive at the lesion area [36], as expected. Administered stem cells have the potential to replace the lost neural connections, to produce and to stimulate the release of trophic factors enhancing brain repair mechanisms [35]. After stroke, neurogenesis in both the SVZ and subgranular zone is increased in order to produce proliferation and migration of neuroblasts to the lesion areas replacing the neuron loss caused by stroke. However, only a few of the neuroblasts differentiate and survive in the long term. It has been reported that stromal cell-derived factor 1 could play an important role in this process, acting as a chemoattractant and improving the survival of these endogenous stem cells [37].

In recent years, several subtypes of cell therapies have been developed using the intra-arterial route in experimental animal models of stroke. Some studies have reported that intra-arterially administered neural stem cells are related to successful recovery after stroke. These observations show that grafted cells do not need to be close to the damaged area to be effective. Neural stem cells are not the only type of stem cell; human umbilical cord blood mononuclear cells, umbilical cord mesenchymal stromal cells [38], and mesenchymal stem cells [35, 36, 39] are also being used for the treatment of stroke lesions in animal models in an attempt to recover functional activity after stroke using an intra-arterial route.

Before translation to clinical trials, many basic details regarding administration require investigation because safety reports after intra-arterial transplantation are still contradictory, with observations ranging from successful recovery to increased mortality of test animals. One report suggests that the mortality of stroke rodents after intra-arterial neural stem cell

grafting is reduced when the cells are delivered via microneedles instead of catheters [40].

In addition, microemboli have been reported in some cases [41]; however, some studies report no adverse effects from the microemboli [35]. Along these lines, cell dose and infusion velocity lead to increased complications after intra-arterial cell administration, as well as cell size. For example, one study showed that a cell dose-related decline in cerebral blood flow was related to an increase in embolic events, and low infusion velocity was associated with an increase in complications [42]. In contrast, another study showed that embolic events could be related to fast infusion velocity [43]. These observations indicate that infusion velocity requires further study because the results of the studies are still contradictory.

Regarding efficacy, there are also contradictory results and issues to solve before clinical implementation. Many studies have reported good stem cell therapy results using the intra-arterial route in an experimental animal model of stroke [35, 36, 38]. However, a study by Mitkari et al. observed that exogenous bone marrow mesenchymal stem cells did not improve recovery in middle cerebral artery occlusion (MCAO), despite effective homing to the infarcted hemisphere and enhanced angiogenesis in rats [44].

These preclinical results suggest that for effective clinical implementation, identification of the best administration route in patients is mandatory for new recommendations on cell-based therapies after stroke [45].

Clinical trials of cell therapies for stroke have used the intra-arterial route. Moniche et al. showed that intra-arterial bone marrow mononuclear cell administration is feasible and appears to be safe [46]. Moreover, the infusion of intra-arterial autologous bone marrow mononuclear cells appears to be safe. This trial showed that 30 % of the patients with moderate to severe acute strokes demonstrated satisfactory clinical improvement and 40 % of the patients had a good clinical outcome at 90 days [47]. In addition, intra-arterial transplantation of autologous bone marrow mononuclear cells in nonacute ischemic stroke showed that cell transplantation is safe and feasible [48].

Intravenous

In terms of clinical feasibility and considering that intravenous cell delivery has proven to be noninferior with comparable protective properties, the intra-arterial cell delivery route is clearly less attractive. Interestingly, comparing intravenous with intra-arterial routes shows both to be equally effective [35].

Many preclinical studies have obtained promising results after intravenous administration of cell-based therapy after stroke. Various types of cells and cell sources have demonstrated efficacy after stroke. For example, intravenously administered exogenous bone marrow stromal cells enter the brain, migrate, survive, and improve recovery [49]; bone marrow mononuclear cells can reduce lesion size and improve functional outcomes in a rat model [50]; and adipose-derived mesenchymal stem cells improve sensorimotor dysfunction, enhance brain plasticity, and attenuate the inflammatory reaction and apoptosis [51, 52]. However, these beneficial effects after cell-based therapy do not take place equally in all situations after stroke. In the case of aged rats, the brain is refractive to growth after damage, showing a loss of regenerative capacity. In this sense, cell administration after stroke might not be sufficient to improve this recovery in an aged brain environment [53]. However, a recent study has provided the first evidence that iPSCs transplanted into the stroke-damaged cortex survive, differentiate into neurons, and improve recovery in the aged brain of rats [54].

