Chapter 8

Vesicular Systems for Intranasal Drug Delivery

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Abstract

Recently, the nasal route for systemic drug delivery has gained great interest. It provides several advantages over other routes of drug administrations. These include rapid absorption, avoidance of the intestinal and hepatic presystemic disposition, and high potential for drug transfer to the cerebrospinal fluid. Unfortunately, the mucociliary clearance, which reduces the residence time of the nasally applied drugs, and the poor nasal permeability made it difficult for many drugs to be delivered through this route. Alternative approaches have been adopted to overcome these problems. These include the use of mucoadhesive formulations or chemical penetration enhancers. Vesicular drug delivery systems provide promising alternative for enhanced and controlled nasal drug delivery.

Alternative terms have been used to describe the vesicular systems. These include liposomes, niosomes, ethosomes, and transfersomes. These systems are morphologically similar but differ in composition and function. Nasal delivery employs liposomes and niosomes, and their corresponding proconcentrates, proliposomes and proniosomes. Encouraging results have been recorded for these systems after nasal application with the possibility of achieving many objectives such as systemic delivery of small and large molecular weight drugs. This review article discusses such systems for intranasal vaccination and for improvement of nasal drug delivery to the central nervous system. The review critically evaluates the potential of such systems for systemic drug delivery after intranasal applications.

Key words: Nasal mucociliary clearance, olfactory route, nose-to-brain delivery, targeted brain delivery, blood–brain barrier, colloidal carriers, polymeric hydrogels, liposomes, mucoadhesion, vaccine delivery.

1. Introduction

The history of nasal drug delivery dates back to earlier topical applications of drugs intended for local effects. The early 1980s saw the introduction of nasal route as a promising systemic delivery alternative to other conventional drug delivery routes (1). Intranasal drug delivery has many advantages over other routes of drug

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administration. It is easily accessible, convenient, and a reliable method, with a porous endothelial membrane, and a highly vascularized epithelium that provides a rapid absorption of compound into the systemic circulation, avoiding the hepatic first pass elimination.

In addition, intranasal drug delivery enables dose reduction, rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, and fewer side effects (2, 3). It was reported that lipophilic drugs are generally well absorbed from the nasal cavity with pharmacokinetic profiles, which are often identical to those obtained after an intravenous injection with a bioavailability approaching 100% (1). The unique characteristic of intranasal drug delivery is the high potential for drug transfer to the cerebrospinal fluid through the olfactory region which is situated in nasal cavity (4). Recent developments in nasal drug delivery have suggested intranasal administration as a safe and acceptable route for brain targeting, especially for drugs with biological effects on the central nerves system (CNS) and limited blood–brain permeability (BBB) (5). The brain targeting research attempts of large molecular weight molecules investigated nerve growth factor, insulin, desmopressin, cholecystokinin, and insulin-like growth factor-1 demonstrated the potential of nose-to-brain pathway (6–9). These advantages have provided the intranasal route some superiority over the parenteral as well as oral routes (10).

The major problems with nasal delivery are the mucociliary clearance, which reduces the residence time of nasally applied dosage forms and the poor nasal permeability of many drugs (11). Several alternative strategies have been employed to overcome these limitations. Bioadhesive polymers, for example, can be used to achieve long residence time on nasal mucosa which results in higher concentration gradient and subsequent increased absorption of the drugs (12). Polymers may widen the tight junctions (**Fig. 8.1**) producing absorption enhancing effect (13).

Successful nasal delivery has been obtained with solutions, powders, gels, and microspheres as delivery systems (14–16). However, these systems suffer from number of disadvantages; solutions show rapid clearance from nasal cavity and do not allow extended drug release (17). In addition, chemical instability problems may be encountered in solutions, particularly in case of peptide or protein drugs (18). Powders and microspheres require sophisticated delivery devices for deposition and accurate dosing (19), with insufficient wetting at mucosa resulting in low bioadhesive force and incomplete drug release (20).

