INTRANASAL DELIVERY- OPPORTUNITIES FOR SYSTEMIC AND BRAIN TARGETING

Vibhu Nagpal; R. N. Saha*

Drug Delivery & Pharmacokinetics Lab, Birla Institute of Technology & Science, Pilani 333031, India

***Corresponding author:**

Dr. Ranendra Narayan Saha, Deputy Director (Research & Education Development),
Birla Institute of Technology and Science, Vidhya Vihar, Pilani- 333031, India.
E-mail: rnsaha@bits-pilani.ac.in, Tel: ++91 9414082463.

ABSTRACT

Advances in pharmaceutical technology have led to development of specialized drug delivery systems that allow drugs to be delivered through the various alternative routes. Mucosal membranes, particularly the nasal mucosa, offer the potential for a rapid absorption of some drugs with a plasma profile closely replicating that from an intravenous bolus injection. The relatively large surface area, the porous epithelial membrane, and the extensive vascularization are factors favoring absorption of nasally administered drugs. This is especially useful in emergency situation with several advantages. Intranasal drug delivery can also be exploited as a better route of entry into the systemic circulation, as well as for direct brain targeting and is appropriate to its clinical application. Targeting the central nervous system (CNS) by intranasal delivery is a promising alternative for oral or parenteral administration, and is investigated to directly target the brain, thereby increasing CNS target and availability and the efficacy of CNS active drugs. Direct delivery of therapeutics from the nasal cavity via the olfactory region into the CNS, bypasses the BBB and provides a better alternative to invasive methods of drug administration. Another application of this nasal delivery can be targeting brain cancer through olfactory pathway by bypassing BBB, particularly drugs having poor permeability to brian. In addition to bypassing the BBB, the advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic firstpass drug metabolism, and elimination of the need for systemic delivery, thereby reducing unwanted systemic side effects. Intranasal delivery also provides painless and convenient self-administration. Although the market share for nasal delivery may never take the number one spot enjoyed by oral controlled release, it remains a drug delivery route with an enormous potential for growth.

Keywords: Intranasal delivery, Brain targeting, Nasal transmucosal delivery, Noninvasive·

INTRODUCTION

The anatomy and physiology of the nasal passage indicate that nasal administration has potential benefits for systemic delivery of therapeutic drugs. The relatively large surface area, the porous epithelial membrane, and the extensive vascularization are factors favoring absorption of nasally administered drugs [1]. Furthermore, nasally absorbed drugs circumvent the

first-pass metabolism in the liver associated with orally administration. Conventionally, most of the drugs are given through oral route. But due to various limitations like stability issues in gastrointestinal fluid, extensive biotransformation, lack of proper biodistribution, variability of drug absorption, problems with patients with nausea, vomiting and swallowing difficulties and to achieve quick onset of action, there is need for alternative delivery systems. Advances in pharmaceutical technology have led to development of sophisticated drug systems, that allow drugs to be delivered through the skin, ocular, transmucosal membranes (nose, buccal or bronchial) [2].

Mucosal membranes, particularly the nasal mucosa, offer the potential for a rapid absorption of drugs with a plasma profile closely replicating that from an intravenous bolus injection. This is especially useful in emergency situation. Intranasal drug delivery exploited as a better route of entry into the systemic circulation, either because the absorption profile of the drug is appropriate to its clinical application, e.g. a quick onset of action for the treatment of migraine with sumatriptan and/ or for those compounds which cannot be given orally [3].

Despite enormous advances in brain research, central nervous system disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined [4]. Direct delivery of therapeutics through the nasal cavity via the olfactory region, intranasal delivery (IN) into the CNS bypasses the BBB and provides an better alternative to invasive methods of drug administration [5, 6]. Patient compliance and risk-benefit ratio put forward the use of this non invasive method of drug delivery over invasive methods. Targeting the central nervous system (CNS) by intranasal delivery is a promising alternative for oral or parenteral administration, and is investigated to directly target the brain, thereby increasing CNS target site bioavailability and the efficacy of CNS drugs.

One of the first to demonstrate the presence of the olfactory pathway for non-microbial and non-viral agents was W.F. Faber, who placed Prussian blue dye in the nasal cavity of rabbits and observed the dye in the perineural space of the olfactory nerve and in the subarachnoid space of the brain as early as 1937 [7].

NASAL ANATOMY AND PHYSIOLOGY

Breathing and olfaction are the prime functions of the nasal cavity. The surface of the nasal cavity is enlarged by three main regions nasal vestibule, the respiratory region and the olfactory region (Figure 1) [8].

