Expert Opinion

Intranasal delivery of stem cells to the brain

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Introduction: Stem cell-based therapy has proved to be a promising treatment option for neurological disorders. However, there are difficulties in successfully administrating these stem cells. For example, the brain–blood barrier impedes the entrance of stem cells into the CNS after systemic administration. Direct transplantation or injection may result in brain injury, and these strategies are clinically less feasible. Intranasal administration is a non-invasive and effective alternative for the delivery of drugs, vector-encoded viruses or even phages to the CNS. Recent studies have in fact demonstrated that stem cells may enter the CNS after intranasal administration. These results suggest that intranasal delivery may provide an alternative strategy for stem cell-based therapy.

Areas covered: This review summarizes current studies that have applied the intranasal delivery of stem cells into the brain. In addition, the distribution and fate of stem cells in the brain and the potential opportunities as well as challenges of intranasal stem cell delivery are also discussed.

Expert opinion: Intranasal delivery of stem cells is a new method with great potential for the transplantation of stem cells into the brain, and it may provide an extraordinary approach to overcoming the existing barriers of stem cell delivery for the treatment of many neurological disorders. This potential benefit emphasizes the importance of future research into intranasal delivery of stem cells.

Keywords: brain–blood barrier, intranasal, neurological diseases, stem cells, stroke

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1. Introduction

Stem cells have been shown to have a great potential for the treatment of many neurological disorders [1-5]. Stem cells retain the ability to differentiate into neurons [6], and they can produce neurotrophic growth factors to protect existing neurons from various cytotoxic insults [7]. Although there are many basic sciences studies and preclinical data suggest that stem cells are a viable option for the treatment of neurological diseases [8,9], substantial clinical applications of stem cell therapy have not been reported. One of the potential barriers to stem cell therapy for the treatment of neurological diseases is the lack of a safe and efficient delivery method. To address this issue, a primary focus of The Stroke Therapy Academic Industry Roundtable (STAIR) is to bring drugs effectively into the brain to further neurological disease research [10]. At present, the routes used for stem cell delivery to the brain are either invasive, such as intracerebroventricular injection, or inefficacious because of the existing blood–brain barrier (BBB), such as intravenous injection. Thus, the development of a new approach is necessary for stem cell-based therapy.

Intranasal administration can deliver peptides [11,12], chemical drugs [13], metals [14,15], viruses [16], plasmid [17], siRNA [18] and bacterial phages [19] into the

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brain directly. Substances following intranasal administration have access to the brain through olfactory, trigeminal, vascular or cervical node routes [20]. Intranasal drugs may prevent living neurons from further damage in ischemic stroke [21] and Alzheimer's disease [22]. However, current drug-based therapies are limited. Without the full complement of neurons, the patient may still experience the underlying symptoms of neurological diseases.

Recent studies have shown that with intranasal delivery, stem cells could also get into the brain to treat hypoxia-ischemia (HI), Parkinson's disease and ischemic stroke, bypassing the BBB [23-25]. In this review, the following are discussed: the detailed method and pathway; applications in neurological diseases; and the limitations and perspectives of intranasal delivery of stem cells to the brain.

2. Stem cell therapy in neurological diseases

2.1 Definition and classification of stem cells

Stem cells have the ability to self-renew and differentiate into other types of cell. At present, stem cells are divided into two types, embryonic stem cells (ESCs) and adult stem cells. ESCs, which are derived from the inner cell mass of the 16-cell stage embryo, have an unlimited capacity to self-renew and differentiate into nearly every cell type of the organism [26]. Among the major concerns of ESCs in clinical application are the inherent ethical problems and potential tumor formation. Adult stem cells exist in the bone marrow and many other organs, including the brain. Bone marrow stem cells (BMSCs) are the most studied among mesenchymal stem cells. BMSCs have been applied for the treatment of hematological and other diseases for many years. Non-hematopoietic BMSCs may exert neuroprotection solely through the excretion of neurotrophic growth factors because BMSCs cannot differentiate into functional neurons [27]. Neural stem cells (NSCs) were first detected in rodents in 1965 and have been defined recently in the adult human brain [28]. NSCs are able to differentiate into neurons and other neural cells; however, they can only be collected in fetal brain tissue, which may limit their therapeutic potential. Induced pluripotent stem cells (iPSs) are ESC-like cells that are derived from fully differentiated somatic cells by the transfection of defined reprogramming genes [29,30]. Thus, iPSs overcome the ethical problem of ESCs, the restricted differentiation of BMSCs and limited sources of NSCs. However, iPSs may generate tumors, suggesting that they need to be evaluated further before clinical application.

