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INTRANASAL ROUTE FOR CNS DELIVERY: OVERVIEW AND RECENT ADVANCEMENTS

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ABSTRACT

This review focuses on the researchers' efforts to explore intranasal route to deliver drugs to CNS. With a brief overview of nasal anatomy, article discusses various possible mechanism through which drugs could be delivered to brain via intranasal route. Physiochemical properties of drugs affecting nasal absorption and role of formulations in brain delivery through intranasal route are discussed. Despite of the potential of nasal route to deliver the drugs to brain, enzymatic activity in nasal cavity, short residence time and higher mucociliary clearance are some of the short-comings and various approaches to overcome these short-comings are discussed. Additional focus on enhancement techniques to deliver proteins and peptides is given. In the final part of the article, some of the recent studies of intranasal delivery to brain for small molecules, protein and peptide are discussed.

KEYWORDS: Intranasal drug delivery, CNS delivery, olfactory pathway, nasal mucosa, protein and peptide delivery, permeation enhancers.

1 INTRODUCTION

Drug delivery to the brain has been difficult to achieve due to the presence of the blood-brain barrier, which is formed by tight junctions within the capillary endothelium of the vertebrate brain. Drugs used against CNS diseases should reach the brain by crossing the BBB. The tight junctures between endothelial cells in brain results in a very high trans-endothelial electric resistance of 1500-2000 W cm^2 compared to 3-33 W cm^2 of other tissues like skin, bladder, colon, lung etc. which significantly affects uptake of drugs by brain.[1,2] However, certain classes of drugs like benzodiazepines such as diazepam, due to its high lipophilicity, readily cross the BBB but the passage of hydrophilic drugs and macromolecules like protein and peptide through BBB is quite difficult. A great deal of efforts, therefore, has been undertaken in developing ways to open, defeat or circumvent the BBB in order to deliver drugs from blood to brain. In recent times, intranasal delivery is explored for its potential to deliver the drugs to CNS. In addition to absorption through respiratory region, the finding of potential of olfactory region to deliver the drugs bypassing the BBB has shown a great potential. However, the enzymatic degradation in the nasal cavity, short residence time, and resistance imparted by mucus are some of the challenges to overcome for effective intranasal delivery. This review takes a look at the basics of intranasal delivery to deliver therapeutic agents to brain, and some of the published work in the field, challenges against optimum delivery and approaches to overcome them.

2 INTRANASAL DRUG DELIVERY FOR CNS DELIVERY

Feeling pleasure on sniffing cocaine and sense of smell with odorants give an idea that there should be some relation between nose and brain. Following this earlier rudimentary finding, the nasal route for the delivery of the CNS drugs to the brain has been widely studied over the last one and half decades.[3,4] In the recent years, the nasal route has been exploited for the systemic delivery of polar drugs, peptides and proteins as well.[5, 6] Many of such drugs are ineffective during oral administration mainly because of first pass metabolism and enzymatic degradation in the gastrointestinal tract, but can be absorbed nasally due its avoidance of the aforementioned problems. Nasal route of drug delivery to CNS also offers several advantages over the other alternative routes. Due to rapid absorption and higher bioavailability in CNS, the dose required for administration is lower than that required in systemic circulation and there is a faster onset of action as compared to oral administration. It is also a non-invasive, painless, and easily administered, does not require the formulation to be sterile, and increases patient comfort, convenience and compliance.[7] The risk of overdose is also reduced because the maximum volume deliverable in the nose is not more than 300µl.[8, 9]

2.1 Nasal anatomy and physiology

The human nasal cavity can be divided into two halves by a nasal septum. Each of the nasal cavities can be subdivided into three regions: the olfactory region, the

respiratory region and the nasal vestibule region. The total nasal surface area is 150 cm^2 and total volume is about 15 mL. The vestibular region is located at the opening of nasal passages, and is responsible for filtering out the air borne particles and thus, is considered to be the least important of the three regions for drug absorption. The respiratory region is the largest region having the highest degree of vascularity and thus, is mainly responsible for drug absorption. Drugs absorbed through respiratory region have to cross blood brain barrier for CNS activity. The olfactory region in man covers an area of about 10 cm^2 and is situated on the superior turbinate and opposite the septum posteriorly. It extends to the nasopharynx, while the nasal vestibule forms the anterior part and opens to the face through the nostril. The intermediate region between the vestibule and the respiratory region is the atrium. The nasal conchae or turbinates forming the respiratory region occupy a major part of the nasal cavity and possesses lateral walls dividing nasal conchae into 3 sections: the superior, middle and inferior nasal turbinates. The presence of these turbinates creates a turbulent airlflow through the nasal passages which ensures a better contact between the inhaled air and the mucosal surface, enabling the air to be warmed and humidified by vasculature and secretions of the epithelium.[5, 10] The anterior one-third of the nasal cavity is covered by squamous and transitional epithelium, the upper part of the cavity by an olfactory epithelium and the remaining portion consists of ciliated, columnar and pseudo stratified epithelium.[5, 10]

