Chapter 7
Intranasal Delivering Method in the Treatment of Ischemic Stroke

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Abstract Ischemic stroke is a leading cause of death and disability worldwide. Advances in early recognition of stroke symptoms and the transport of patients to specialized stroke centers has been a major step in improvement of mortality and morbidity. Speed is essential since current intravenous thrombolytic treatments can only be delivered in a very narrow therapeutic window. Intravenous therapy requires specialized skills, subjects the medication to first pass metabolism, and the issue of blood to brain transport is a major problem.

An alternate approach, the intranasal route, could deliver medication to the target in a quick manner and overcome the blood brain barrier to the central nervous system while avoiding first pass metabolism. Intranasal medication also requires minimal skill to administer in a hospital or in the field.

This chapter will address the pathway through which substances travel from the nasal epithelium to various regions of the central nervous system. This includes multiple substances for intranasal administration for the potential treatment of ischemic stroke, such as proteins and peptides, stem cells, gene vectors and nanoparticles. The chapter will conclude with the merits and potential issues of intranasal administration, as well as future directions.

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Ischemic stroke is still one of the leading causes of death across the world. Thrombolytic therapy is available for ischemic stroke management, which can only be delivered in a very narrow therapeutic window within 4.5 h [1]. Neuroprotective agents have been tested effective in various animal models, however, none of them was able to achieve to be a clinical neuroprotectant. One of the potential causes is that these agents can only be delivered to the stroke patients after the arrival to the hospital, losing the opportunity to be delivered in a quicker manner. To develop an intranasal approach could potentially overcome some of the barriers to deliver the medication to the target in a quick manner especially when there is no intravenous route available. Another major benefit to develop the intranasal formulation is that it could potentially overcome the blood brain barrier (BBB) to deliver the macromolecules to the central nervous system (CNS) when the traditional intravenous approach will not work at all. It may also bypass the first pass effect from intranasal administration. To avoid intravenous administration disadvantages, intranasal administration has other critical benefits including lack or reduction of systemic side effects and increasing the efficacy by avoiding metabolism or hydrolysis in the blood for certain medications.

7.1 Pathways for Intranasal Administration

The pathway through which substances travel from the nasal epithelium to various regions of the CNS has not been fully investigated. However, previous studies reported that intranasally delivered macromolecules could bypass the BBB to elicit biological and pharmacological effects rapidly in the brain and spinal cord in rats and monkeys [2, 3]. There are at least three sequential transport steps as described as follows.

7.1.1 Transport Across the Olfactory or Respiratory Epithelial Barriers

Substances intranasally administered initially crossed the olfactory or respiratory epithelium either by intracellular or extracellular pathways. Intracellular pathways across the olfactory or respiratory epithelium include endocytosis into olfactory sensory neuron (OSN) or trigeminal neuron processes and subsequent intracellular transport to the olfactory bulb or the brainstem [4–12]. As for the extracellular pathways, substances cross the epithelium to assess the underlying lamina propria

potentially by paracellular diffusion. Horseradish peroxidase (HRP) has been shown to reach the olfactory bulb not only through intracellular uptake by OSN, but also by passing through open intercellular clefts [13]. The expression of the tight junction (TJ) proteins in the epithelium significantly determines the paracellular permeability [14, 15].

7.1.2 Transport from the Nasal Lamina Propria to Sites of Brain Entry

After entering the nasal laminal propria across the olfactory and respiratory epithelium via intracellular and extracellular pathways mentioned above, substances are to reach the brain entry sites (olfactory bulbs and brainstem) through both olfactory and trigeminal nerves. This transport may via intracellular pathways and extracellular pathways. The former is endocytosis and intraneuronal transport with OSN or trigeminal ganglion cells, while the latter happens when substances diffuse within perineural, perivascular or lymphatic channels associated with olfactory and trigeminal nerve bundles extending from the lamina propria to the olfactory bulbs and brainstem, respectively [5–7]. Fates of substances reaching the extracellular environment in the lamina propria are different and includes: (a) absorption into blood vessels and entry to systemic circulation; (b) absorption into lymphatic vessels and entry to deep cervical lymphatic nodes; (c) diffusion around nerve bundles and entry to brain. However, the travel time of different mechanisms varies. Some researchers have simulated and estimated the travel time of these mechanisms. It seems that the extracellular bulk convection is the most plausible mechanism for the rapid transport [16]. It takes at most 30 min, which fits the experimental results well.