2.2 Stem cell application in neurological diseases

Stem cells may treat neurological disorders by means of cell replacement or the production of neurotrophic factors. Basic researches and clinical trials of stem cells have concentrated mainly on the treatment of cerebral vascular diseases, traumatic injury, neurodegenerative diseases and immune system-mediated neurological diseases.

2.2.1 *Cerebral vascular diseases*

Cerebral vascular diseases have typically consisted of ischemic and hemorrhagic stroke, which are the leading cause of death and long-term disability in adults worldwide [31]. A recent study by Jin *et al.* [1] investigated the potential of using stem cells for the treatment of ischemic stroke by injecting neural precursor cells (NPCs) directly into the ischemic regions of rat brains and found that these stem cells differentiated into neurons. This neural regeneration resulted in a decreased infarct volume of 50% and an improvement of rat sensorimotor and cognitive functions. Similarly, Lee *et al.* [32] reported an improved functional recovery after the transplantation of human NSCs overexpressing brain-derived neurotrophic factor (BDNF) into mice with hemorrhagic stroke.

2.2.2 *Traumatic brain injury*

Traumatic brain injury (TBI) is a devastating injury that is characterized by the progressive loss of neurons and lifelong neurological defects. Unfortunately, it has been shown that NSCs placed around the area of injury remained in the tissues 2 weeks after placement and significantly improved motor function in rats [2]. Apart from differentiating into neurons, grafted stem cells expressed and released glial-cell-line-derived neurotrophic factor (GDNF), which protects neurons from secondary damage and contributes to neural regeneration of the host [33].

2.2.3 *Neurodegenerative diseases*

Parkinson's disease is a model neurodegenerative disease for stem cell therapy because of the progressive loss of dopaminergic neurons in Parkinson's disease patients. A promising study utilizing a hemiparkinsonian rat model demonstrated that transplanted stem cells survived, integrated and differentiated into dopaminergic neurons, which ultimately alleviated behavioral motor asymmetry [3]. Recently, a clinical trial of stem cell therapy for amyotrophic lateral sclerosis (ALS) revealed that the transplantation of BMSCs into the end of the brain stem and the anterior part of the spinal cord alleviated symptoms of 9 out of 13 patients when compared with their preoperative status during the 1-year follow-up [4].

2.2.4 *Immunity-related disease*

Multiple sclerosis is an inflammatory autoimmune disease of the CNS. Transplantation of ESCs into the ventricles of mice with experimental autoimmune encephalomyelitis (EAE) significantly alleviated the clinical signs of EAE. This therapeutic effect most probably occurred through an immunosuppressive neuroprotective mechanism because the differentiation of ESCs to mature oligodendrocytes and the remyelination of the white matter in EAE were negligible [5]. A recent clinical trial demonstrated that non-myeloablative autologous hematopoietic stem cell transplantation in patients with relapsing-remitting multiple sclerosis reversed neurological deficits [9].

2.3 The routes of stem cell delivery to the brain

A key step for progressing stem cell-based therapy from the bench to the bedside is to find routes that target the CNS both safely and efficiently. Several routes have been designed for the delivery of stem cells to the CNS. The advantages and disadvantages of these routes are summarized in **Table 1**.

Direct transplantation of stem cells was first developed for stem cell delivery to the brain [34]. Typically, surgery is needed to transplant the grafts or stem cells to the desired location in the CNS. Therefore, excess injury induced by the surgery precludes this method from being utilized in the clinic. Indeed, it is almost completely abandoned except for the investigation of stem cell mechanisms *in vivo*.

Intracerebroventricular (ICV) or intracerebral parenchyma injection is another technically difficult method [35], which requires special equipment and experienced surgeons. Stem cells scarcely moved beyond the injection site, which limits the usefulness of this method because the lost neurons in some neurological disorders are distributed throughout the entire brain.