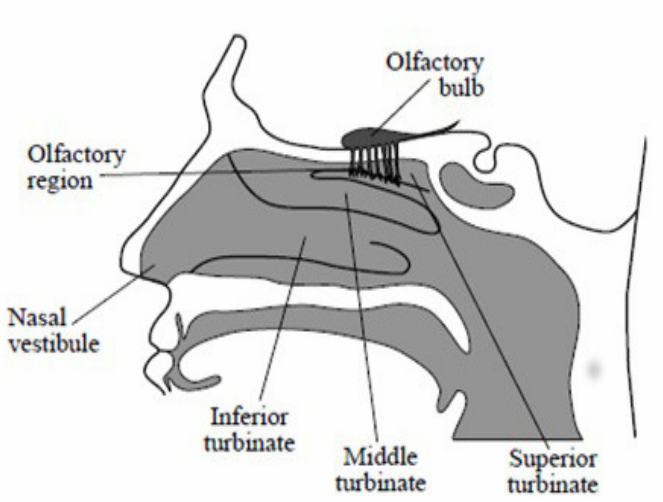

Figure 1 Anatomy of the nose. To the left is the lateral wall of the nasal cavity with the olfactory region at the roof of the cavity, just below the cribriform plate of the ethmoid bone.

The total surface of nasal cavity is about 150 cm^2 of which the respiratory epithelium covers about 130 cm^2 (large inferior turbinate) and the olfactory region about 2 cm^2 to 10 cm^2 (superior turbinate). The nasal cavity is covered with a mucous membrane which can be divided into nonolfactory and olfactory epithelium areas [9]. The non-olfactory area includes the nasal vestibule, which is lined with skin-like cells, and the respiratory region, which has a typical airway epithelium. The intense blood flow in the arteriovenous

anastomosis and the large surface of the respiratory epithelium favors transmucosal nasal drugs absorption. On the other side, drug absorption in the olfactory region is possibly resulting in direct nose to brain-transport through the nervus olfactorius [10].

The Respiratory Region

The nasal respiratory epithelium is described as a pseudo-stratified ciliated columnar epithelium. This region is considered to be the major site for drug absorption into the systemic circulation. The four main types of cells seen in the respiratory epithelium are ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells (Figure 2) [11].

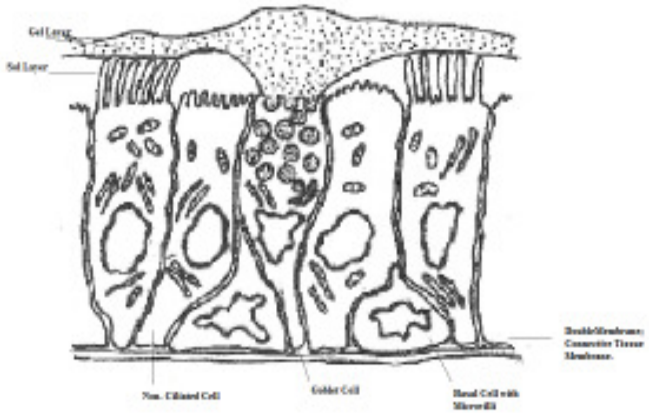

Figure 2: Schematic illustration of the various cell types in the nasal respiratory epithelium.

The proportions of these four cell types vary in different regions of the nasal cavity. In the lower turbinate area, about 15-20% of the total numbers of cells are ciliated and 60-70% are non-ciliated epithelial cells. The numbers of ciliated cells increase towards the nasopharynx with a corresponding decrease in non-ciliated cells [12]. The role of the ciliated cells is to transport mucus towards the pharynx.The high number of non ciliated cells indicates their importance for absorption across the nasal epithelium.Both columnar cell types have numerous microvilli (about 300–400 per cell) [13]. The presence of large number of microvilli increased the effective surface area and enhanced absorptive capacity of the nasal membrane.

The Olfactory Region

In humans, the olfactory region is located on the roof of the nasal cavity, just below the cribriform plate of the ethmoid bone, which separates the nasal cavities from the cranial cavity (Figure 1) [8]. Humans have relatively simple nose, since the primary function is breathing, while other mammals have more complex nose better adapted for the primary function of olfaction. In a morphometric analysis of rodent nasal cavities, Gross et al. [14] indicated that, in mice and rats, about 47% and 50% of the total nasal epithelium consists of olfactory epithelium respectively. In humans, however, the neuroepithelium covers an area of 2-10 cm^2, i.e. around 3% [15]. The olfactory epithelium composed of a thick connective tissue, lamina propria, which contains blood vessels, olfactory axon bundles and Bowman's glands. Like the epithelium of the respiratory region, the olfactory epithelium comprises pseudo-stratified columnar cells of three principal types: olfactory receptor cells, supporting cells and basal cells (figure 3) [16].