The internal and external carotid arteries supply blood to the nasal mucosa which is vital for the systemic delivery of drugs. Circulation to the lateral and medial wall of the nasal chamber is provided by the terminal branch of the maxillary artery which supplies the sphenopalatine artery. The anterior and posterior ethmoid branches come from the ophthalmic artery, a branch of the carotid artery. These vessels supply blood to the anterior portion of the nose. The vestibule and the anterior portions of the septum are supplied by branches from the facial artery. Some vessels from the greater palatine artery pass through the incisive canal of the palate to reach the anterior part of the nose. The veins of the nasal cavity drain into the sphenopalatine formmen and then into the pterygoid plexus. Some other veins accompany the ethmoid arteries and join the superior ophthalmic vein. Veins which are anterior in the nose drain into the facial vein. The presence of high blood vasculature, low resistance to drug permeability and the presence of neurons in the olfactory region allows not only systemic availability of the drug but also access to the CSF after intranasal administration.[11]

2.1.1 Enzymatic activity in the nose

The metabolic capacity in nasal cavity is lower than that in liver or GI tract.[12] Drugs administered through the nasal route avoid the liver, thus bypass the first pass metabolism but are susceptible to the nasal defensive enzymatic barrier which prevents against the entry of these xenobiotics. Biotransformation in the nasal mucosa is through cytochrome P450, some oxidative phase one enzymes and conjugative phase 2 enzymes.

2.1.2 Nasal mucociliary clearance

Approximately 100000 submucosal glands subdivided into mucus cells and serous cells, secrete most of the nasal mucus in humans which consists of mucus gel and a water fluid respectively.[13,14] Mucus secretion is composed of approximately 95% water, 2% mucin, 1% salts, 1% of proteins such as albumin, immunoglobulins, lysozyme and lactoferrin, and <1% of lipids. The nasal mucus performs a number of physiological functions such as it covers the mucosa, and physically and enzymatically protects it; it acts as adhesive and transports particulate matter towards the nasopharynx; the mucus has water-holding capacity; it exhibits surface electrical activity; and it permits efficient heat transfer.[15] About 1.5-2 L of nasal mucus is produced daily and forms a blanket about 5 µm thick, consisting of two layers. The mucus layer exists as a double layer consisting of perciliary sol phase wherein the cilia beat and a superficial layer of gel is moved forward by the tip of the cilia. Clearance of this mucus and the adsorbed/dissolved substances into the GIT is called the Mucociliary clearance (MCC). The permeability of drug through nasal mucosa is affected by viscosity of nasal secretions. If the sol layer of mucus is too thin, the viscous surface layer will inhibit the ciliary beating, and if the sol layer is too thick, MCC is impaired because contact with cilia is lost. There are approximately 300 cilia per cell, each cilia is 0.1-0.3 µm wide, 5-10 µm long and beats at a frequency of about 20 Hz. The MCC rate has been estimated at 6 mm/min. Pathological conditions, formulation factors, environmental conditions can affect MCC and hence impart influence on drug permeability. MCC can be controlled to an extent that sufficient time is allowed for drug to get absorbed and this can be achieved by using mucoadhesive polymers which can aid in localizing the formulation in the nasal cavity for extended period of time and increase absorption of the drug.[15]

2.2 Transport pathways and mechanisms - intranasal drug delivery to the CNS

The exact mechanisms underlying intranasal drug delivery to the CNS are not entirely understood, but various investigations and experiments demonstrate that pathways involving nerves connecting the nasal passages to the brain and spinal cord are important. Additionally, pathways involving the vasculature, cerebrospinal fluid, and lymphatic system also play a role in the transport of molecules from the nasal cavity to the CNS. It is likely that a combination of these pathways is responsible for intranasal drug delivery to the CNS while one pathway may predominate, depending on the properties of the drug, formulation characteristics, and the delivery device used.[16]

2.2.1 Olfactory nerve pathways

Olfactory pathways arise in the upper portion of the nasal passages, in the olfactory region, where olfactory receptor neurons (ORNs) are interspersed among supporting cells (sustentacular cells), microvillar cells, and basal cells. Drugs which are administered intranasally reach the CNS via extracellular or intracellular mechanisms of transport along olfactory nerves. Extracellular transport mechanisms involve the rapid movement of molecules between cells in the nasal epithelium or within the channels created by the olfactory ensheathing cells (OECs). Intracellular mechanisms, while important for certain therapeutics, are not likely to be the predominant mode of transport into the CNS.[16] A study carried out by R.thorne et el. studied quantitative analysis of olfactory pathway for drug delivery to the brain by using horseradish peroxidase (HRP) and the conjugate wheat germ agglutinin-horseradish peroxidase (WGA-HRP). HRP is a 40 kDa enzyme- protein that lacks binding sites on the plasmalemma whereas, 62 kDa WGA-HRP is capable of binding cell surface glycoproteins of olfactory sensory neurons and subsequently undergoes adsorptive endocytosis.[17] Higher concentration of WGA-HRP was found in the olfactory bulb compare to HRP followed by intranasal delivery. It was concluded that selectivity of WGA-HRP to the olfactory region compare to HRP that lacks associated receptors on the surface of the olfactory sensory neuron could contribute to the higher concentration of WGA-HRP in olfactory bulb.[17]