Intra-arterial administration uses catheterization to implant stem cells into the carotid artery or Willis circle [36]. Intra-arterial administration has a greater biological distribution than intravenous delivery [37]. However, expensive equipment and experienced interventionalists are also required for the catheterization, and stem cells in the artery may form microemboli, which could block blood vessels and cause an ischemic stroke [38].

Intravenous delivery of stem cells is the most widely used method because of limited complications during the process of administration [39]. However, like other high-molecular-mass substances, stem cells cannot cross the BBB. Moreover, the biological distribution of intravenous injected stem cells is poor [40].

Intrathecal administration of stem cells is done by lumbar puncture [41]. This method of administration has the advantage of being able to deliver the cells directly into cerebrospinal fluid (CSF). Complications from lumbar puncture arise in almost one-third of patients after the procedure [42]. Furthermore, the stem cells will ultimately be washed away by the CSF.

Intranasal delivery is a new administration of stem cells to the brain and will be discussed in detail below.

3. Intranasal delivery

3.1 Blood–brain barrier

The main benefit of intranasal delivery of stem cells is the ability to circumvent the BBB and directly target the CNS. The vascular BBB occurs at the level of tight junctions between adjacent brain endothelial cells that are encircled by the basement membrane and sheathed by the astrocytic end feet [43]. Drugs are able to cross the BBB through lipid-mediated free diffusion, carrier-mediated transport systems, active efflux transport and receptor-mediated transport. Although many substances such as glucose, amino acids and regulatory proteins could access to the brain, > 98% of hydrophilic and almost 100% of high-molecular-mass drugs might not cross the BBB successfully. Therefore, it is necessary to develop an alternative drug delivery method to bypass the BBB. For details on the BBB, please see [44].

3.2 Anatomy, histology and physiology of nasal cavity

The nasal cavity consists of the vestibule, atrium, respiratory region and olfactory region. The respiratory region is the largest area in the nasal cavity, and it is covered by columnar non-ciliated cells, columnar ciliated cells, goblet cells and basal cells. The olfactory region is located in the roof of the nasal cavity and contains specialized olfactory receptor cells. Olfaction and conditioning of inspired air for travel to the lungs are the two major functions of the nasal cavity. For details on the nasal cavity, see [45,46].

3.3 Intranasal delivery of drugs to the brain

Delivering drugs through the nasal cavity has occurred for a very long time and can be traced back to AD 150 [47]. The first intranasal delivery of drugs to the brain was first established by Dr William II Frey in 1989. Intranasal delivery should be utilized in the following four circumstances: intranasal delivery validation; intranasal delivery pathway elucidation; delivery of various therapeutics for the treatment of neurological diseases; and enhancing the efficiency of intranasal delivery.

3.3.1 Intranasal delivery of different substances to the CNS

Since Dr Frey proved the existence of the route from the nose to the brain in 1989, there has been accumulating evidence verifying this unique technology [48]. Peptides [11,12], chemical drugs [13], metals [15], viruses [16], plasmid [17], bacterial phages [19] and cells [25,49] have been administrated to the brain through intranasal delivery. Furthermore, intranasal delivery has also been applied in some clinical trials [50-56]. Born *et al.* [52] intranasally administered three peptides, melanocortin, vasopressin and insulin, to 33 healthy humans (9 female and 27 male) and found that these peptides moved to the CSF within 30 min, bypassing the bloodstream. Other researchers have demonstrated that the intranasal delivery of insulin improves the memory of humans, which could be used to treat Alzheimer's diseases [53-57]. However, some researchers have claimed that there is no significant increase in drug concentrations in the CNS after intranasal delivery when compared with systemic delivery [58].

3.3.2 The pathways of intranasal delivery

Although the exact mechanism of intranasal delivery is not understood, there is a great deal of evidence suggesting that the olfactory nerve pathways, trigeminal nerve pathways, vascular pathways and lymphatic pathways are involved.

Table 1. Routes of stem cell to the brain.