7.1.3 Transport from the Brain Entry Sites to Widespread Sites Within the CNS

Substances may be distributed to widespread sites of CNS from the entry sites through the bulk flow in the perivascular spaces of cerebral blood vessels [17, 18]. Many studies attempted to elucidate the flow's direction and characteristics. However, different groups get different results [17–19]. It has been shown that rats with high blood pressure and heart rate displayed a larger distribution of adeno-associated virus 2, fluorescent liposomes, and bovine serum albumin, which suggests that fluid circulation within the CNS through the perivascular space is the primary mechanism [20]. What's more, following intranasal administration, uptake of [^{125}I]-calcitonin and [^{125}I]-erythropoietin (EPO) are abolished with surgical transection of the rostral migratory stream (RMS), the pathway along which neuronal

precursors migrate to the olfactory bulb. This provides evidence of the vital role of the RMS in the CNS delivery of intranasally administered agents [21]. In addition, some of the substances could be lost in the nasal-associated lymphoid tissues [22]. Another study indicates that oxytocin (OT) reached the CSF within 1 h after intranasal administration [23]. Following tracer application, substances rapidly enter into the CSF along perivascular spaces [24, 25] while limited distribution along perivascular spaces [26, 27]. The mechanism about the perivascular distribution following intranasal administration to target requires further research.

7.2 Multiple Substances for Intranasal Administration for Ischemic Stroke

Studies have demonstrated that intranasal delivery of multiple substances can effectively prevent the ischemic brain injury in animal models. The substances can be divided into four types, which were listed in Table 7.1: (1) proteins and peptides; (2) stem cells; (3) gene vectors; (4) nanoparticles.

7.2.1 Proteins and Peptides

Numerous proteins and peptides have been suggested to have a robust neuroprotective effect in focal cerebral ischemia via intranasal administration.

Administration of insulin-like factors (IGF-1) by the intranasal approach has shown significantly reduced infarct volume and improved motor-sensory and somatosensory functions in rats. Also, intranasal IGF-1 after middle cerebral artery occlusion (MCAO) decreased neuronal apoptosis in the ischemic ipsilateral hemisphere [28]. Intranasal delivery of granulocyte colony-stimulating factor (G-CSF) also decreased infarct volume, increased recovery of neurological function and promoted angiogenesis and neurogenesis following ischemia in rats [31]. What's more, study has provided evidence that intranasal administration of exendin-4 exerted a neuroprotective effect mediated by anti-apoptotic mechanism in MCAO mice and protected neurons against ischemic injury through the glucagon-like peptide 1 receptor (GLP-1R) pathway [32]. In addition, high mobility group box 1 (HMGB1) binding heptamer peptide [33], tissue plasminogen activator (tPA) [34, 35], exogenous recombinant human erythropoietin (rHu-Epo) [36], Neuro-erythropoietin (Neuro-EPO) [38, 39], transforming growth factor-beta1 [40], Wnt-3a [41], Apelin-13 [42], nerve growth factor [43] also have a positive impact on ischemic stroke through the intranasal administration.

Table 7.1 The intranasal delivery of multiple substances on animal models of ischemic stroke

Catalog	Substances	Functions	Animal model	References
Peptide and protein	IGF-1 and insulin	Reduced infarct volume Decreased apoptosis Improved neurologic functions	Rats and mice MCAO	[28–30]
	G-CSF	Reduced neuronal damage Promoted angiogenesis and neurogenesis	Rats MCAO	[31]
	Exendin-4	Exerted neuroprotective efforts by anti-apoptotic mechanism	Mice MCAO	[32]
	HBHP	Inhibit HMGB1 to reduce damages	Rats MCAO	[33]
	Recombinant tPA	Improved nervous functions Reduced the cortical stimulation threshold Enhanced neurogenesis increased the level of mature brain-derived neurotrophic factors	Rats TBI and MCAO	[34, 35]
	rHu-Epo	Promoted neuroprotection	Rats chronic hypoxia	[36, 37]
	Neuro-EPO	Reduced delayed neuronal death	Mongolian gerbil CCAO	[38, 39]
	TGF-beta1	Reduced infarct volume Improved functional recovery Enhanced neurogenesis	Mice MCAO	[40]
	Wnt-3a	Reduced infarct volume by enhancing the cerebral blood flow Enhanced neurogenesis	Mice MCAO	[41]
	Apelin-13	Reduced inflammation Decreased cell death Increased angiogenesis	Mice MCAO	[42]
	NGF	Reduced infarct volume Improved neurologic function	Rats MCAO	[43]
	PAI-1	Reduced the extravascular toxicity of tPA Reduced brain atrophy Attenuated neuroinflammation	Rat pups HI	[44]
	Caspase-9 inhibitor	Reduced neuron death	Rats MCAO	[45]
	IL-1RA	Promoted neuroprotection	Rats MCAO	[46]
	OPN	Reduced infarct volume Reduced inflammation Ameliorated neurological deficits	Rats MCAO	[47, 48]