2.2.2 Trigeminal nerve pathways

It is an important pathway connecting the nasal passages to the CNS involving the trigeminal nerve, which innervates the respiratory and olfactory epithelium of the nasal passages and enters the CNS. Intranasal studies with some proteins and peptides, including interferon-b1b (IFN-b1b),hypocretin-1, and peptoids, found similar results of high levels of radioactivity in the trigeminal nerve.[18-20] A study carried out by R.G. Thorne et.el. for the investigation of CNS delivery of insulin-like growth factor-I (IGF-I), a 7.65 kDa protein neurotrophic factor following intranasal administration and the possible pathways and mechanisms underlying transport from the nasal passages to the CNS showed the presence of both olfactory and trigeminal pathway for intranasal transport of drugs for brain delivery.[21]

2.2.3 Vascular pathways

Nasal mucosa is highly vascular because it receives its blood supply from branches of the maxillary, ophthalmic and facial arteries, which arise from the carotid artery. The absorbed drug can reach the CNS by entering into the systemic circulation and crossing the blood brain barrier, especially for small lipophilic drugs compared to hydrophilic drugs, peptides and proteins. It is also possible that rather than being distributed throughout the systemic circulation, drugs can enter the venous blood supply in the nasal passages where they are rapidly transferred to the carotid arterial blood supply feeding the brain and spinal cord, a process known as counter-current transfer. Researchers also suggest perivascular channels also involved in drug transport to the CNS.

2.2.4 Cerebrospinal fluid and lymphatic pathway

Pathways connecting the subarachnoid space containing CSF, perineurial spaces encompassing olfactory nerves, and the nasal lymphatics provide access for intranasally applied therapeutics to the CSF and other areas of the CNS. A study performed by Ved et al, displayed evidence that a polar antiviral compound, zidovudine could preferentially transfer into the CSF via these pathways.[22]

2.3 Physicochemical properties of drugs affecting nasal absorption

Odorants are volatile chemical compounds that are carried by inhaled air to the regio olfactoria (olfactory epithelium) located in the roof of the two nasal cavities of the human nose, just below and between the eyes. The odorant must possess certain molecular properties in order to provide sensory properties such as some water solubility, a sufficiently high vapor pressure, low polarity, some ability to dissolve in fat (lipophilicity), and surface activity. And to date, no known odorant possesses a molecular weight greater than 294.[23] These properties should provide some idea about the drugs those should be ideal candidate for nasal absorption.

2.3.1 Molecular weight and size

Regardless of the mechanism of absorption of drug across the nasal mucosa, the main factor determining the bioavailability of large molecular weight compounds (>1000 kDa) is their molecular weight.[24] M. D. Donovan and Y. Huang (1988) studied a large number of therapeutic agents such as peptides and proteins and found that for compounds >1 kDa, molecular weight is mainly responsible for the bioavailability of the drug.[24] The bioavailability of these large molecules ranges from 0.5 to 5%.

In 1987, Fisher et al studied five water soluble compounds having molecular weights ranging from 190 to 70000, which was a 368 fold increase in molecular weight.[25] The absorption of these drugs across the nasal mucosa decreased 43 fold from the lowest to the highest molecular weight.[25] According to the results, the authors inferred that the nasal absorption of water soluble drugs is through aqueous channels in the nasal mucosa. Literature data compiled by McMartin et al (1987) shows data on absorption of 32 different compounds with molecular weights ranging from 160 to 34000, over a range of about 213-fold. The percentage absorbed followed an inverse relationship with the molecular weight, varying from 83% to 0.60% from lowest to the highest molecular weight.[26] Apart from molecular weight other possible mechanisms cannot be overlooked because multiple factors are involved in determining absorption and they are discussed below.

2.3.2 Drug solubility and dissolution rate

Drug solubility regulates the drug absorption and also limits the formulation scientist's ability to formulate the product.[27] From a mechanistic and thermodynamic of standpoint, it is important to learn about the relationship between saturation of drug solubility and its absorption. While dissolution rate becomes an important factor in determining drug absorption for powder inhalations or suspensions where the particles deposited in the nostrils needs to be dissolved prior to absorption or they will eventually be cleared by the mucociliary clearance.