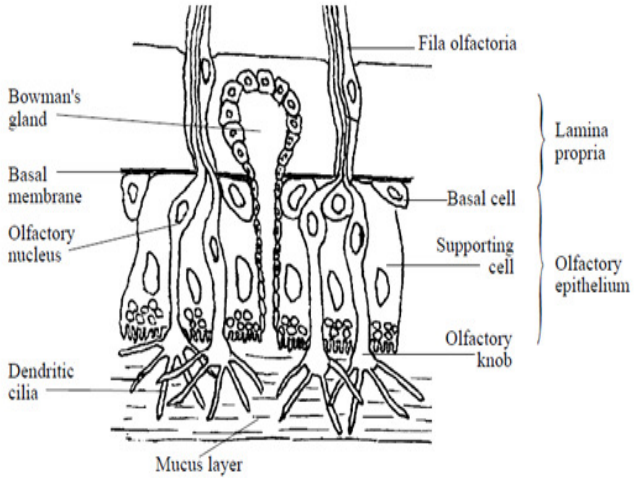

Figure 3 The olfactory epithelium of the nasal cavity showing the three principal cell Types[16]

The olfactory pathways have been reviewed by several authors [16,17,18]. Mathison *et al.* broadly classified the pathways into two possible routes from the olfactory mucosa in the nasal cavity into the CNS along the olfactory neurons: the olfactory nerve pathway (axonal transport) and the olfactory epithelial pathway. Agents that are able to enter the olfactory receptor cells, by endocytotic or pinocytotic mechanisms, could utilise the olfactory nerve pathway and thus be transported by intracellular axonal transport to the olfactory bulb [17]. Mouse hepatitis [19] and vesicular stomatitis viruses [20] and agglutinin-conjugated horseradish peroxidase [21] have been found to enter the brain by axonal transport. In the olfactory epithelial pathway, the substance must first cross the olfactory epithelium. The substance could be absorbed by passive diffusion through the supporting cells or Bowman's glands or it could be transported by a paracellular route through the tight junctions between the supporting. After entering the lamina propria, adjacent to the olfactory neurons, the substance could then enter the perineural space and reach the CNS [16]. By directly targeting the brain, it has been hypothesized that IN delivery can enhance the CNS target site bioavailability and the efficacy of CNS drugs [22].

FACTORS AFFECTING NASAL DRUG ABSORPTION

The extent of absorption of a drug from the nasal cavity depends partly on the size of the drug molecules, a factor that is most important for hydrophilic compounds. It has been reported by several workers, that there is an almost linear but inverse relationship between the molecular weight and the bioavailability of water soluble drugs (190–70 000 Da) and dextran of different weights (1260–45 500 Da) [23, 24]. McMartin *et al.* (1987) linked the extent of absorption of compounds with their molecular weight. The nasal route appears to be suitable for the efficient rapid delivery of molecules of molecular weight <1000 [25]. This means that the bioavailability of larger polypeptides like insulin will be too low when they are administered nasally. This factor also decides that whether drug will be or not transported along the olfactory pathway. In studies in rats, Sakane and co-workers have demonstrated an inverse linear relationship between the transport of compounds from the nose into the CSF and their molecular weight [26], but directly proportion to degree of dissociation [27] and lipophilicity [28]. These studies demonstrated the usefulness of dextrans as molecular weight markers and confirmed the inverse relationship between molecular size and nasal absorption for highly water soluble compounds. In these studies, direct uptake into the CSF of various molecular weights of dextrans labelled with fluorescein isothiocyanate after nasal administration was dependent on molecular weight. Dextrans with molecular weights d"20 kDa were directly transported to the CSF, while those weighing 40 kD were not found in the CSF.

However, formulation additives like absorption enhancers may increase the bioavailability of these compounds, and several research groups are now engaged in the search for suitable enhancer systems for larger molecules. The main problem is to achieve high absorption enhancement without causing irreversible damage to the nasal cavity, such as affecting the cell membrane or altering the defence mechanisms in the nose. The nasally administered drugs will normally be cleared rapidly from the nasal cavity into the gastrointestinal tract by the mucociliary clearance system. Therefore, the use of absorption enhancers and the design of suitable dosage formulations, such as mucoadhesive delivery systems, is necessary to enhance the nasal bioavailability [29, 30]. Combination of absorption enhancer and mucoadhesive polymers such as methyl cellulose, polyacrylic acid, sodium alginate, chitosan, hyaluronan etc. can potentially increase the delivery of drugs, into the system as well as CNS via the olfactory pathway [31, 32]