In the past, it was thought that transplanted cells replaced lost brain cells to carry out their functions. Therefore, the cells initially administered were neural progenitors or cells more differentiated to neurons. After intravenous transplantation of neural stem cells in rodents with intracerebral hemorrhage, these exogenous cells migrated and differentiated into neurons and astrocytes. In Jeong et al. study, the authors observed the survival and migration of the administered cells, and the rodents showed improved functional recovery [55]. In another study, a small percentage of the neural stem cells injected had accumulated in the infarct boundary zone after ischemic stroke. Most of these exogenous cells remained undifferentiated, however, up to 30 days after intravenous administration [56].

On the other hand, the neural stem cells administered were observed to migrate primarily to the spleen instead of the brain. This treatment reduced inflammation, edema formation, and apoptosis after damage. Because these effects were not observed in splenectomized rats, the study suggested that it provided this protection by interrupting splenic inflammatory responses [57]. Similarly, a recent study observed that intravenous human bone marrow stem cells (4×10^6) administered 60 days after stroke migrated to the spleen more than the brain, attenuated the stroke-induced inflammation, and decreased the infarct area in the striatum. This study thus demonstrated that intravenous stem cells used as a treatment for post-acute stroke could possibly work by abrogating inflammation-plagued secondary cell death [58].

In the clinical context, there are several studies that have used cell-based therapy with intravenous administration. Bang et al. observed that intravenous infusion of autologous mesenchymal stem cells was a safe treatment that could improve neurological deficits, based on five patients with severe stroke [59]. Another study was later performed to evaluate the safety and efficacy of autologous intravenous mesenchymal stem cell administration to a larger population and demonstrated that this treatment was safe for stroke patients based on 5 years of follow-up [60]. In another study, administration of autologous human mesenchymal stem cells expanded using

autologous human serum demonstrated the safety of these cells and reported that the mean lesion volume was reduced by greater than 20 % at 1 week after cell administration [61].

There are many studies on other types of cells administered intravenously, such as bone marrow mononuclear cells, which evaluated whether this therapy was feasible and safe in patients with cerebral infarct [62, 63]. After autologous transplantation in patients in the chronic phase of stroke, researchers observed an improved modified Barthel Index and increased brain plasticity without adverse effects [64]. Another recent study showed neurological recovery and an improvement in cerebral blood flow [65]. All the above-mentioned studies indicated that autologous mononuclear stem cell administration was safe and feasible in stroke patients.

There are currently 17 ongoing and completed studies in clinical trials [29] using the term "intravenous administration of stem cells in stroke". One is a phase 1 study in which autologous bone marrow mononuclear cells are administered in a peripheral intravenous infusion after acute ischemic stroke (NCT00859014). Another phase 1 study is using autologous bone marrow administration intravenously and intra-arterially (NCT00473057). There are several phase 2 clinical trials in acute ischemic stroke: one study use intravenous bone marrow mononuclear cells to study the safety, feasibility, and efficacy (NCT01501773). Our group is performing an ongoing phase 2a clinical trial named AMASCIS-01, in which intravenous, allogenic mesenchymal stem cells obtained from adipose tissue are delivered in the acute phase of stroke (NCT01678534). A phase 3 trial is ongoing, named STARTING-2 (NCT01716481), and it is the first to analyze the efficacy of autologous mesenchymal stem cells expanded in autologous ischemic serum obtained from patients with ischemic stroke to enhance therapeutic efficacy. The results obtained in this trial might show better evidence for the effectiveness of mesenchymal stem cell treatment [66].

Intraperitoneal

A recent study compared two routes of stem cell administration: intraperitoneal and intravenous. The authors reported that the route of administration influenced cell distribution after brain injury. The intravenous route demonstrated a greater number of cells in the lung and brain in the mesenchymal stem cells and in the spleen, liver, and lung using mononuclear cells compared with the intraperitoneal group. Therefore, the route of administration might influence the effect of the cells [67].

Intranasal

Intranasal delivery is one of the newest routes used in the treatment of cell-based therapy in stroke. Little research has been performed to date and has only used experimental animal models. In this sense, a mouse cerebral ischemia model analyzed a delayed treatment of bone marrow mesenchymal stem cells by intranasal delivery. The authors observed that intranasal administration enhanced cell homing to the ischemic zone and optimized therapeutic efficacy [68]. Another group observed that intranasal bone marrow mesenchymal stem cell in neonatal stroke in rats reduced lesion area and blood-brain barrier disruption, enhanced brain plasticity, and improved local cerebral blood flow, as well as showed better functional recovery [69]. Regarding intracerebral hemorrhagic stroke, treatment with bone marrow mesenchymal stem cells improved behavioral performance and increased neurogenesis in mice [70].