2.3.3 pKa and partition coefficient

The most important factors affecting the transport mechanisms are pH, pKa and partition coefficient of the drugs. Hirai et al. (1981) measured the pH of the surface of the nasal mucosa to be 7.39.[28] The authors found that the nasal absorption of weak electrolytes such as salicylic acid and aminopyrine in rats was highly dependent on degree of ionization. It was also suggested that aminopyrine could be absorbed through the nasal mucosa via a passive transport of the unionized form. For salicylic acid, the absorption rates decrease with the pH and a substantial deviation of the experimental data from the theoretical profile was seen. The absorption rates for salicylic acid are much higher than the predicted values. One reason could be that salicylic acid enhances its own absorption by the nasal mucosa.[29] The effects of dose, pH and osmolarity on the nasal absorption of secretin in rats were studied by Ohwaki et al. (1985). It was observed that the absorption of the drug was much higher below the pH of 4.79, with the maximal absorption at pH 3 and minimal absorption at pH 7.[30, 31] Conversely, the observations were not because of pH-partition theory but due the structural changes in the nasal mucosa due to the decrease in pH of the test formulations.[30, 31]

Corbo et al compared the effect of lipophilicity of progesterone with the absorption rate constant in the nose, rectum and vagina of rabbits. It was found that the absorption rate constant increased linearly with the increase in partition coefficient and maximum absorption was seen in the nasal mucosa. Hence, it was concluded that nasal mucosa was more permeable to drugs than the other membranes and absorption rate increased with an increase in the lipophilicity of the drug.[32] However, Huang et al. found that ionized benzoic acid and polar quaternary ammonium compounds were efficiently absorbed through the nasal mucosa, in such cases log P of drug might not be the only major factor determining the extent of nasal absorption. The drug absorption phenomenon through biological membranes is so complex that no single factor can explain the dynamics of drug absorption.[33]

2.3.4 Prodrugs

Prodrug approach has been widely applied to improve the drug delivery. Ester prodrugs can be chemically synthesized by simple esterification of hydroxyl or free carboxylic groups to form derivatives resulting in more favorable partition coefficients and thereby in better drug transport. Due to high esterase activity, these ester linkages are cleaved either in the nasal mucosa or in the blood to provide the original/parent compound. This kind of approach has been implemented on drugs like acyclovir[34], 5-iodo-2′-deoxyuridine and L-dopa. On the other hand, this kind of approach is not much preferred as the prodrug may be considered as a new chemical entity by the regulatory authorities and will require undergoing the same tests and evaluations for safety and efficacy as the parent drug, which is a tedious process.

2.4 Role of formulation properties on nasal drug delivery

2.4.1 Type of dosage forms and delivery systems

2.4.1.1 *Nasal drops*

It is the simplest and most convenient form of administering drug formulations into the nose. However, a disadvantage of this dosage form is that an exact amount of the formulation cannot be delivered. This dosage form was most popular in the past because no metered dose nasal devices were available. For most prescription type drugs, this may not be the desired dosage form.

2.4.1.2 *Solutions*

Because of sophisticated drug delivery devices like metered dose nasal actuators, solution formulations were packaged in such delivery devices. Aerosol type systems using propellants were also available but due to detrimental effects of propellants on the environment, mechanical pumps/actuators were developed. Metered dose nasal actuator systems can precisely deliver actuation volumes as low as 25µl.[11]

2.4.1.3 *Suspensions*

In suspension sprays the delivery device employed is the same as solution but modified for accommodating the particle size and morphology of the drug particles.

2.4.1.4 *Cosolvent system*

Cosolvent system provides an advantage over aqueous systems when drug solubility and stability may be insufficient to deliver a dose in a small volume of 300 µL of the solution. Yu et al developed a cosolvent system for hexapeptide, hexarelin which is a growth hormone releasing peptide used in the treatment of growth hormone deficiency.[35] L. Li et al developed a hydroalcohol-glycolic cosolvent system for rapid onset delivery of diazepam for treatment of epilepsy.[36]

2.4.1.5 *Microspheres*

Bioadhesive microspheres help to increase the residence time of the formulation and slowly release the drug over a period of time. They are available in particle size range of 10-300 µm but in nasal drug delivery microspheres with size less than 180 µm have been used.[37] By incorporating drugs into the microspheres, it is possible to control the rate of clearance of the delivery system

from the nose and thereby provide potential for increasing the bioavailability of the drug.[38] Elisabetta Gavini et al developed alginate/chitosan spray-dried microspheres and suggested that it has promising properties for use as mucoadhesive nasal carriers of an antiemetic drug metoclopramide.[39] Y. Huh et al reported that the bioavailability of fexofenadine.HCl after nasal administration of the microsphere formulation to rabbits was increased up to about 48% while that of the control solution was only about 3%.[40]

2.4.1.6 *Nanoparticles*

Dalargin a hexapeptide Leu-enkephalin analogue with the sequence Tyr-d-Ala-Gly-Phe-Leu-Arg was the first drug that was transported to BBB using nanoparticles.[41] Gao et al. prepared lectin conjugated PEG-PLA nanoparticles because lectins have affinity for N-acetyl-D-glucosamine and sialic acid, both of which are found profusely on the olfactory epithelium. They also demonstrated an increased uptake of vasointestinal peptide when incorporated into the poly (ethylene glycol)-poly (lactic acid) nanoparticles modified with wheat germ agglutinin.[42, 43]