Lipophilic drugs like propranolol [33] and nicotine [34] are well absorbed from the nasal cavity, providing plasma concentration-time profiles similar to those obtained after intravenous administration. A linear relationship between the rate constant of absorption and the log P (octanol/water) has been demonstrated earlier with progesterone [35] in rabbits. In case of transport through olfactory pathway, for drugs with comparatively low lipophilicity, transport into the CSF is also dependent on the partition coefficient. In a reported study, the concentrations of various sulphonamides in the CSF found to be increased linearly with the partition coefficient [28]. Similar results were reported in a study of distribution of local anaesthetics in rats with similar chemical structures [36]. The rank order of these local anaesthetics, according to the ratios of the area under the concentration-time curve (AUC) values in the CSF for the two administration routes (nasal/parenteral), correlated well with their ranking by distribution coefficient.The pK_a of a substance and the pH in the surrounding area vehicle are the two factors that decide the ratio of dissociated to undissociated molecules of a drug. Several studies have shown that the amount of absorbed drug is increased with an increase in fraction of undissociated molecules [37]. Nasal administration of sulphasomidine in perfusions of varying pH resulted in more extensive transport of undissociated drug molecules into the CSF [27]. The ratio of the drug concentration in the CSF to that in the nasal perfusion fluid was dependent on the unionised fraction of the drug, i.e. drug transport from the nasal cavity into the CSF conforms to the pH partition theory.

APPLICATIONS OF NASAL DELIVERY

Topical and Systemic Bioavailability of Nasally Applied Drugs

Topical drug delivery describes the application of a drug directly on the target organ. The term nasal drug delivery refers to topical and systemic nasal drug delivery. For diseases of the nasal mucosa, such as infectious rhinitis, allergic rhinitis, and nasal polyposis, the topical nasal administration delivers drug directly to the target site [38]. Nasal applications of topical decongestants or anti-inflammatory drugs are therefore the most popular topical nasal drug deliveries.

Whereas, nasal drug delivery for systemic effect means transmucosal drug delivery leading to, direct access to the systemic circulation or to the brain. As discussed earlier, transmucosal nasal drug delivery has been found to be suitable alternative route for drugs with poor systemic bioavailability after oral administration. Due to the rapid therapeutic action that can be achieved, medications used in emergency medical situations make ideal candidates for nasal drug delivery. One such drug, apomorphine is the drug of choice for treatment of on/off-syndrome in patients suffering from Parkinson's disease. Aqueous solution of the compound is reasonably well absorbed following nasal administration with a relative bioavailability of 45% [39]. It has been demonstrated in several studies that the pharmacokinetic profiles of apomorphine after nasal administration may be improved following incorporation of mucoadhesive polymers like polyacrylic acid, carbopol and carboxymethylcellulose [40]. In using mucoadhesive polymers for nasal drug delivery, it is significant to demonstrate that mucoadhesion is the predominant mechanism responsible for improved drug absorption. Many antibiotics are still exclusively administered via parenteral routes. Recently a few studies have examined the potential of the nasal route for systemic delivery of antibiotics using mucoadhesive polymers. In a preliminary study, Lim et al. prepared and evaluated mucoadhesive microspheres of hyaluronic acid and chitosan for nasal delivery of gentamicin and other drugs [41]. This study showed that hyaluronic acid and chitosan may be employed for nasal

administration of antibiotics to obtain a high bioavailability and prolonged release. For drugs extensively metabolized in the gastrointestinal tract or in the liver, such as proteins, peptides and steroid hormones (estradiol, progesterone and testosterone), nasal administration is a convenient alternative [42, 43]. Table 1 gives an overview of compounds tested for transmucosal nasal drug delivery.

Table 1: Compounds Studied for System Delivery by Nasal Route

Compound	Indication	Compound	Indication
apomorphine	Parkinson's disease (on-offsymptoms)	human growth	hormone growth hormone deficiency
buserelin	prostate cancer	insulin	diabetes mellitus
calcitonin	Osteoporosis	ketamine, norketamine	Analgesia
cobalamin	(vitamin B12) substitution of vitamin B12	L-dopa	Parkinson's disease
desmopressin	diabetes insipidus centralis, enuresis nocturna	melatonin	jet-lag
diazepam	sedation, anxiolysis, statusepilepticus	midazolam	sedation, anxiolysis, statusepilepticus
estradiol	substitution of estradiol	morphine	Analgesia
fentanyl	analgesia, postoperative painand agitation in children	progesterone	infertility, amenorrhea
sildenafil	erectile dysfunction	propranolol	hypertonia
testosterone	substitution of testosterone		

Targeting to the CNS

The nose-brain pathway, as a conduit for transmission of agents into the CNS, is an area of ongoing research. Table 2 lists drugs and drug-related compounds that are reported to reach the CNS after nasal administration. In one of the first studies by Sakane *et al.* [44], the authors compared the uptake into the CSF after intranasal, intraduodenal and intravenous administration of the water soluble antibiotic cephalexin in a rat model. The plasma concentrations were similar after 15 and 30 minutes for the three routes but the levels of the drug in the CSF were found significantly higher at both time points after nasal administration. Because of the higher concentration in CSF after 15 minutes, Sakane *et al.* postulated that cephalexin was transported from the nasal cavity to the CSF by passive diffusion, i.e. via the olfactory epithelium pathway.