Vesicular drug delivery systems provide promising alternatives with many advantages over the conventional systems. Various pharmaceutical approaches can be employed to render their final formulation more effective. Liposomes are preferred over other

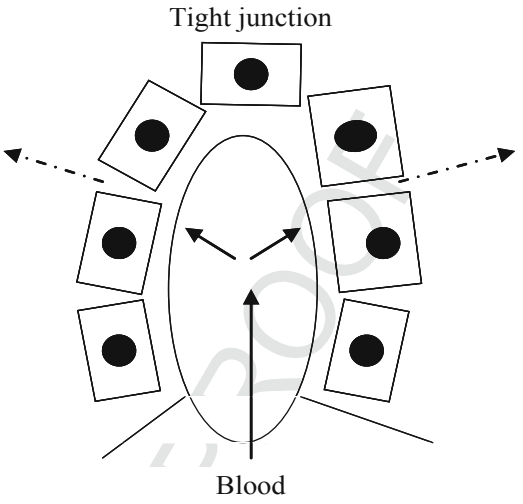

Fig. 8.1. Methods of brain selective drug delivery.

vesicular systems in nasal drug delivery. Liposomes are known to sustain the release of the entrapped drug and in the case of nasal administration are able to decrease mucociliary clearance due to their surface viscosity. The action of liposomes on nasal mucosa is related to the incorporation of phospholipids in the membrane, opening "new pores" in the paracellular tight junctions (21).

2. Nasal Anatomy and Physiology

The nasal cavity (**Fig. 8.2**) is subdivided along the centre into two halves by the nasal septum. The two cavities open to the facial side through the anterior nasal apertures and to the rhinopharynx via the posterior nasal apertures and each of two nasal cavities can be subdivided into different regions: nasal vestibule, inferior turbinate, middle turbinate, superior turbinate, olfactory region, frontal sinus, sphenoidal sinus, and cribriform plate of ethmoid bone. The nasal cavity also contains the nasal associated lymphoid tissue (NALT), which is mainly situated in the nasopharynx (22). The NALT contains specialized M-like cells similar to those present in the Peyer's patches in the gut. However, mucosal lymphoid tissue is located immediately under the nasal mucosa, where B and T lymphocyte follicles, macrophages, and dendritic cells are present (23). The cells are capable of taking up antigen and processing these for immune stimulation. It is generally recognized that

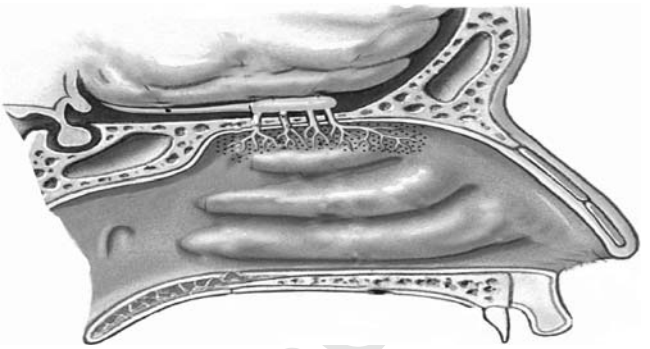

Fig. 8.2. A model for intraarterial drug delivery to the brain. Model of intracarotid drug delivery, C1 C2 and V1 V2 are concentrations and volumes in the brain (1) and remainder of the body (2), respectively. Q is the regional blood flow and CL1 and CL2 are cerebral and remaining body clearances. Modern microcatheters can restrict intraarterial interventions to tumor tissue. See Dedrick 1988 for details.

soluble antigens can penetrate the whole of the nasal mucosa and reach the superficial cervical lymph nodes to produce mainly a systemic immune response (24).

The total surface area of the nasal cavity in human adult is about 150 cm^2 and total volume is about 15 ml. The olfactory region in men covers an area of about 10 cm^2 and is positioned on superior turbinate on opposite septum (25). The respiratory region contains three nasal turbinates: superior, middle, and inferior which project from the lateral wall of each half of the nasal cavity. The presence of these turbinates creates a turbulent airflow through the nasal passages ensuring a better contact between the inhaled air and the mucosal surface (1). The nasal epithelial membrane provides a significant barrier to the free diffusion of substance across them.