2.4.1.7 *Microemulsions*

Some of the drugs with extreme aqueous solubility, and poor penetration resulting in lesser concentration in the brain are ideal candidates to be delivered with microemulsions. Shah et. al. formulated microemulsion (ME) and mucoadhesive microemulsions (MMEs) of rivastigmine (anti Alzheimer's drug) for nose to brain delivery. Developed formulation was studied in-vitro, ex-vivo and bio-distribution was studied as well.[44] Some other reported studies include mucoadhesive microemulsion based ibuprofen delivery for brain targeting[45], targeted nose to brain delivery of neurocysticercosis via microemulaion[46] and microemulsion-based drug delivery system for transnasal delivery of Carbamazepine for treatment of epilepsy.[47] Recently, intranasal to brain delivery via nanoemulsion have been reported.[48, 49] Researchers have studied the nanoemulsion using design of experiments for optimization of the formulation for optimal delivery. However, there is limited research reported for optimization in the field of intranasal to brain delivery, with wide usage of it for optimization in other delivery area [50-52], we are expecting more and more usage of it to optimize formulation.

2.4.2 Drug concentration, dose and volume of administration

The effect of drug concentration on its absorption depends on the pathway it follows for absorption. For instance, when the rate of nasal absorption of 1-tyrosyl-L-tyrosine was examined, it showed to increase with the drug concentration in ex vivo nasal perfusion experiment in rats by Huang et al. (1985). Studies performed by Hirai et al., different effects of drug concentration in rats for two compounds were indentified.[53] Aminopyrine was found to absorb at constant rate as a function of its concentration while the nasal absorption of salicylic acid was observed to decline with its concentration. The outcome of salicylic acid could be argued to be due to detrimental effect on the nasal mucosa by increasing concentration of the drug, whereas that of aminopyrine could be due to an active transport system in the nose which has been saturated. Thus, drug concentration effects on nasal absorption actually reveal that if the primary mechanism of absorption was passive, there should be a clear positive relationship between absorption and drug concentration. Such a relationship is not always observed. However, it may not be necessarily mean that nasal absorption does not occur via passive mechanism. There are many other confounding factors which can influence the nasal membrane transport mechanism and provide a modified absorption profile.

In general, higher nasal absorption or therapeutic effect was observed with increasing dose. It is important to note how the dose is varied. If the dose is increased by increasing formulation volume, there may be a limit as to what extent nasal absorption can be increased. The nostrils can retain only a limited volume, beyond which a formulation will drain out of the nasal cavity. The ideal dose volume range is 0.05-0.15 mL with an upper limit of 0.30mL. Different approaches have been explored to use this volume effectively including the use of solubilizers, gelling, or viscosity modifying agents.[11]

2.4.3 Formulation pH

The pH of the formulation should be maintained between 4.5-6.5 as the pH of the nasal surface is 7.39, and that of the nasal secretions is 5.5-6.5 in adults and 5.0-6.7 in infants and children. A pH on acidic side should help keep the drug in the un-ionized form and prevents infiltration by pathogenic bacteria. Lysozyme present in the nasal mucosa is capable of dissolving certain bacteria in acidic conditions. Therefore, the pH should be maintained on the acidic side to prevent the deactivation of lysozymes which will ultimately lead to microbial infections.[11]

2.4.4 Viscosity

A higher viscosity of the formulation helps to increase contact time between the drug and the nasal mucosa thereby increasing the time for permeation. At the same time, highly viscous formulations interfere with the normal functions like ciliary beating or mucociliary clearance and thus alter the permeability of drugs.[54]

2.4.5 Physical form of formulation

Nasal drops are regarded as one of the simplest and most convenient dosage form but the exact amount that can be delivered cannot be easily quantified and often results in overdose. Moreover, rapid nasal clearance is a problem with drops. Solution and suspension sprays are preferred over powder sprays because powder results in mucosal irritation. A powder form was found to be more effective than liquid formulations in delivering insulin in rabbits.[55] Hardy et. al studied the nasal delivery of

human serum albumin in humans by administering a spray versus 1-3 drops and the drops were found to deposit albumin in the nostril more efficiently than did the spray. The nasal bioavailability of renin inhibitor was found to be higher from an emulsion formulation than from a PEG 400 solution.[56]

Gels help to reduce the postnasal drip and anterior leakage and localize the formulation in mucosa. Microemulsions, gels, microspheres, liposomes increase the residence time of the formulation and thereby prolonging the contact time between the drug and the mucosal membrane. Promising results have been shown for novel drug delivery system for insulin lipid emulsion[57], microparticulate system for gentamicin.[58]

2.5 Permeation enhancers

An ideal permeation enhancer should possess following characteristics: It should be pharmacologically inert, non-irritating, non-toxic and non-allergenic. Its effect should be transient and reversible and should have no taste and offensive odor. It should be compatible with drug and formulation adjuvants should be easily metabolized if absorbed along with the drug.[59] It should be potent (it should have a good absorption promoting efficiency and hence requiring only small amounts) and readily availability should be a plus.