CSF drainage via the nasal route in man *post mortem* was demonstrated by Löwhagen, P. et al., and a few studies showing access to the human brain after nasal administration of drugs have been published [45]. Functional evidence of the facilitated access of arginine-vasopressin [46] and cholecystokinin-8 [47] into the brain by this route has been reported by researchers. Intranasal administration of angiotensin II to healthy volunteers showed that the drug directly influences the CNS regulation of blood pressure [48]. It was shown that the blood pressure profiles differed with the route (intravenous or intranasal) of administration of angiotensin II, and that the plasma concentrations of vasopressin were increased after intranasal but not after intravenous angiotensin II administration. The same research group also showed that nasal administration of insulin [49], an active fragment of adrenocorticotrophin [50], and a corticotrophin-releasing hormone [51] resulted in effects not seen after intravenous administration assuming a direct deliver into the CNS of the compounds. Table 2 lists drugs and drug-related compounds that are reported to reach the CNS after nasal administration in different species.

Table 2: Drugs and drug-related compounds reported to reach the CNS after nasal administration in different animal models

Drug	Species	Sample	Method
Adenoviral lacZ vector	Mouse	–	Histochemical
â-Alanine(as carnosine)	Hamster Mouse	Brain tissue	Autoradiography,Biochemical analysis Radioactivity counting counting
Albumin (labelledwith Evans blue)	Mouse	–	Light microscopy Fluorescence -microscopy Electron microscopy
Bupivacaine	Rat	CSF	HPLC
Cephalexin	Rat	CSF	HPLC
Chlorpheniramine	Rat	CSF	HPLC
Cocaine	Rat	Brain tissue	HPLC
D4T	Rat	CSF	HPLC
Dextrans(FITC labelled)	Rat	CSF	HPLC
Dihydroergotamine	Rat	Brain tissue	Radioactivity counting
Dopamine	MonkeyMouse	CSFBrain tissue	Radioactivity countingAutoradiography
Estradiol	MonkeyRabbit	CSF	Radioactivity counting
Fibroblast growthfactor	Mouse	–	Motor activityDopamine activity
L-dopa	Rat	–	MicrodialysisActivity in neostriatum
Lidocaine	Rat	CSF	ECVHPLC
Nerve growthfactor	Rat	Brain tissueCSF	ELISARadioactivity counting
Sulphonamides	Rat	CSF	HPLC
Tetracaine	Rat	CSF	HPLC
Triprolidine	Rat	CSF	HPLC
WGA-HRP	MouseRatMonkey	–	HistochemicalLight microscopyElectron microscopy

D4T = 2', 3'-didehydro-3'-deoxythymidine, WGA-HRP = wheat germ agglutinin-horseradish peroxidase, FITC =fluorescein isothiocyanate, ELISA = enzyme-linked immunosorbent assay, HPLC =high performance liquid chromatography, ECF= extracellular fluid

New therapeutic approach for this nasal delivery is targeting brain cancer through olfactory pathway by bypassing BBB. The blood-brain barrier is a substantial obstacle for delivering anticancer agents to brain tumors, and new strategies for bypassing it are greatly needed for brain-tumor therapy. Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain and could provide an alternative to intravenous injection and convection-enhanced delivery. Recently, anticancer agents such as methotrexate [52], 5-fluorouracil [53] and raltitrexed [54] have been delivered to the CNS and/or CSF using intranasal delivery. However, these chemotherapeutic agents do not discriminate between tumor and normal tissues. Thus, the concentrations of drug required to kill tumor cells can also lead to toxicity in normal neural tissues. To achieve therapeutic efficacy without toxicity to normal tissues, the drugs must preferentially target brain tumor while sparing normal tissues from damage. Because telomerase is expressed in the vast majority of GBMs but not in normal brain tissues [55], inhibition of telomerase provides a therapeutic strategy for selectively targeting malignant gliomas. One group of researchers has adminstered 3'-Fuorescein isothiocyanate (FITC) - labeled GRN163 intranasally every 2 min as 6 ìl drops into alternating sides of the nasal cavity over 22 min. FITC-labeled GRN163 was present in tumor cells at all time points studied, and accumulation of GRN163 peaked at 4 h after delivery. Moreover, GRN163

delivered intranasally, daily for 12 days, significantly prolonged the median survival from 35 days in the control group to 75.5 days in the GRN163-treated group. Thus, intranasal delivery of GRN163 readily bypassed the blood-brain barrier, exhibited favorable tumor uptake, and inhibited tumor growth, leading to a prolonged lifespan for treated rats compared to controls. This delivery approach appears to kill tumor cells selectively, and no toxic effects were noted in normal brain tissue. These data support further development of intranasal delivery of tumor-specific therapeutic agents for brain tumor patients [56]