2.1. Nasal Epithelium

2.1.1. Respiratory Region

The respiratory region is considered as the major site for drug absorption into systemic circulation. The mucosa consists of an epithelium resting on a basement membrane and a lamina propria. The anterior part of respiratory region is covered with squamous epithelium, while the posterior part covered by a pseudostratified columnar epithelium. The cells of respiratory epithelium are covered by about 300 microvilli per cells (25, 26).

The respiratory epithelium consists of four dominated cell types; ciliated columnar cells, non-ciliated columnar cells, goblet cells, and basal cells. The basal cells are situated on the basal membrane and do not extend to the apical epithelial surface, as do the other three cell types. The main function of the goblet cells is the secretion of mucus. The respiratory cells are covered by layer of long cilia of size 2–4 μm; the cilia move in a coordinated way to propel mucus across the epithelial surface toward the pharynx with

a clearance half time of approximately 15–20 min. The mucus layer consists of low viscosity sol layer that surrounds the cilia and a more viscous gel layer forming a layer on the top of the sol layer and covering the tips of the cilia. The epithelial cells are closely packed on apical surface, surrounded by intercellular junction whose specialized sit and structural components are commonly known as junction complex (22, 25). The presence of tight junction between neighboring epithelial cells prevents the free diffusion of hydrophilic molecules across the epithelial by the paracellular route (27). The normal diameter of the tight junctions is considered to be of the order of 3.9–8.4 Å (28). Tight junctions are located at the boundary between apical and boslateral domains in epithelial cell periphery. On a molecular level, tight junctional complex consists of a number of complexes of transmembran proteins (occluding, claudine, and junction adhesion molecule) and cytoplasmic proteins (27).

2.1.2. The Olfactory Region

The olfactory region is situated between the nasal septum and the lateral walls of each of the two nasal cavities and just below the cribriform plate of the ethmoid bone separating the cranial cavity from nasal cavity (25). The olfactory epithelium is a pseudostratified epithelium, comprising olfactory sensory neurons and two types of cells; basal cells that are able to differentiate into neuronal receptor cells and sustentacular cells (supporting cell) that provide mechanical support by ensheathing neuronal receptor cells and maintain the normal extracellular potassium level for neuronal activity (22). The olfactory epithelium is covered by a dense and viscous layer of mucus, which is secreted from the tubuloalveolar Bowman's glands and the supporting cells. The olfactory epithelium constitutes only about 5% of the total area of the nasal cavity in man (26), but is of considerable interest in drug delivery because it bypasses the BBB, delivering therapeutic drugs to CNS (29).

It should be emphasized that the blood supply to the nasal mucosa is pertinent with regards to systemic drug delivery. The arterial blood supply to the nasal cavity is derived from both the external and internal carotid arteries. The blood that is supplied to olfactory region by anterior and posterior ethmoidal branches come from the ophthalmic artery supply, which is a branch of carotid artery. These vessels supply the anterior portion of the nose. The venous drainage is as for respiratory system via sphenopalatine foramen into the pterygoid plexus or via superior ophthalmic vein (30). When the drug is administered intranasally, it can enter into the brain via three different paths (31). The first one is the systemic pathway by which the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing BBB (especially lipophilic drug). The others are the olfactory region and the trigeminal neural pathway by which the drug is transported directly from the nasal cavity to CNS (cerebrospinal

fluid and brain tissue) (32). The trigeminal nerve receptors which are present in the nasal cavity are responsible for most chemoperception and are suggested to transport the drug directly to CNS (33).

The deep and superficial cervical lymph nodes were of special interest in intranasal drug delivery because they are known to receive lymphatic afferents from portions of the nasal passages and nasolabial areas, respectively (34). The exact pathway from nasal cavity to lymph node is uncertain, but similar delivery from nasal cavity to lymphatics has been observed. This pathway is thought to mediate the efflux of large molecules and/or immune cells from sites within the CNS to the lymphatic system (35). The connection between the brain and nasal lymphatics may offer a direct pathway from the brain interstitial fluid to the nasal submucosa that excludes direct contact with the cerebrospinal fluid (36).