2.5.1 Mechanism of action of permeation enhancers

The major permeation-enhancing mechanism of the chemical enhancers is to overcome the physicochemical barrier of nasal epithelium. Some enhancers alter the drug solubility or partition coefficient or have weak ionic interactions with the drug. This mechanism is preferred because it has least potential of toxicity. Some enhancers modify the nasal mucosal surface which may or may not be harmful.

2.5.2 Bile salts and surfactants

Bile salts such as sodium cholate, sodium glycocholate, sodium taurocholate, sodium taurodeoxycholate and sodium glycodeoxychloate are the most widely used surfactants for nasal absorption. Plausible mechanisms by which these classes of compounds exert their effects include increasing permeability of the membrane structure, inhibition of proteolytic enzymes, formation of aqueous pore type transport pathways, and solubilization of drugs in the aqueous vehicle. Duchateau et al. (1986) proved experimentally that gentamicin could not be absorbed in the absence of absorption enhancers, but the absorption was markedly increased in the presence of these bile salts, sodium cholate and sodium taurocholate being the most active promoters showing 41% and 34% absorption respectively. Dihydroxy bile salts were found to be more toxic than trihydroxy bile salts. Deoxycholate is extremely ciliotoxic, ciliary arrest occurred within 1 min at a concentration of 5 mM.[60]

2.5.3 Cyclodextrins

Cyclodextrins are cyclic oligomers of glucose and form inclusion complexes with any drug or molecule which can fit into the lipophilic cavities of the cyclodextrins molecules. These complexes are formed without any covalent bonds but they modify the physicochemical properties of the drugs, thus improving its formulation as well as absorption properties. Amongst various forms of cyclodextrins studied (α-cyclodextrin, β- cyclodextrin, γ-cyclodextrin, methyl cyclodextrin, dimethyl β-cyclodextrin and hydroxyl propyl β- cyclodextrin) dimethyl β- cyclodextrin has drawn great amount of interest due its significant enhancing effect and good safety profile. Proposed mechanism of action for cyclodextrins could be an interaction with lipids and divalent cations on membrane surface or a direct effect on the paracellular pathway by a transient effect on tight junctions.[59] Schipper et al. showed that for insulin (5.8 kDa) the absolute bioavailability after nasal administration in rats was increased from no absorption (3-5% bioavailability) without absorption enhancer, to about 100% with dimethyl-β-cyclodextrin.[14]

2.5.4 Fusidic acid derivatives

Sodium taurodihydrofusidate is a derivative of fusidic acid originally developed as an efficacious antibiotic with excellent safety profile in humans. The sodium salts of fusidic acid and its taurine and glycine conjugates have similar physicochemical properties to those of the free and conjugate bile salts. Sodium taurodihydrofusidate has a critical micellar concentration of 2.5 mM, a critical micellar temperature lower than 0°C and a lower pK_a. In a study by Baldwin et al., 0.5% sodium taurodihydrofusidate improved the bioavailability of human growth hormone by 11 folds in rats and rabbits, and 21 fold across the sheep nasal mucosa.[61]

2.5.5 Phosphatidylcholines

Lysophospholipids are surface active amphiphiles and present in very low concentration in most biological membranes. They are even active at low concentrations and are converted in the cell to normal cell components. O'Hagan et al. (1990) reported the absorption enhancement for biosynthetic human growth hormone in rats after intranasal absorption and compared it with several absorption enhancers, including lysophosphatidylcholines. Lysophosphatidylcholines used at a concentration of 0.2% gave the highest peak concentrations with an increase in a peak height of 450% and a relative bioavailability of 25.8%, as compared to plamitoyl-DL- carnitine hydrochloride, *N*-acetyl-L-cysteine, amastatin hydrochloride, bestatin hydrochloride, o-phenylenediamine hydrochloride and human serum albumin.

2.6 Enhancement techniques for intranasal delivery of proteins and peptides to brain

Nasal route is superior to the oral route of administration for protein and peptide delivery due to lack of proteolytic

enzymes and extreme pH conditions. However, several other barriers exist to hamper nasal protein and peptide uptake, e.g. rather tight cellular compositions and the presence of peptidases, proteases and proteinases in nasal cavity. In addition, shorted residence time of drugs in the nasal cavity further limits the effective absorption. Following are some of techniques explored by researchers to overcome these barriers.