Route	Advantages	Disadvantages
DT	Getting to the brain directly	The trauma to the normal brain; need experienced surgeons
IA	Greater biological distribution than intravenous; less invasive	Microemboli formation
IV	Less invasive	Hardly crossing the BBB
IS	Getting to the CSF directly	Hardly crossing the BBB
ICV	Getting to the brain directly	The trauma to the normal brain; need experienced surgeons and equipment
IN	Less invasive; convenient	Some barriers in the nasal cavity

BBB: Blood–brain barrier; CSF: Cerebrospinal fluid; DT: Direct transplantation; IA: Intra-arterial; ICV: Intracerebral ventricle; IN: Intranasal delivery; IS: Intra-subarachnoid; IV: Intravenous.

Jansson and Bjork [59] used fluorescein dextran to visualize olfactory uptake and transfer and found that drug concentrations were highest in the olfactory bulbs; furthermore, there was a positive correlation between drug concentrations in the olfactory epithelium and the olfactory bulbs [60]. The results indicated that the olfactory nerve pathway was involved in intranasal delivery. Thorne *et al.* [61] used high-resolution phosphor imaging of brain and spinal cord after intranasal delivery of [^{125}I]-radiolabeled insulin-like factor I and found a widespread distribution in the CNS, which was highest in the trigeminal regions. There is increasing evidence of the vascular pathway being involved in intranasal delivery [62-64]. The vascular pathway is composed of the outermost layer of blood vessels and the basement membrane of the surrounding tissue. Essentially, the vascular pathway acts as a lymphatic system for the brain. The underlying mechanisms of rapid drug transportation following intranasal administration may be a result of bulk flow mechanisms and arterial pulsations, which are known as 'perivascular pump'. The vascular pathway was first implicated in intranasal delivery because substances such as amyloid beta or radiolabeled tracers could be cleared from brain interstitial fluid by entering the perivascular channels of cerebral blood vessels. Similarly, intranasally delivered drugs appeared in the perivascular spaces after removal of blood by saline perfusion. Furthermore, it has been found that intranasal delivery of [^{125}I]-IGF-I targeted the deep cervical lymph nodes to a much greater extent than intravenous administration. This distribution resulted in 18 times more [^{125}I]-IGF-I in the deep cervical lymph nodes than in the final blood sample following intranasal administration [61]. All four pathways participate in the process of intranasal delivery, and the dominant pathway depends on the properties of drugs, the formulation and the administration technique.

3.3.3 *Application of intranasal delivery in diseases of the nervous system*

The authors' and other laboratories have shown that intranasal delivery of drugs is effective at treating many diseases of the nervous system. Liu *et al.* [65] demonstrated that intranasal insulin-like growth factor I (IGF-I) improved neurological function after ischemic stroke in rats. This result was recently confirmed by Jiang *et al.* [21]. In addition, there have been promising results for the treatments of epilepsy [66], tumor [67], Alzheimer's disease [22,57], ALS [68] and multiple sclerosis [69] following intranasal delivery of drugs.

3.3.4 *Enhancing the efficiency of intranasal delivery*

Drugs can gain direct access to the brain following intranasal delivery; however, there are some barriers in the nasal cavity. The most probable delivery obstacles are mucosa barriers, the nasal mucociliary clearance, efflux transport proteins and drug-metabolizing enzymes in the nasal cavity [20]. Various approaches to overcome these barriers have included improved drug solubility through microemulsion [70] and nanoemulsion [71], increased membrane permeability via permeation enhancers and devices [72], and increasing the time at which a drug is at the delivery site by using the chitosan-based drug delivery system [73].

4. Intranasal delivery of stem cells to the brain

4.1 The procedure of intranasal delivery of stem cells to the brain

4.1.1 *Preparation of stem cells*

At present, all researchers tend to choose BMSCs as the model stem cell for intranasal delivery [25,49]. BMSCs are derived from the mononuclear cell fraction in the bone marrow after plastic adherence. However, the number of BMSCs in the bone marrow is very limited. Harvested cells were cultured *in vitro* (37°C, 5% humidified CO_2) in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum. BMSCs retain the ability to differentiate into myeloid and hematopoietic cells; therefore, low cytometry was used to characterize BMSCs with specific antigens. A fluorescent dye is introduced into the stem cells 1 day before harvesting them for intranasal delivery. Accordingly, this dye serves as a marker to trace stem cells in the brain that originated from the nose. Commonly used dyes for this purpose are Hoechst 33342, PKH-26 and

carboxyfluorescein diacetate. Cells are harvested and resuspended in phosphate-buffered saline (PBS). The final destiny of stem cells in the authors' laboratory and others is ~ 10^4 cells/μl [23].