There are different mechanisms by which the drugs cross the olfactory membrane to reach CNS. The first mechanism involves direct transfer of the drug to primary neurons of the olfactory epithelium and transport to the olfactory bulb by intracellular axonal transport with subsequent possible distribution into more distant brain tissues. The second mechanism depends on drug permeation across the olfactory sustentacular epithelial cells, either by transcellular or paracellular mechanisms followed by uptake into CNS. The last one employs pinocytosis by olfactory neurons (26). The drug can cross olfactory lobe by one or combination of pathways.

3. Factors Influencing the Absorption of Drugs Across the Nasal Epithelium

The factors influencing nasal absorption are related to nasal physiology, the physicochemical characteristics of the drug, and the properties of specific drug formulation.

3.1. Physiological Barrier

3.1.1. Mucociliary Clearance

The function of mucociliary clearance (MCC) system is to remove foreign substances and particles from the nasal cavity, consequently preventing them from reaching the lower airways (37). Nasal clearance proceeds at an average rate of about 5–6 mm/min (4). Nasally administered formulation can be cleared from the nasal cavity with a half-life of clearance of about 15 min with the result of limiting the time available for absorption (38). The normal mucociliary transit time in humans has been reported to be 12–15 min (11).

Rapid mucociliary clearance of drug formulations that are deposited in the nasal cavity is thought to be an important factor underlying the low bioavailability of intranasally administered

drugs (11). Some drugs, hormonal changes in the body, pathological conditions, environmental conditions, and formulation factors especially rheology are reported to affect the mucociliary clearances and in turn exert significant influence on drug permeability (39). In isotonic solution, the ciliary beat frequency is best preserved. Optimal ciliary beat frequency was observed between pH values of 7 and 11. Values outside this range can result in a larger decrease in the beat frequency (40). Ciliary beat frequency measurements have shown to be a good indicator of the effects of substances on nasal tissue morphology (11).

3.1.2. Enzymes

Despite avoiding the hepatic first pass metabolism, nasally administered drugs can be subjected to a broad range of metabolic enzymes in nasal mucosa with possible reduction in the bioavailability of some drugs, especially those containing peptides or proteins (41). In spite of this possibility, the nasal route is still considered to be superior to the oral route. Various approaches have been used to overcome these degradations, which include the use enzymatic inhibitors of protease and peptidase such as bacitracin, amastatin, boroleucin, and puromycin which have been reported to improve the absorption of many drugs (42) or design prodrug (43).

3.2. Physicochemical Characteristics of the Drug

The physicochemical characteristics of the administered drug include molecular weight, solubility, dissolution rate, charge, partition coefficient, pKa, particle size, and the presence of polymorphism and these can influence drug absorption.

The permeation of drugs having molecular weight of less than 300 Da is not significantly influenced by the physicochemical properties of the drug as they will mostly permeate through aqueous channels of the membrane. In contrast, the rate of permeation is highly sensitive to molecular size for compounds with molecular weight >300 Da (44). The bioavailability of intranasally administered peptides and proteins including insulin may be low because of high molecular weight and hydrophilicity (45).

As for other routes of administration, the nasal absorption can take place only after the drug's dissolution. The dissolution rate is important in determining nasal absorption of powder and suspensions dosage forms. Rapid dissolution is critical for the drug particles after nasal administration otherwise the particles will be subjected to rapid clearance from the airway with subsequent reduction of the bioavailability (30).

The rate and extent of absorption of a drug across a biological membrane is influenced by its lipophilicity. Normally, the permeation of the compound through nasal mucosa increases with increasing the lipophilicity (46). Low molecular weight lipophilic drugs are absorbed quite efficiently across the nasal epithelium, whereas larger hydrophilic drugs, such as peptides and proteins, have substantially lower bioavailability (45).