Moreover, van Velthoven et al. performed a comparative study between intranasal application of mesenchymal stem cells and BDNF-secreting mesenchymal stem cells in neonatal hypoxic-ischemic brain injury in rats. Both treatments attenuated ischemic brain damage, reducing gray and white matter loss and motor deficits and promoting cell proliferation after damage [71]. Other approaches to cell-based therapy, such as the administration of cell culture-condition medium, have been used to avoid possible tumorigenic effects and improve the low survival rates of cells administered intranasally. A recent study on rats observed that intranasal administration of cell-culture medium derived from human umbilical cord mesenchymal stem cells improved functional outcome, enhanced blood-brain barrier functional integrity, and contributed to vascular remodeling after stroke [72].

In terms of this type of administration in human patients, there are no current clinical trials using cell-based therapy [29].

Advantages and Disadvantages of Routes of Administration in Stroke Treatment

In this section, we describe the various advantages and disadvantages of the routes of administration after stroke (Fig. 1).

Intracerebral, Intraventricular, and Subarachnoid Route

One of the advantages of the intralesional route is that the transplanted cells attempt to reconstruct the cytoarchitecture of damaged tissue after stroke. It has been proven that these cells can at least partially replace the neurons lost to stroke [6, 20]. This type of administration route allows us to study the mechanisms underlying the differentiation process of the stem cell. The disadvantage of using this route is that it requires the survival of transplanted cells in an inhospitable milieu [3, 4]. To improve graft cell survival, proliferation, migration, and differentiation, various hydrogels such as hyaluronic acid, collagen gels, or Matrigel have been assessed as cell-seeded