4.1.2 Intranasal delivery

Until now, intranasal delivery of stem cells to the brain has been carried out in mice or rats [23,25,49]. First, the animals are held with a small roll of gauze under the dorsal neck, which allows the animals to recline on their backs while also immobilizing the skull. According to previous research [74], the head position is kept at an angle of 70° or 90°. Different head positions may alter stem cell absorption into the CNS following intranasal administration. A supine position with the head angle at 70° or 90° was found to give most efficient delivery. Second, a drop containing the cell suspension was carefully placed on one nostril, allowing it to be snorted. The volume of the drops was 3 and 6 μl for mice and rats, respectively. If the volume of the drop is too great, cell deposition will occur in the nasopharynx, which may lead to respiratory distress; if the volume of the drop is too small, deposition will occur primarily in the respiratory epithelium in the nasal cavity. During the placement of each drop, to facilitate snorting of the drops into the nasal cavity, the contralateral naris is occluded gently. The total volume of the stem cell suspension used for intranasal delivery depends on the species, with 24 for 12 μl for rats and mice, respectively. As discussed in Section 3.3.4, the nasal cavity has many protective barriers to prevent the entrance of foreign substances into the brain. To overcome the membrane barrier in the nasal cavity, hyaluronidase was used to promote access of stem cells to the brain following intranasal administration. Hyaluronidase [25] has the ability to loosen the barrier function of the nasopharyngeal mucosa and facilitate invasion [75]. This process enhances passage of BMSCs to the brain.

4.1.3 Distribution of stem cells in the brain following intranasal delivery

Stem cells were detected in the subarachnoid space, different layers of the olfactory bulb, thalamus and cerebral cortex post-intranasal delivery [25]. Further study showed that stem cells were predominantly in the glomerular layer of the olfactory bulb. There were no stem cells labeled with fluorescent dye in the subventricular zone, which is recognized as the location of NSCs in the adult [49]. There was a very interesting phenomenon in two studies that more stem cells administrated intranasally were detected in the damaged brain area. Van Velthoven *et al.* [49] demonstrated that there were more BMSCs in the damaged ipsilateral hippocampus in a mice model of HI and Wei *et al.* [23] showed that Hoechst-labeled BMSCs were found in and around the ischemic area in the middle cerebral artery occlusion (MCAO) mouse model. The mechanisms underlying the phenomenon were not very clear. Previous studies have suggested that damaged brain tissues express many substances, including growth factors such as GDNF and BDNF, which may drive stem cells into the damaged area. This hypothesis needs to be tested further. However, a large number of stem cells remained in the upper nasal cavity 1 h after intranasal administration even in the presence of hyaluronidase. This result indicated either that intranasal delivery of stem cells needs > 1 h to complete, or the efficiency of intranasal delivery is low and requires further optimization.

4.1.4 Fates of stem cells in the brain following intranasal delivery

Intranasally delivered stem cells are foreign objects to the host brain, which may result in a host versus graft reaction. Microglia are the most immunocompetent cells in the brain and labeled by CD45 when activated. It was found that intranasal stem cells labeled by carboxyfluorescein diacetate in the brain were separate from CD45-positive cells in the cortex. This result suggests that stem cells applied intranasally may escape the defense of the immune system [25]. However, it was found that the surviving BMSCs in the brain may not have differentiated into mature neurons, astrocytes or microglia because the cells did not express any of the corresponding markers. The results may be explained in two ways. First, the number of stem cells in the brain after intranasal administration may be relatively low and may not be detected by the technique used in the literature. Second, as described previously, BMSCs were used as the source stem cells for intranasal delivery in all the studies, and BMSCs have a very limited ability to differentiate into neural cell types [76]. One question still remaining is how long stem cells will stay in the brain, to which there is no conclusive answer. The longest recorded time that intranasally delivered stem cells have existed in the brain was 18 days.