Highly lipophilic corticosteroids drugs are absorbed more quickly by the nasal mucosa and may preferentially partition into the systemic tissue providing a large volume of distribution at steady state (47). Prodrug technique has been employed to increase the lipophilicity. The aliphatic prodrug of acyclovir provides a classical example of this process, which resulted in an increased drug bioavailability. However, it should be noted that the 140-fold increase in partition coefficient of the drug was only associated with 30% increase in bioavailability. It should be also emphasized that the ester form of the prodrug can show greater increase in transnasal drug transport but premature hydrolysis of such ester in the nasal cavity provides the main limitation of this technique (48).

Water-soluble prodrugs of 17β-estradiol have been evaluated after intranasal administration. These prodrugs were capable of producing high levels of estradiol in the cerebrospinal fluid (CSF), compared to an equivalent intravenous dose. These data suggest that the drug can reach the CSF via a direct pathway through the nasal cavity and as a result may have a significant value in the treatment of Alzheimer's disease (49).

4. Types of Vesicular Drug Delivery Systems

Vesicular systems have been employed as drug delivery carriers for many decades. They were adopted to achieve many objectives which included targeted drug delivery, enhanced drug transport through various biological membranes or prolonging and controlling drug release. Alternative terminologies have been used to describe such vesicular systems throughout these investigations (50). These included liposomes, niosomes, transfersomes, ethosomes, vesosomes, colloidosomes, and pharmacosomes. These vesicles are similar to the standard liposomes in morphology but may differ in function and composition. Only vesicles employed in the nasal drug delivery will be discussed in the subsequent paragraphs, and for detailed methods of preparation and characterizations, the readers are strongly encouraged to refer to the constructive practical approaches published in 1990 by New (51).

4.1. Liposomes

Liposomes are spherical microscopic vesicles composed of one (unilamellar) or more (multilamellar) concentric lipid bilayers, arranged around a central aqueous core (**Fig. 8.3**). They are made of natural, biodegradable, nontoxic, and natural constituents such as phospholipids and may mimic naturally occurring cell membranes. They may contain cholesterol as a membrane stabilizer and may include trace amounts of charging agents (51, 52). Having these desirable structure features, liposomes can encapsulate drugs with widely varying lipophilicities, with the lipophilic ones being located in the lipid bilayer and the hydrophilic ones

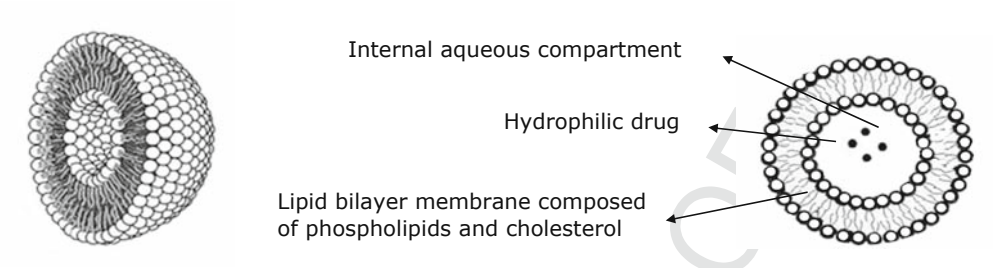

Fig. 8.3. Liposomes structure.

being retained in the aqueous core. Amphiphilic drugs can be adsorbed at the head group region of the bilayers (53). Liposomes have been investigated as carriers of various pharmacologically active agents such as antineoplastic, antimicrobial drugs, chelating agents, steroids, vaccines, and genetic materials (54). Liposomes provide an efficient drug delivery system because they can alter the pharmacokinetics and pharmacodynamics of the entrapped drugs. Table **8.1** provides an overview of the reported liposomal administered drugs.

Table 8.1.
Liposome-encapsulated drugs studied for nasal administration

Drug	Results	Reference
Diphenhydramine	Increased drug retention in the nasal	(55)
HIVgp160-encapsulated hemagglutinating virus	HIV specific humoral and cellular immunity in mucosal and systemic sites	(56)
Meningococcal OpaB and OpaJ proteins	Induced highly significant anti-Opa responses	(57)
Influenza virus hemagglutinin from 3 viral strains	Provides an almost total prevention of virus shedding combined with a high level of immunological protection against homologous virus challenge	(58)
Trivalent influenza A/H1N1-proteosome	Produced high antibody titers in serum as well as in nasal secretions	(59)
Salmon calcitonin	Ultra-flexible liposomes significantly enhanced the hypocalcemia effect than conventional liposomes	(60)
Ovalbumin in an archaeal lipid mucosal vaccine adjuvant and delivery (AMVAD)	Eliciting robust antigen-specific mucosal and systemic immune responses	(61)
Tetanus toxoid antigen	Effective mucosal immune responses and high mucosal secretory IgA titers	(62)
M. tuberculosis vaccines (DNA-hsp65)	Effective protection against TB with a single dose vaccination	(63)