(continued)

Table 7.1 (continued)

Catalog	Substances	Functions	Animal model	References
Stem cells	BMSC	Reduced infarct volume Induced long-lasting cell proliferation	Rats; Mice [49] MCAO	[49–51]
Gene vector	HMGB1 siRNA	Inhibited HMGB1 to reduce damages	Rats MCAO	[52]
	iNOS siRNA	Reduced infarct volume Reduced neurologic deficits	Rats MCAO	[53]
Small molecule and others	Salvinorin A	Protected the brain and improved neurological outcome	Mice MCAO	[54]
	Polycation-shielded $Ca^{(2+)}$/nucleotide nanocomplexes	Reduced infarct volume	Rat MCAO	[55]
	bFGF nanoliposomal	Improved accumulation of bFGF	Rats MCAO	[56]
	Progesterone	Decreased the mortality rate Improved motor function Reduced infarct volume Decreased the early BBB disruptions	Mice MCAO	[57]
	GRb1	Reduced infarct volume	Rats MCAO	[58]
	DFO	Reduced infarct volume	Rats MCAO	[59]
	CPA	Decreased ischemic damages	Rats MCAO	[60]
	Z-LIG	Enhanced protection against ischemic injury	Rats MCAO	[61]
	Xingnaojing mPEG2000-PLA modified microemulsion	Used for ischemic treatment	Mice MCAO	[62]

IGF-1 insulin-like growth factor 1, *G-CSF* granulocyte colony-stimulating factor, *HBHP* HMGB1 binding heptamer peptide, *HMGB1* high mobility group box 1, *tPA* tissue plasminogen activator, *rHu-EPO* recombinant human erythropoietin, *TGF-beta1* transforming growth factor-beta1, *NGF* nerve growth factor, *PAI-1* plasminogen activator inhibitor-I, *IL-1RA* interleukin-1 receptor antagonist, *BMSCs* bone marrow mesenchymal stem cells, *OPN* osteopontin, *bFGF* basic fibroblast growth factors, *GRb1* Ginsenoside Rb1, *DFO* deferoxamine, *CPA* N(6)-cyclopentyladenosine, *Z-LIG* Z-Ligustilide, *XNJ-M* xingnaojing microemulsion, *TBI* traumatic brain injury, *CCAO* common carotid artery occlusion, *HI* hypoxia and ischemia

7.2.2 Stem Cells

Stem cell intranasal delivery is a promising treatment possibility for ischemic stroke due to their potential ability to deliver neurotrophic factors to damaged cells. Intranasal bone marrow mesenchymal stem cells (BMSCs) transplantation after neonatal stroke in rats has neuroprotection and great potential as a regenerative therapy to enhance neurovascular regeneration and improve functional recovery

observed at the juvenile stage of development [50]. Meanwhile, mesenchymal stem cells (MSCs) and MSC over-expressing brain-derived neurotrophic factor (MSC-BDNF) significantly reduced infarct size and gray matter loss and induced long-lasting cell proliferation in the ischemic hemisphere in rats [51]. Furthermore, delayed intranasal delivery of hypoxic-preconditioned BMSCs significantly enhanced cell's homing to the ischemic region, optimized the therapeutic efficacy, decreased cell death in the peri-infarct region and reduced infarct volume in mice. All of these results provide promising therapeutic strategy for stroke.

7.2.3 Gene Vectors

Intranasal delivery of HMGB1 siRNA markedly reduced infarct volume in the post-ischemic rat brain (maximal reduction to 42.8 ± 5.6% at 48 h after 60 min MCAO). In addition, this protective effect was manifested by recoveries from neurological and behavioral deficits, which indicated that intranasal delivery of HMGB1 siRNA confers robust neuroprotection in the post-ischemic brain [52].

7.2.4 Small Molecules and Others

Our recent study showed that salvinorin A, a kappa opioid receptor (KOR) agonist, could potentially protect the brain and improve neurological outcome via blood brain barrier protection, apoptosis reduction and inflammation inhibition in a mouse MCAO (Middle Cerebral Artery Occlusion) model [54]. Importantly, as a non-invasive and quick method for treatment compared with other methods such as the intravenous or intramuscular injection, we demonstrated that intranasally applied molecules may bypass the blood–brain barrier and the viability of the nasal pathway to the CNS along olfactory or trigeminal associated extracellular pathways. A total volume of 10 μL of the SA solution (25% DMSO as the solvent) was administered intranasally over approximately 5 min using pipette tips. The tip was inserted into each naris so that the solution was administered to the upper nasal passage in the general area of the olfactory epithelium. However, further study was needed to show the delivery efficiency or use the absorption enhancers to improve the efficiency. Furthermore, novel drug formulations for effective brain targeting and clinical usage have to be developed.