4.2 The pathway of stem cells getting to the brain following intranasal delivery

Stem cells have been found in brain tissues as early as 1 h post-intranasal administration. This rapid presentation of stem cells suggested the potential pathway from nose to brain. The ways from the nose to the brain could be divided into two sections, one from the nasal mucosa to the brain and another intracerebral pathway.

4.2.1 From nasal mucosa to brain

The first potential pathway from the nasal mucosa to the brain was the olfactory nerve pathway. Previous research indicated that drugs applied intransally may enter the brain by means of the intracellular or extracellular olfactory nerve pathway. To travel the intracellular pathway, stem cells would first need to be internalized in the olfactory neuron and then transported through axonal flow. It was hypothesized that the procedure may take several hours or much longer, and the stem cells in the olfactory neuron may be digested by the enzyme. However, it is probable that other routes exist because it has been shown that stem cells could appear in the brain as early

as 1 h after intranasal delivery. The extracellular channels are made up of olfactory ensheathing cells, which surround the olfactory nerves. Stem cells could access the cerebrospinal fluid and olfactory bulbs much more rapidly through this route without being digested by enzymes. The second route is the vascular pathway. Solutes could be cleared from the CNS via perivascular spaces [62] and some drugs present in the walls of cerebral vessels and carotid arteries without entering the bloodstream after intranasal delivery. Bovetti *et al.* [77] demonstrated that new neurons derived from the subventricular zone (SVZ) used blood vessels as a scaffold for their migration through an interaction with the extracellular matrix and perivascular astrocyte foot processes. The results from these studies suggested that intranasal stem cells may use the perivascular route as a highway from the nasal mucosa to the brain.

4.2.2 *Intracerebral pathway*

Intranasal stem cells arrived in the olfactory bulb first and then were widely distributed throughout the brain, which indicated that they might be migrating through an intracerebral pathway. Normally, administered stem cells migrate from the SVZ to the olfactory bulb to form olfactory receptor neurons (ORNs) in neonates and adult by means of the rostral migratory stream (RMS). Administered stem cells in the RMS pathway surrounded by glial fibrillary acid protein-positive cells formed a chain [78]. Thus, there could be a possibility of stem cells migrating in the opposite direction. For example, Moore *et al.* [79] injected BMSCs into the olfactory bulb and found BMSCs within the turbinate neuroepithelium, olfactory bulb and frontal lobe. Most of the administered stem cells that had traveled from the implantation site adopted an elongated, arborizing morphology consistent with cellular extensions arrayed in the direction of the RMS. However, there is no direct evidence on how intranasal stem cells migrate in the brain.

4.3 The application of intranasal delivery of stem cells

4.3.1 *Hypoxia-ischemia*

Neonatal HI, which results in brain damage, is a major cause of neonatal disability and mortality; however, both options for effective treatment of HI are unsatisfactory [80]. Stem cells significantly improve the outcome of HI in rodents. Intranasal administration of BMSCs improved the outcome and reduced neuronal and white matter loss in a model of HI brain damage. BMSCs increased the levels of many growth and differentiation factors, such as neuronal growth factor, and fibroblast growth factor 2, which stimulated endogenous repair mechanisms and suppressed the expressions of pro-inflammatory factors, such as IL-1 and IL-6 [49].

4.3.2 *Ischemic stroke*

Stroke is a leading cause of death and adult long-term disability worldwide, with most cases come from ischemic stroke [31]. Although good progress has been made in the last few decades, there are not many effective treatments. As the authors described previously, stem cell therapy may provide new insights for ischemic stroke. However, the BBB and other barriers inhibit the access of cells to the brain. Transplantation of cells directly to the brain may cause great damage to the host, while intranasal delivery has an invasive character. Wei *et al.* [23] intranasally delivered Hoechst-labeled BMSCs into mice 1 day after the ischemia. BMSCs were found as early as 3 h post-intranasal delivery in multiple brain regions and in and around the stroke area 2 days after intranasal administration. Whether intranasal stem cells decrease the infarct volume and improve the outcome of ischemic stroke in mice is under investigation.