Conclusion and Future perspectives

The advantages of administering drugs nasally compared to oral or parenteral route have been described in this review. Exploitation of these unique advantages could lead to a fast track product development. This is proven by the increasing number of nasally administered drugs mentioned in Table 1 & 2 as well as companies either entirely specialized in Nasal Drug Delivery (NDD) or have strong presence in NDD research. The possibility of increased drug absorption will allow product development of nasal peptides and small proteins, while this would not be possible with the oral route and eliminates the use of injections. The increased absorption (due to high epithelial permeability/porosity) together with low enzyme activity will act synergistically towards increasing drug absorption. Controlled release and targeted CNS delivery by bypassing BBB is also the best advantage after nasal administration. Pharmaceutical companies have looked increasingly towards drug delivery companies for help in lifecycle management of drugs on the market and with promising yet hard-to-deliver drugs. However, the potential for growth in this sector is extensive, pending the successful delivery of proteins and peptides as an alternative to parenteral delivery. Currently, many nasal drug products on the market are indicated for the treatment of local diseases such as allergic rhinitis, infectious rhinitisand nasal polposis. However, this is likely to change soon. There are a number of nasally delivered, systemically as well as CNS targeting on the market in different therapeutic categories, with a growing number of products in the pipeline. There are many reasons for this change, including improved patient compliance (elimination of needles), avoidance of ûrstpass metabolism, decreased dose which leads to minimum side effects and rapid onset of action. Migraine is a key area where a nasal system (Imitrex® nasal spray, GlaxoSmithKline) has provided rapid relief, avoidance of taking an oral formulation while nauseated, and pain-free administration circumventing the need for an injection. Other therapeutic areas where nasal delivery could provide an alternative to current dosage forms are crisis situations (seizure and heart attack), erectile dysfunction, pain management, motion sickness and psychotropic drugs. New therapeutic area for this nasal delivery is targeting brain tumors. Despite the development of drugs that preferentially target tumor cells without harming normal tissues, delivery of these drugs to brain tumors remains a major challenge because of difficulty in penetrating the blood-brain barrier (BBB). Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain because of the unique anatomic connection provided by the olfactory and trigeminal nerves. Further development of intranasal ligand based delivery as a potential therapy for braintumor patients and perhaps as a means for treating multifocal brain tumors and/or pediatric brainstem tumors, which are less amenable to potentially risky surgical procedures. As well as delivering tumor-specific agents intranasally for the treatment of intracranial neoplasms. Although the market share for nasal delivery may never take the number one spot enjoyed by oral controlled release, it remains a drug delivery route with an enormous potential for growth.

REFERENCES

[1] P. Arora, S. Sharma, S. Garg, Permeability issues in nasal drug delivery, Drug Discovery Today. 7(18) (2002):967-975.

[2] R. Zimmer, A. Michael Ashburn, Noninvasive Drug Delivery, Comp Ther. 27 (2001) 293–301.

[3] A.M. Hillery, A.W. Lloyd, J. Swarbrick, Drug delivery and targeting. In: Nasal drug delivery. (Lansley A. B., Martin G. P., Eds.), Taylor and Francis, Londan and New York, 2001, pp. 237-268.

[4] Fact Sheets, CNS disorders, [Cited 2010 Aug 11]. Available from: http://www.who.int/mediacentre

[5] D.S. Dhanda, W.H. Frey, D. Leopold, U.B. Kompella, Approaches for drug deposition in the human olfactory epithelium, Drug Delivery *Technol. 5* (2005) 64-72.

[6] W.H. Frey, Bypassing the blood-brain barrier to delivery therapeutic agents to the brain and spinal cord, Drug Delivery Technol. 5 (2002) 46-49.

[7] W.F. Faber, The nasal mucosa and the subarachnoid space, Am. J. Anat. 62 (1937) 121-148.

[8] Y.W. Chien, K.S.E. Su, S.F. Chang, Nasal systemic drug delivery. Drugs and the pharmaceutical sciences. New York, 1989. Marcel Dekker Inc.

[9] N. Geurkink, Nasal anatomy, physiology and function, J. Allergy Clin. Immunol.72 (1983) 123-128.

[10] S. Gizurarson, The relevance of nasal physiology to the design of drug absorption studies, Advanced Drug Delivery Reviews. 11(3) (1993) 329-47.

[11] B. Petruson, H.A. Hansson, G. Karlsson, Structural and functional aspects of cells in the nasal mucociliary system. Arch. Otolaryngol. 110 (1984) 576-581.

[12] J.A. Popp, J.T. JMartin, Surface topography and distribution of cell types in the rat nasal respiratory epithelium:Scanning electron microscopic observation, Am.J. Anat. 169 (1984) 425-436.

[13] N. Mygind, Scanning electron microscopy of the human nasal mucosa, Rhinol.13 (1975) 57-75.