2.6.1 Protease inhibitors

The nasal epithelial mucosa is covered with numerous microvilli, resulting in a large surface area available for drug absorption and transport. However, the ability of the nasal tissues to metabolize is something that cannot be overlooked. The nasal cavity contain both exopeptidase and endopeptidase which cleave proteins and peptides from terminal and internal peptide bonds respectively which includes amino peptidase, carboxypeptidase, dipeptidyl peptidase, thiol proteinase, postprolyl cleaving enzyme, and endopeptidase. In addition, leucocytes and enzymes released from lysosomal vesicles into the nasal secretions as a part of the nasal defense mechanism may cause proteolytic degradation of some protein and peptide drugs. Various peptidase inhibitors such as Aprotinin, Bastatin, Chymostatin and Trypsin inhibitor have been used to overcome this problem and to enhance absorption. Aminopeptidases have been indicated in the degradation of leucine enkephalins and use of aminopeptidase inhibitors such as boroleucine, bestatin and puromycin have been reported.[62] Interestingly, bile salts have been employed to inhibit nasal peptide activity against leucine enkephalin in vitro.[63,64]

2.6.2 Mucolytic agents

Mucus is a complex secretory material comprising glycoprotein (including mucins), other proteins (including enzymes and immunoglobulin), lipid, inorganic salts and water which forms a more firm gel at the surface of the layer and works as an obvious physical barrier for drug absorption. Use of potent mucolytic agent N-acetyl-l-cysteine was shown to increase the nasal absorption of human growth hormone in rats.[65] Bile salts (by reducing the viscosity of the mucus) and chelating agents (by chelating with Calcium ions involved in ciliary action) could be used to increase the absorption. Use of chelating agents including EDTA, citric acid, salicylates have been reported to improve the absorption of peptides such as human insulin, 1-deamino-8 D-arginine vasopressin and powder of interferon by chelating with calcium and magnesium ions to open up the “tight” junctions. Various bile salts such as sodium deoxycholate, sodium glycocholate, sodium taurocholate, sodium taurohydrofusidate, sodium glycodihydrofusidate have been used to increase the absorption by damaging the membrane surface and opening up the junctions.

2.6.3 Prodrug approach

Prodrugs are also an important way to increase the absorption by reducing metabolism and prolonging residence time in the nasal. Use of prodrug 4-imidazolidinone derivatives of enkephalin has been reported to protect N-terminal amino acid residue of enkephalins against cleavage by aminopeptidases and to obtain transport forms with improved lipophilicity.[34,66]

2.6.4 Use of mucoadhesive agents

Even if the surface area of the nasal mucosa is relatively small (150 cm^2), it is highly vascularised and has a relatively permeable membrane. But, lower residence time due to rapid mucociliary clearance (the residence half-life between 15-30 mins) in nasal cavity calls for usage of bio-adhesive systems to increase the contact time between drug and the sites of absorption. Mucoadhesive agents like chitosan, carbomer derivatives and modified cellulose derivatives are expected to increase residence time of the formulation in the nasal cavity which could result in better transnasal transport/bioavailability.

2.6.5 Enhanced and specific delivery to the olfactory region

Therapeutic agents reach to brain after nasal delivery via either systemic route or olfactory route. Due to vast region in the nasal cavity, most of the absorption of therapeutics occurs through respiratory route but these therapeutics have to cross BBB for CNS delivery. Whereas, olfactory region possess only a small fraction of the entire nasal mucosa which limits the amount of drug reaches to the olfactory bulb, however, drugs do not need to pass BBB for CNS delivery. The olfactory fraction of the nasal mucosa is about 50% of the total surface areas in rats whereas, in humans, the olfactory part of the nasal mucosa is only 3–8%.[67] Thus, if the contact time of therapeutic agents to olfactory region can be specifically enhanced, improvement in delivery to CNS is expected. Prolonging residence time in the nasal cavity by bioadhesion for indirect improvement in absorption through olfactory region and surface modification of drug carriers for specific binding the olfactory region are some of the approaches have been used to enhance delivery to olfactory region.

2.7 Intranasal drug delivery to brain

2.7.1 Delivery of small molecules to the brain via intranasal route

Researchers have widely explored the potential of intranasal delivery to deliver the drugs to brain from last 2-3 decades. While there is surge in exploring the potential for protein and peptide delivery, researchers are working on using this pathway for delivery of small molecules. Various reports of intranasal to brain delivery have reported with different formulations such as microemulsions, micelles, microspeheres, nanoparticle and also with targeting techniques. In such study, thermo-sensitive gels containing lorazepam microspheres were developed and characterized for intranasal brain