Opioids are commonly used in critically ill patients. However, the role of opioid in modulating brain ischemia was not clear and has to be elucidated. In our study, we found that intranasal administration of Salvinorin A could reduce the infarct volume and improve the neurological outcome via inhibiting the apoptosis and the inflammation. Unlike other KOR agonists, salvinorin A does not belong to the opioid family, which produces dysphoric effects. Many intrinsic characters of the compound, i.e. naturally available from abundant plant, quick onset, lipid soluble, easy

to pass blood brain barrier, sedative and antinociceptive effect, negative pathological finding in vital organs with high dose or prolonged exposure (non-toxic) and no respiratory depression, make it a potential therapeutic medication as a non-opioid KOR agonist for various neurological conditions.

Besides, in order to further improve the therapeutic effect of substances, studies have been conducted with using of gelatin nanoparticles (GNPs). One group showed that the use of GNPs as a carrier for intranasal delivery of osteopontin (OPN) peptide allowed for a 71.57% reduction in the infarct volume and extended the therapeutic window to at least 6 h post-MCAO [63]. Also, the treatment efficacy of intranasal iNOS siRNA encapsulated in GNPs delivery was investigated. Suppressed infarct volume and reductions in neurological and behavioral deficits were observed. Importantly, therapeutic potency of iNOS siRNA/GNPs was greater and sustained longer than that of bare siRNA [53]. Another group designs polycation-shielded Ca^{2+}/nucleotide nanocomplexes with simple mixing, which produce 10–25 nm sized particles. The nanocomplexes release nucleotides in response to acidic pH, which enhance cell survival rates under unfavorable conditions such as low temperature or hypoxia. Critically, the nanocomplexes reduce cerebral infarct volume in a post-ischemic rat model [55]. Previous study supported that compared with free basic fibroblast growth factor (bFGF), nano liposomal therapy was able to improve the accumulation of bFGF in brain tissues including the most affected penumbra regions which could be rescued [43].

7.3 Merits and Issues of Intranasal Administration in Stroke

Intranasal administration is a useful method to deliver drugs to the brain of ischemic stroke. We have mentioned many substances above with intranasal administration, which are potent in the treatment of ischemic stroke. Here we conclude the merits and potential issues of intranasal administration, as well as the future direction.

7.3.1 Merits

First, intranasal administration provides a direct route into the brain, which could bypass the BBB and first-past effect of liver and gastrointestinal degradation. This increases the bioavailability of drugs, especially for peptides and substances of large mass weight, if with the help of absorption enhancers [64–67]. Large surface area for absorption (human ~160 cm^2) also promotes the absorption of the drug [68].

Besides, as the drugs directly entering the brain, systemic side effects like gastrointestinal irritation will be minimized [64, 67] and the substances will stay in higher concentration (100-fold for IGF-1 [29]) with less time to reach the targeted area (about 30 min or less [67]), compared with systemic administration [69].

What's more, from clinical prospect, intranasal administration also has its own advantages. It can:

1. Expand window for thrombolytic therapy. There is only 3- or 4.5-h time window for robust thrombolytic therapy [70]. Current stroke therapy usually fails to reach patients because of delays following stroke onset, which limits the recovery of patients. However, owing to its operability, intranasal administration could be easily deployed at home or in the ambulance to extend the time window for thrombolytic treatment. Dodecafluoropentane emulsion (DDFPe) has been used to extend time window for tPA therapy in a rabbit stroke model [71]. Extended time window is crucial for the rescue of stroke, which may allow more patients more complete stroke recovery.
2. Be effective and convenient in the chronic treatment and recovery. Since peptides, proteins and other macromolecules could be transported to the brain directly through intranasal administration, substances such as PEG-TFG-alpha [21] could improve neurogenesis and the behavioral deficit in a chronic stroke model, which may be also effective in human. Additionally, as a non-invasive and painless method for treatment, intranasal administration will increase patients' compliance. When compared with other parenteral medications, its convenience makes self-medicine and quick dose adjustment possible, which is important for the treatment of chronic phase and recovery of the patients [32, 72]. Intranasal administration also reduced risk of disease transmission from application due to its non-invasiveness [68].