[14] E.A. Gross, J.A. Swenberg, S. Fields, J.A. Popp, Comparative morphometry of the nasal cavity in rats and mice, J. Anat. 135 (1982) 83-88.

[15] E.E. Morrison, R.M. Costanzo, Morphology of the human olfactory epithelium, The Journal of Comparative Neurology. 297 (1990) 1-13.

[16] S. Mathison, R. Nagilla, U.B. Kompella, Nasal route for direct delivery of solutes to the central nervous system: fact or fiction?, J. Drug Target. 5 (1998) 415-441.

[17] L. Illum, Transport of drugs from the nasal cavity to the central nervous system, Eur. J.Pharm. Sci. 11 (2000) 1-18.

[18] R.T. Jackson, J. Tigges, W. Arnold, Subarachnoid space of the CNS, nasal mucosa, and lymphatic system, Arch. Otolaryngol. 105 (1979) 180-184.

[19] E.M. Barnett, S. Perlman, The olfactory nerve and not the trigeminal nerve is the major site of CNS entry for mouse hepatitis virus, strain JHM, Virology. 194 (1993) 185-191.

[20] B.S. Huneycutt, I.V. Plakhov, Z. Shusterman, S.M. Bartido, A. Huang, C.S. Reiss, C. Aoki, Distribution of vesicular stomatitis virus proteins in the brains of BALB/c mice following intranasal inoculation: an immunohistochemical analysis, Brain Res. 635 (1994) 81-95.

[21] R.G. Thorne, C.R. Emory, T.A. Ala, W.I. Frey, Quantitative analysis of the olfactory pathway for drug delivery to the brain, Brain Res. 692 (1995) 278-282.

[22] L. Graff, Gary M. Pollack, Nasal drug administration: potential for targeted central nervous system delivery, Journal of Pharmaceutical Sciences. 94 (6) (2005) 1187–1195.

[23] A. Fisher, K. Brown, S. Davis, G. Parr, D. Smith, The effect of molecular size on the nasalabsorption of water-soluble compounds in the albino rat, J. Pharm. Pharmacol. 39 (1987)357-362.

[24] A.N. Fisher, L. Illum, S.S. Davis, E.H. Schacht, Di-iodo-L-tyrosinelabelled dextrans as molecular size markers of nasal absorption in the rat, J. Pharm. Pharmacol. 44 (1992) 550-554.

[25] C. McMartin, L.E.F. Hurchinson, R. Hyde, G.E. Peters, Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity, J. Pharm. Sci.76(1987) 535-540.

[26] T. Sakane, M. Akizuki, Y. Taki, S. Yamashita, H. Sezaki, T. Nadai, Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the molecular weight of drugs, J. Pharm. Pharmacol. 47 (1995) 379-381.

[27] T. Sakane, M. Akizuki, S. Yamashita, H. Sezaki, T. Nadai, Direct transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the dissociation of the drug. J. Pharm.Pharmacol. 46 (1994) 378-379.

[28] T. Sakane, M. Akizuki, M., Yamashita, S., Nadai, T., Hashida, M. and Sezaki, H., The transport of a drug to the cerebrospinal fluid directly from the nasal cavity: the relation to the lipophilicity of the drug, Chem. Pharm. Bull. 39 (1991a) 2456-2458.

[29] M.I Ugwoke, R.U. Agu, N. Verbeke, R. Kinget, Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives, Adv. Drug Deliv. Rev. 57 (2005) 1640–1665.

[30] S. Khafagy, M. Morishita, Y. Onuki, K. Takayama, Current challenges in noninvasive insulin delivery systems: a comparative review, Adv. Drug Deliv. Rev. 59 (2007) 1521–1546.

[31] Y. H. Liao, A. Jones, B. Forbes, G.P. Martin, M.P. Brown, Hyaluronan: pharmaceutical characterization and drug delivery, Drug Deliv. 12 (2005) 327–342.

[32] D. Jiang, J. Liang, P.W. Noble, Hyaluron in tissue injury and repair, Annu. Rev. Cell Dev. Biol. 23 (2007) 435–461.

[33] A. Hussain, S. Hirai, R. Bawarshi, Nasal absorption of propanolol from different dosage forms by rats and dogs, J. Pharm. Sci. 69 (1980) 1411-1413.

[34] B.H. Jung, B.C. Chung, S.J. Chung, M.H. Lee, C.K. Shim, Prolonged delivery of nicotine in rats via nasal administration of proliposomes, J. Controlled Release. 66 (2000) 73-9.

[35] D.C., Corbo, Y.C. Huang, Y.W. Chien, Nasal delivery of progestational steroids in ovariectomized rabbits. II Effect of penetrant hydrophilicity, Int. J. Pharm. 50 (1989a) 253-260.

[36] K.J. Chou, M.D. Donovan, The distribution of local anaesthetics into the CSF following intranasal administration, Int. J. Pharm. 168 (1998a) 137-145.