4.3.3 *Model of brain tumor*

Danielyan *et al.* [25] also investigated whether intranasal tumor cells could gain access to the brain. They labeled the human T406 glioma cell lines with PhiYellow and intranasally delivered 10^5 cells into rats. The glioma cells appeared in the olfactory bulb, frontal cortex and hippocampus 1 h after intranasal administration. These results suggested that carcinoma may metastasize into the brain through the nasal route.

4.4 The limitations and perspective of intranasal delivery of stem cells

Current studies on the intranasal delivery of stem cells to the brain are just emerging and are limited to several laboratories. There may be some limitations of this technology, which needs further attention.

4.4.1 *More sorts of stem cell need to be verified*

All the current studies use BMSCs as the model for intranasal delivery. Although BMSCs exert neuroprotection via the secretion of nerve growth factors, they rarely differentiate into functional neural cells, which is a major limitation because impaired cells need to be replaced in neurological disorders. Stem cells having the ability to differentiate into neurons such as ESCs and NSCs need to be intranasally delivered and tested. Thus, intranasal delivery of other stem cells will determine whether the nasal route is specific for BMSCs or whether it is an unspecific pathway for all types of stem cell.

4.4.2 *The pathway of intranasal stem cells to the brain*

None of the available studies has provided definite evidence on the exact pathways of intranasal delivery of stem cells to the brain. Current studies could only speculate that olfactory nerve pathways are the route of stem cell migration because stem cells are found in the olfactory bulb after intranasal delivery. Knowledge of how stem cells are transferred from the olfactory bulb to other brain areas is also limited. Previous studies revealed that drugs are also distributed in the trigeminal nerve and cervical lymph nodes following intranasal administration; however, whether stem cells could distribute

in a similar pattern is a question that still needs to be answered. In other words, the question could be interpreted as whether intranasal stem cells could enter the brain only through the olfactory bulb route. In addition, people obtained the data from animals that had been killed without monitoring the stem cell distribution in live animals. Therefore, future studies should explore: i) whether stem cells will emerge in the trigeminal nerve and cervical lymph nodes; and ii) how stem cells enter the brain and distribute themselves through intracerebral pathways in living animals.

4.4.3 *Inspection of the efficiency of intranasal delivery of stem cells in more neurological disorders*

Only three diseases, HI, Parkinson's disease and ischemic stroke, have been investigated so far as potential diseases that could be ameliorated by the intranasal delivery of stem cells. Stem cells have proved effective at treating a variety of neurological disorders. Many neurological disorders have an intact BBB, which prevents stem cells from entering the brain. Future study is needed to validate whether the various neurological diseases, with the exception HI, Parkinson's disease and ischemic stroke, could benefit from intranasal delivery of stem cells. These studies will expand the applicable ranges of intranasal delivery of stem cells.

4.4.4 *Differences among species*

The current studies were carried out in rodents (mice and rats), although previous studies have shown a variety of differences in the nasal anatomy and physiology between rodents and humans. For rodents, the total area of the olfactory epithelium occupies 50% of the nasal mucosal area, whereas only 12% of the nasal cavity surface area is the olfactory region in human beings. In addition, many current studies on the intranasal delivery of stem cells have been carried out on anesthetized animals, and anesthesia may influence the efficiency of intranasal delivery. Therefore, it is of great importance to investigate the effect of intranasal delivery of stem cells in other unanesthestized animal models and human beings in the future.

4.4.5 *Investigating the side effects of intranasal delivery of stem cells*

The authors went through all of the previous studies carefully and found that there were no data on the side effects of intranasal delivery of stem cells. However, the density of stem cells used in the experiments was usually 10^4 per microliter or larger, with most of the cells not reaching the brain through the nasal route. These off-target stem cells may end up causing side effects, such as tumors, which is a potential risk for all stem cell-based therapy. However, previous research demonstrated that the intranasal delivery of insulin improved the outcome Alzheimer's disease without changing blood glucose. In any case, further studies still need to be carried out to identify the side effects of intranasal delivery of stem cells.