According to their size, liposomes can be classified as either small unilamellar vesicles (SUV) 10–100 nm or large unilamellar vesicles (LUV) 100–3000 nm. If more than one bilayers are present, then they are referred to MLV (64). These characteristics can be controlled by proper selection of the method of preparations (51). Liposomal formulations should have high entrapment efficiencies, narrow size of distributions, long-term stabilities, and ideal release properties (based on the intended application). These require the preparation method to have the potential to produce liposomes using a wide range of ingredient molecules, e.g., lipids/phospholipids that promote liposome stability (65).

Liposomes can be formulated as dry powder or a suspension, as an aerosol or in a semisolid form such as a gel or cream. In vivo, they can be administered topically or parenterally. In the systemic circulation, liposomes can be recognized as foreign particles and consequently endocytosed by reticuloendothelial system reaching the liver and spleen (66). MLV are considered the largest type of liposomes which are capable of entrapping large percent of drugs. Once they are infused, they are rapidly recognized by the immune system and taken up by macrophages which subsequently remove them from the circulation. LUVs (intermediate size liposomes) have a better opportunity of escaping the reticuloendothelial system (RES) and so have the ability to stay in the circulation for a longer period. The small liposomes SUV show the shortest circulation time in blood due to capillary extravasations (52).

The activity of liposomes as carriers for drugs depends upon various factors such as encapsulation efficiency, stability, release rates, body distribution after administration, size surface charge, and rigidity. The properties of liposomes can be varied and controlled by incorporating different types of lipids and by varying the preparation methods (67). Poor liposomal stability is the major problem in liposome research. The instability problem arises from chemical degradation of the liposome components in addition to physical stability problems which are manifested as loss of entrapped drug and size change upon storage. Loss of entrapped material can be minimized by increasing the rigidity of the bilayer membrane or reducing the water content of liposome formulations producing the so-called proliposomes (52). Furthermore, addition of appropriate cryoprotectants allows storage of liposomes in frozen or lyophilized state.

4.2. Niosomes

These are similar to liposomes in morphology but with different compositions; they are formed from the self-assembly of non-ionic amphiphilic in combination with other lipidic surfactants in aqueous medium (68–71). Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed from admixture of

non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media resulting in closed bilayer structures (72). Like liposomes, aqueous dispersions of niosomes may exhibit fusion, aggregation, leaking, or hydrolysis of encapsulated drugs, therefore limiting the shelf life of the dispersion (73).

Niosomes are widely studied as an inexpensive alternative of non-biological origin to liposomes. Niosomal surfactants are biodegradable, biocompatible, and non-immunogenic. It has greater accessibility, superior chemical stability, and relatively low cost of niosomes compared with liposomes, resulting in easier storage leading to the exploitation niosomes as alternatives to phospholipids. Theoretically, niosomal formulation requires presence of a particular class of amphiphile and an aqueous system (74).

Niosomes improve the therapeutic performance of drug molecules by delaying clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells (75). Like liposomes, the surface of niosomes can be attached to hydrophilic moieties by incorporation of hydrophilic groups such as PEG, concanavalin A, and polysaccharide producing stealth or long circulating niosomes (76). Similar to liposomes, niosomes can be classified into MLV, SUV, and LUV. Non-ionic surfactant-based vesicles were also investigated as potential non-viral carriers for non-invasive topical delivery of plasmid DNA encoding HBsAg (77).