targeting with a dual purpose of prolonged drug release and enhanced bioavailability. The results of this study showed that the release rate followed a prolonged profile dispersion of the microspheres in the viscous media, in comparison to the microspheres alone. In addition, histopathological studies proved that the optimised formulation does not produce any toxic effect on the microscopic structure of nasal mucosa.[68] The potential use of chitosan nanoparticles as a delivery system to enhance the systemic and brain targeting efficiency of didanosine following intranasal administration was investigated in another published report. Results of this study concluded that both the intranasal route of administration and formulation of didanosine in chitosan nanoparticles increased delivery of didanosine to CSF and brain suggesting an attractive route against infections caused by aids viruses.[69] In another study, nanoemulsion (NE) was developed for intranasal delivery for enhanced bioavailability and CNS targeting of saquinavir mesylate (SQVM) which is a protease inhibitor, widely used as antiretroviral drug but a poorly soluble drug, with oral bioavailability is about 4%. Results of in vivo biodistribution studies show higher drug concentration in brain after intranasal administration of NE than intravenous delivered plain drug suspension. Gamma scintigraphy imaging of the rat brain conclusively demonstrated transport of drug in the CNS at larger extent after intranasal administration as NE.[70] Another microemulsion based intranasal delivery was reported for tacrine hydrochloride to assess its pharmacokinetic and pharmacodynamic performances for brain targeting and for improvement in memory in scopolamine-induced amnesic mice. The results demonstrated rapid and larger extent of transport of tacrine into the mice brain and fastest regain of memory loss in scopolamine-induced amnesic mice after intranasal microemulsion based delivery.[71] There are some other reported formulation strategies for delivery of tacrine hydrochloride including transdermal delivery.[72-75] Another study was aimed to prepare a nanoparticulate drug delivery system of Olanzapine (a second-generation or atypical antipsychotic) using poly (lactic-co-glycolic acid) (PLGA) for direct nose-to-brain delivery to provide brain targeting and sustained release. These results proved that Olanzapine could be transported directly to the brain after intranasal delivery of PLGA nanoparticles, enhanced drug concentration in the brain and would therefore be effective in improving the treatment of central nervous system disorders.[76]

2.7.2 Delivery of protein and peptides to the brain via intranasal route:

In the age of advanced peptide, protein, and vaccine research, nasal administration of such compounds provides an attractive delivery route. Investigational studies in human have provided evidence of direct delivery of macromolecules to the CNS following nasal administration. CNS effects of intranasal corticotrophin releasing hormone (CRH) without altering plasma cortisol or CRH levels has been demonstrated. Results of this study confirmed the notion of a pathway for CRH from the nose to the brain, initiating, via central nervous mechanisms, inhibition of gastric acid secretion and a change of mood in humans.[77] In another study, intranasal administration of wheat germ agglutinin horseradish peroxidase resulted in a mean olfactory bulb concentration in the nanomolar range.[78] Recent evidence of direct nose-to-brain transport and direct access to CSF of three neuropeptides including insulin, leptin, and oxytocin bypassing the bloodstream has been demonstrated in human trials, despite the inherent difficulties in delivery.[79] In an important study it has been noted that intranasal administration of insulin like growth factor-I (IGF-I) circumvent the blood-brain barrier and protects against focal cerebral ischemic damage. The study confirmed that IGF-I does not cross blood-brain barrier efficiently however, can be delivered to brain directly by intranasal administration.[80]

Research in humans has also provided evidence for direct delivery of therapeutic agents to the CNS from the nasal cavity. Demonstration of much greater brain evoked potential changes with intranasal than with intravenous AVP, the cholecystokinin analog CCK, ACTH4-10 and desacetyl-a-MSH have been shown in studies in human.[81, 82] CNS effects of intranasal insulin in humans without altering plasma glucose or insulin levels and of intranasal corticotropin-releasing hormone (CRH) without altering plasma cortisol or CRH levels has been observed.[80] It has been reported that intranasal growth hormone-releasing hormone (GHRH) not only increased rapid eye movement sleep and slow wave sleep in humans, but also decreased growth hormone, which is likely due to a CNS effect of the GHRH following direct delivery to the brain.[83] The action of intranasal ACTH (4-10) on human event-related brain potential and attention was due to direct delivery of the peptide from the nose to the brain and did not require prior resorption into the blood.[84] It has been reported that following intranasal administration of MSH/ACTH (4-10) to humans, MSH/ACTH (4-10) increased significantly in the CSF but not in the blood, and concluded that the peptide directly entered the CNS from the nasal cavity. They also found that intranasal MSH/ACTH (4-10) significantly reduced body fat and body weight presumably by acting on the hypothalamic melanocortin system.[84]

3 SUMMARY

As we have discussed, many sophisticated and effective approaches to CNS drug delivery have emerged in recent years. Direct transport of drugs, proteins and peptides through the olfactory pathway to the CNS has generated an immense interest in devising strategies and methodologies to exploit this approach as a portico for CNS drug delivery. The problems arise due to the physiological status in terms of nasal function and accompanying pathologies and pharmaceutical challenges with respect to CNS drug delivery, i.e., low bioavailability, local irritation and toxicity upon long-

term usage. Synthesis of more lipophilic analogues (prodrug approach), enzyme inhibitors, permeation enhancers, colloidal and bio-adhesive novel drug delivery modalities could help to eliminate few of the problems to some extent. Targeting olfactory region for improved delivery to the brain looks prominent but more research has to be done in this area. It is needless to say that the nasal route with all its inherent advantages has been heralded as the most promising means for the delivery of drugs to the CNS in the near future.

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