[37] A.A. Hussain, R. Bawarshi-Nassar, C.H. Huang, Physiochemical conciderations in intranasal drug administrations. In: Chien, Y.W. (Ed.) Transnasal systemic medications. Fundamentals, developmental concepts and biomedical assassments, Elsevier, Amsterdam. (1985) 121-137.

[38] R.J. Salib, P.H. Howarth , Safety and tolerability profiles of intranasal antihistamines and intranasal corticosteroids in the treatment of allergic rhinitis, Drug Saf. 26(12) (2003) 863-93.

[39] E. Sam, A.P. Jeanjean, J.M. Maloteaux, N. Verbeke, Apomorphine pharmacokinetics in parkinsonism after intranasal and subcutaneous application, Eur. J. Drug Metab. Pharmacokinet.20 (1995) 27–33.

[40] M.I. Ugwoke, E. Sam, G. Van Den Mooter, N. Verbeke, R. Kinget, Bioavailability of apomorphine following intranasal administration of mucoadhesive drug delivery systems in rabbits, Eur. J. Pharm. Sci. 9 (1999) 213– 219.

[41] S.T. Lim, G.P. Martin, D.J. Berry, M.B. Brown, Preparation and evaluation of the in vitro drug release properties and mucoadhesion of novel microspheres of hyaluronic acid and chitosan, J. Control. Release 66 (2000) 281–292.

[42] M. P. van den Berg, J.C. Verhoef, S.G. Romeijn, F.W. Merkus, Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats, *Eur J Pharm Biopharm* 58(1) (2004)131-5.

[43] A.A. Hussain, R. Kimura, C.H. Huang, Nasal absorption of testosterone in rats, J Pharm Sci. 73(9) (1984) 1300-1.

[44] T. Sakane, M. Akizuki, M., Yoshida, S. Yamashita, T. Nadai, M. Hashida, M. H. Sezaki, Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity, J. Pharm. Pharmacol. 43 (1991b) 449-451.

[45] P. Löwhagen, B.B. Johansson, C. Nordborg, The nasal route of cerebrospinal fluid in man: A light-microscope study, Neuropathol. Appl. Neurobiol. 20 (1994) 543-550.

[46] R. Pietrowsky, C. Strüben, M. Mölle, H.L. Fehm, J. Born, Brain potential changes after intranasal vs. intravenous administration of vasopressin: evidence for a direct nose-brain pathway for peptide effects in humans, Biol. Psychiatry. 39 (1996a) 332-340.

[47] R. Pietrowsky, A. Thiemann, W. Kern, H.L. Fehm, J. Born, A nose-brain pathway for psycotropic peptides: evidence from a brain evoked potential study with cholestokinin, Psychoneuroendocrinology. 21 (1996b) 559-572.

[48] I. Derad, K. Willeke, R. Pietrowsky, J. Born, H.L. Fehm, Intranasal angiotensin II directly influences central nervous regulation of blood pressure, American Journal of Hypertension. 11 (1998) 971-977.

[49] W. Kern, J. Born, H. Schreiber, H.L. Fehm, Central nervous system effects of intranassaly administered insulin during euglycemia in men, Diabetes. 48 (1999) 557-563.

[50] R. Smolnik, M. Mölle, H.L. Fehm, J. Born, Brain potentials and attention after acute and subchronic intranasal administration of ACTH4-10 and desacetyl-á-MSH ni humans, Neuroendocrinol. 70 (1999) 63-72.

[51] W. Kern, B. Schiefer, J. Schwarzenburg, E.F. Stange, J. Born, H.L. Fehm, Evidence for central nervous effects of corticotropin-releasing hormone on gastric acid secretion in humans, Neuroendocrinol. 65 (1996) 291-298.

[52] F. Wang, X. Jiang, W. Lu, Profiles of methotrexate in blood and CSF following intranasal and intravenous administration to rats, Int J Pharm. 263 (2003) 1–7.

[53] T. Sakane, S. Yamashita, N. Yata, H. Sezaki, Transnasal delivery of 5-fluorouracil to the brain in the rat, J Drug Target. 7 (1999) 233–240.

[54] D. Wang, Y. Gao, L. Yun, Study on brain targeting of raltitrexed following intranasal administration in rats, Cancer Chemother Pharmacol. 57 (2006) 97–104.

[55] S. Le, J.J. Zhu, D.C. Anthony, C.W. Greider, M. Black, Telomerase activity in human gliomas, Neurosurgery. 42 (1998) 1120–1125.

[56] Rintaro Hashizume, Tomoko Ozawa, Sergei M. Gryaznov, Andrew W. Bollen, Kathleen R. Lamborn, William H. Frey II and Dennis F. Deen, New therapeutic approach for brain tumors: Intranasal delivery of telomerase inhibitor GRN163,=. 10 (2) (2008) 112-120.