4.4.6 *Exploring the therapeutic mechanisms of stem cells*

Although significant progress has been made in basal experiments and preliminary clinical trials, clinical applications of stem cells in neurological disorders are very limited. The main reason could be that we do not understand stem cells completely. Intranasal delivery of stem cells to the brain depends greatly on the understanding of the stem cell being used. In the future, more studies should be carried out concerning: the mechanisms of differentiation and proliferation; the mechanisms of treatment of disease; the side effects of stem cells; and how to obtain stem cells safely and without ethical problems.

5. Conclusion

Recent research has demonstrated that stem cells applied intranasally could circumvent the BBB and provide access to the CNS while also yielding new insights into stem cell-based therapy for neurological disorders.

6. Expert opinion

The transport of stem cells to the brain from the nose to the brain seems to be almost a fiction. However, the intranasal delivery of drugs actually has a long history, and intranasal delivery of drugs to the brain has been developed for a decade. Recently, Dr Frey, a pioneer of intranasal delivery of drugs to the brain, demonstrated that stem cells or tumor cells applied intranasally targeted the CNS and were distributed in the whole brain. This interesting phenomenon was confirmed by other teams, and some neurological diseases such as HI and stroke were alleviated by stem cells applied intranasally. Owing to its efficiency at bypassing the BBB without invasion, this technology will facilitate the clinical applications of stem cells in treatments of neurological diseases because conventional delivery routes are either invasive or prone to be blocked by the BBB.

It must be pointed out here, however, that there are not many studies of stem cells in neurological diseases using intranasal delivery as the route of stem cell delivery to the brain. The major concern for researchers in adopting intranasal delivery might be the pathway of the stem cells from nose to brain not being completely clear. The authors reviewed all the published papers carefully and found that all of the available work could not exclude the possibility that stem cells might be absorbed into the circulation and cross the BBB to enter the brain. Though the probability is very small, as discussed before, the study design of all the previous work did not compare intravenous delivery with the intranasal delivery of stem cells in the same study. There are four possible routes for substances migrating from the nose to the brain, which are through the olfactory bulb, trigeminal nerve, vascular pathway and cervical lymph nodes. To determine the role of the four routes, it would be advisable to focus on one route with the

other three blocked or excluded at the same time. The olfactory bulb, trigeminal nerve, vascular pathway and cervical lymph node pathways could be blocked using olfactory bulb ectomy, trigeminal neurectomy, nasal atherectomy and cervical lymphadenectomy, respectively. The stem cells can also be labeled using new dyes, to detect the stem cells in live animals.

A very interesting phenomenon observed was that stem cells applied intranasally may target the insult area. In the mouse model of focal ischemia, intranasally delivered stem cells were located in and around ischemic area. Furthermore, in the model of HI by right common carotid artery occlusion, many stem cells were present in the severely damaged ipsilateral hemisphere, whereas no stem cells were detected in the contralateral hippocampus. On the one hand stem cells applied intranasally may have presented where they were needed; on the other hand, previous study has demonstrated that transplanted stem cells survive and grow only in particular locations known as 'niches'. These niches may occur in the same location as the disorders, which would result in an inaccurate conclusion that stem cells migrate to injured areas. Some researchers [81] have used multi-potent astrocytic stem cells maintained in a hydrogel biomaterial tissue scaffold from oligomeric gelatin and copper-capillary alginate gel and injected them into the brain of a neonatal rat pup. After a week *in vivo*, viable cells were retained within the injected scaffolds, and some delivered cells migrated into the surrounding brain tissue. It needs to be elucidated whether stem cells can be maintained in the scaffold and enter the brain through intranasal administration.

Before clinical application of intranasal delivery of stem cells in neurological diseases, the following questions should be addressed: Do all stem cells enter the brain through the nasal route? What are the exact pathways of stem cell migration from the nose to the brain? Are there any side effects from the intranasal delivery of stem cells? Can different neurological diseases be treated with this method? Are the stem cells detected in live animals when applied intranasally? How do the stem cells treat neurological disorders? The answers to these questions will help to transfer the use of intranasally delivered stem cells for the treatments of neurological disorders from the bench to the bedside.

In the authors' opinion, intranasal delivery is a new route with great potential for the transplantation of stem cells to the brain, and the route will promote the clinical application of stem cells in neurological diseases. To make this clinical use a reality, future studies are required.

Declaration of interest

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