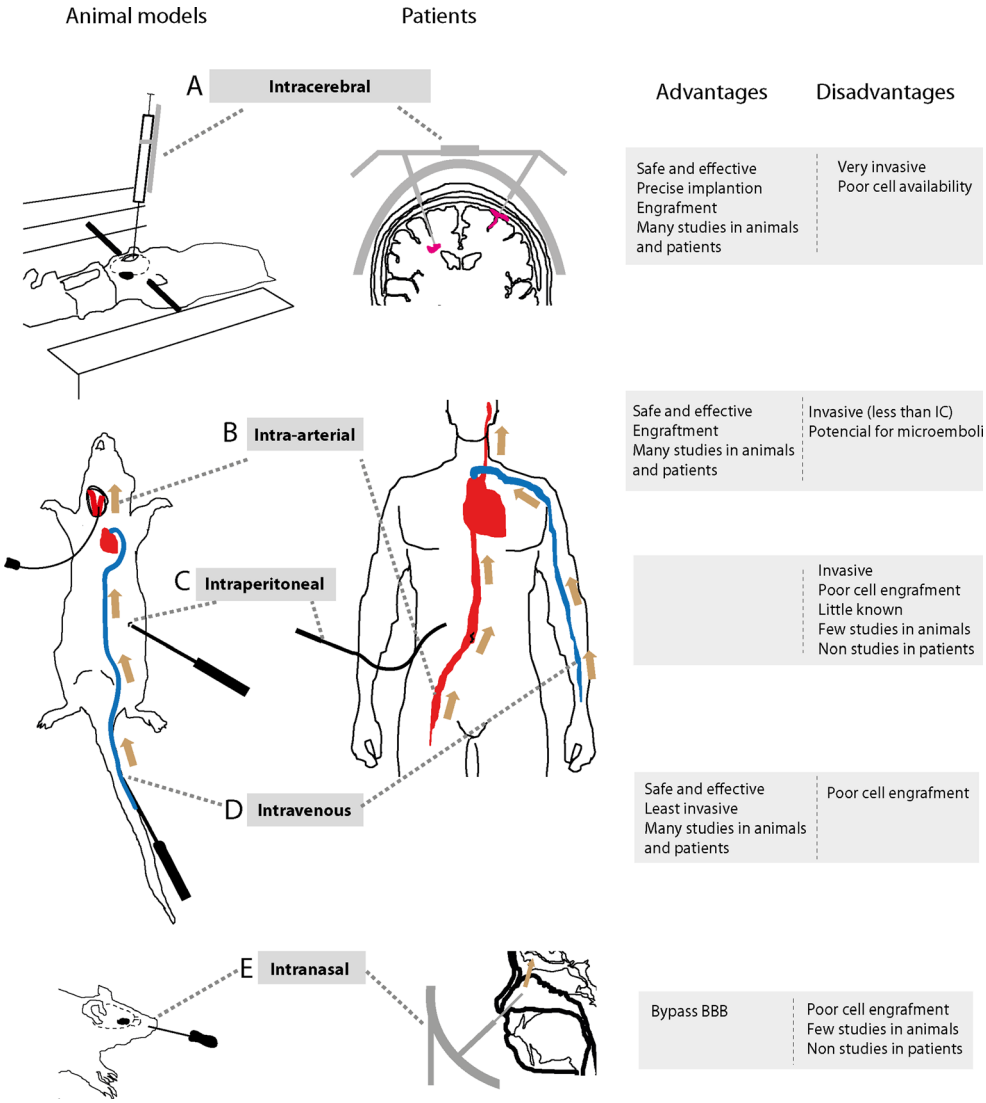

Fig. 1 Routes of administration of stem cells in preclinical studies and clinical applications. The most adequate route of administration for stroke is currently unknown. All present advantages and disadvantages for its clinical translation to stroke patients (*A*) intracerebral, (*B*) intra-arterial, (*C*) intraperitoneal, (*D*) intravenous, and (*E*) intranasal

scaffolds injected at chronic stages [73] to optimize functional recovery after stroke [74].

The brain is susceptible to damage from surgical intervention. Therefore, the use of these invasive surgical approaches in humans would have possible structural and functional consequences; thus, a balance between safety and efficacy is needed before clinical application of the intralesional route [75]. Currently, few clinical trials use these types of routes in the acute phase due to the risk of hemorrhagic transformation. Reducing the total volume of administration could be a good strategy to overcome this challenge. However, in the chronic phase, the risk of hemorrhagic transformation decreases significantly compared with the early phase. Thus, some clinical trials [30, 76] have used this intracerebral route during the delayed phases because it is safer and more appropriate for clinical applications.

Intra-arterial Route

A less invasive route compared with the intracerebral delivery, such as the intra-arterial, is a promising option for stem cell therapy in patients with stroke. Exogenous stem cells can also reach the brain using this type of delivery, showing efficacy in terms of functional recovery. However, some studies report a risk of vascular occlusion linked to the large size of the cells [77] or microemboli [41]. The vessel blockage occurs at the precapillary level, with obstruction of blood flow [75]. In order to avoid aggregates, a careful shaking of the cells prior to administration could improve this disadvantage. In this sense, another study did not demonstrate adverse effects from microemboli after intra-arterial administration [35]. The systemic route of administered stem cells could trigger vascular occlusion, given most cells do not actively extravasate. Thus, strategies to

increase extravascular activity from the vascular lumen to the parenchymal brain [78] or to target cells through overexpression of molecules [79] could increase engraftment of these cells to the brain. These strategies could minimize the formation of microemboli.

On the other hand, current treatments for stroke, such as thrombectomy, include an intra-arterial procedure that shows efficacy until 8 h after stroke [80]. Treatments including intra-arterial intervention represent an opportunity to combine that procedure with intra-arterial administration, possibly offering an advantage in terms of clinical translation.

Intravenous Route

In terms of clinical feasibility and considering that intravenous cell delivery has proven to be noninferior to other routes and having shown itself to be equally effective, the intravenous route of cell delivery is more attractive because it is less invasive for stroke patients. In fact, the most ongoing clinical trials use intravenous administration [73]. Using this route, the administered cells are confined to the peripheral organs, leading to low cell concentrations to the infarct zone [75]. Nevertheless, there are no reports detecting adverse effects linked to this trapping or to tumor formation.

Intraperitoneal

Because it is a novel administration route, further studies need to be conducted on experimental animal models to demonstrate feasibility, safety, and efficacy as a prior step to clinical application.

Intranasal Route

The intranasal route is incipient and could represent a less invasive, feasible, safe, and effective administration route. Some administered cells bypass the blood-brain barrier and maximize distribution to the central nervous system [81]. The cells migrate from the nasal and cross the cribriform plate through various routes, such as the olfactory bulb or the cerebrospinal fluid [82]. More experimental studies concerning minimal effective doses are needed to demonstrate the nonformation of clumps and others adverse effects. Injecting cells really slowly could reduce the clumping.

Summary

As previously described, various routes have been tested in experimental studies. The effects of stem cell therapy and distribution could vary depending on the route of administration. Cell-based therapy can be administered directly into the brain, requiring invasive techniques (intracerebral, intraventricular, subarachnoid), hematic (intra-arterial and intravenous) techniques, or intraperitoneal or intranasal administration. All these routes have been shown to be relatively safe with no major complications. Thus, currently, there are no reasons to discard any of them; however, less invasive routes offer several advantages for clinical applications.

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Compliance with Ethical Standards

Conflict of Interest All authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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