7.3.2 Potential Issues

Though intranasal administration is promising, potential issues still remain to be solved before its use in clinic. We will discuss the issues in the following three parts: (1) factors affecting delivery, (2) mechanism and anatomy, (3) local side effect.

7.3.2.1 Factors Affecting Delivery

Physiochemical properties of drugs, nasal environmental factors and formulation factor affect the permeation of drugs through intranasal route. Physiochemical properties of drugs include the molecular weight (MW), solubility, lipophilic-hydrophilic balance and pKa [64, 72]. Previous study observed that absorption was the highest for the compound of least molecular weight and least lipophilicity [72]. Nasal absorption also depends on the pKa of the drug and the pH of the nasal cavity, which may determine the partition of the drugs and thus affect the absorption. Modification will be needed for better and effective delivery.

Nasal environmental factors such as drug degrading enzymes [73], mucociliary clearance (MCC) [74] and pathological conditions also affect the delivery effect. The first two factors are the self-cleaning mechanisms of respiratory tract to defend inhaled heterogeneous substances, which may also prevent drugs into the nasal laminal propria. It is about 30 min before substances depositing in the nasal cavity are removed [75]. Pathological conditions such as cold and rhinitis may change the MCC and affect the permeation of drugs by hyper-secretion of nasal mucosa.

Formulation factors involve pH, viscosity, osmolarity and type of dosage form. The pH (nasal mucosa pH is 4.5–6.5 [72]) and osmolarity (close to 308 mOsmol/L [76]) of the formulation should be modified well to improve the permeation and reduce mucosal irritation. High viscosity may increase the permeation time for drugs but may also interfere with the function of normal mucosa. Dosage form may vary as different usage with different excipients to enhance absorption. Therefore, better design should be considered for the usage and purpose of drugs.

7.3.2.2 Anatomy Difference Between Rodents and Human

As we mentioned above, the mechanisms of substances delivering to brain have not been fully understood, which hurdles the further usage and modification of drugs. Besides, there are some anatomical differences between rodents and human [29, 46], which may be the problem for human test. The nasal cavity in rodents is easier to access than in human. Moreover, while the olfactory tissue covers over 50% of the nasal cavities lining in rodents, human olfactory tissue is restricted to 3–5% [77]. Therefore, drug delivery through the nasal cavity and olfactory tissue in rodents are likely to be more efficient than in human. In addition, the differences in CSF volume in rodents and human and the turnover time for CSF in these species also lead to reduced efficiency of intranasal delivery in human when compared with rodents. All of the anatomical differences might hurdle the translational research from animal to human and sophisticated models for experiment should be developed [29].

7.3.2.3 Local Side Effect

Local administration may cause local side effect, especially for prolonged medication. The histological toxicity of absorption enhancer and other excipients is not clearly demonstrated, which may be nasal irritation or disrupt the nasal membrane functions. Improper technique of administration may also deliver the drugs to other respiratory regions like lung [64], causing the loss of the dosage and irritating these regions. These restrictions will require better design of drugs and further investigation of delivery methods.

7.3.3 Future Direction

Research now mainly concentrates on the absorption. Prodrugs are utilized to get higher hydrophilic character to enhance absorption. Various prodrug formulation of L-dopa was produced and the solubility increased significantly [78]. Co-solvent, enzymatic inhibitors, muco-adhesive agents and absorption enhancer can be added in the formulation to promote the absorption by either improving permeability or reducing degradation. The use of hyaluronidase has become a routine procedure in intranasal delivery of therapeutics to increase permeability [42].

Over the last few years, novel drug formulations for effective brain targeting have been developed to improve delivery. New formulations such as liposomes, nanoparticles, microsphere and nanoemulsions [72] are very encouraging. Liposome can improve permeation of various drugs and protect them from degeneration. Nanoparticles can easily permeate to nasal lamina propria owing to their small size. Microsphere and nanoemulsions also have advantages in intranasal administration. What's more, some human experiments by intranasal administration have been done to explore the plausibility in human treatment. NeuroEPo was given to healthy volunteers by intranasal administration [79]. The side effects were well tolerated and the products may be effective in human as in rodents.

7.4 Conclusion

In brief, intranasal delivery is a potential strategy to overcome obstacles due to the BBB and is an attractive route for its non-invasiveness and quickness. Although the mechanisms involved in the delivery of different molecules from the nasal to the CNS are not yet completely understood, intranasal administration should be considered in the future for both pre-clinical and clinical studies for the treatment of ischemic stroke.

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