4.3. Proliposomes and Proniosomes

These are dry, free-flowing particles which immediately form vesicular dispersions upon addition of water. Their free-flowing particulate properties permit the fabrication of these nano-aggregates into solid dosage forms, which then is converted to liposomes/niosomes on contact with water or biological fluids (69, 71, 78). In general, they are prepared by penetrating a solution of drugs and phospholipids in volatile organic solvents into the microporous matrix of water-soluble carrier particles, followed by evaporation of the organic solvents. Drugs and phospholipids are thus deposited in the microporous structure of the carrier materials, thus maintaining the free-flowing surface characteristics of the carrier materials. Because of the characteristics of these preparations, the sterilization of proliposomes can be achieved without influencing their intrinsic characteristics (79). They have several advantages over their corresponding liquid formulations. These include the minimization of physical instability problems, such as aggregation, fusion, and leakage. In addition, they provide ease of transportation, distribution, storage, and dosage. Proniosomes have shown equal or greater efficacy in drug release performance when compared with conventional niosomes (78).

5. Pharmaceutical Applications

5.1. Drug Therapy

Vesicular systems play an important role in nasal drug delivery into the systemic circulation by overcoming limitations of the nasal route such as ciliary clearance and breakdown by nasal peptidase enzyme. They showed promising results not only with small molecular but also with large molecules. Liposomes are one of the vesicular systems that offer better absorption and drug retention in nasal mucosa, e.g., desmopressin and insulin (80, 81). The superiority of liposomes was indicated after comparing the permeability of liposomes entrapping insulin through nasal mucosa of rabbit with the permeability of insulin from solution with or without pretreatment with sodium glycocholate (82). Intranasal administration of insulin in liposomes composed of dipalmitoylphosphatidylcholine and sterylglucoside showed a greater reduction in blood glucose level with the effect lasting for 8 h (80). Jain et al. studied the usefulness of multivesicular liposomes as a mucoadhesive to prolong the release of insulin via nasal and ocular route. In their study, the multivesicular liposomes were shown to be marginally effective after nasal administration compared to ocular route although better therapeutic profile as the hypoglycemic effects were prolonged until 72 h (83).

The loading and leakage characteristics of the desmopressin-containing liposomes and the effect of liposomes on the nasal mucosal permeation were investigated. The increase of permeation of the antidiuretic, desmopressin, through the nasal mucosa was in the order of positively charged liposomes > negatively charged liposomes > solution (81).

Calcitonin liposomes with different charges were administered to rabbits to evaluate the effect of liposomes charges on nasal absorption. The bioavailability of intranasally administered calcitonin liposomes was in the following order: positively charged liposomes > negatively charged liposomes > calcitonin solution. The significant bioavailability enhancement of the positively charged calcitonin liposomes may be due to interaction of positively charged liposomes with the negatively charged mucosal surface. The retention of positively charged liposomes on the negatively charged nasal mucosa resulted in an increase in the residence time of calcitonin and thus increased bioavailability (84).

Nasal administration of acyclovir mucoadhesive liposomes has been demonstrated to have good permeability characteristics with enhanced nasal penetration of acyclovir in comparison to free drug suspended in gel. Fifteen rabbits were used in this study and were divided into three groups. The first group received acyclovir liposomes as nasal gel. The second group received the free drug as nasal gel (control). The third group received an intravenous

injection of acyclovir solution. Intranasal preparations were administered by nasal droppers with a wide orifice inserted about 5 mm into the nostril of the rabbit while in a supine position. The AUC values of acyclovir mucoadhesive liposomal gel and acyclovir suspended in gel were 1.91175 and 21.90264 (μg h ml), respectively. The differences in the AUC values are due to the variations in the drug profile of acyclovir suspended in gel (**Fig. 8.4**). The improvement of bioavailability of the prepared liposomal formulations in comparison to free drug suspended in gel could be attributed to both encapsulation and incorporation of acyclovir in nasal mucoadhesive gel. Nasal bioavailability of acyclovir was 60.72% calculated relative to the serum acyclovir levels over a period of 8 h after intravenous injection of acyclovir. Liposomes have been demonstrated to have good permeability characteristics to enhance nasal penetration of many drugs (85).

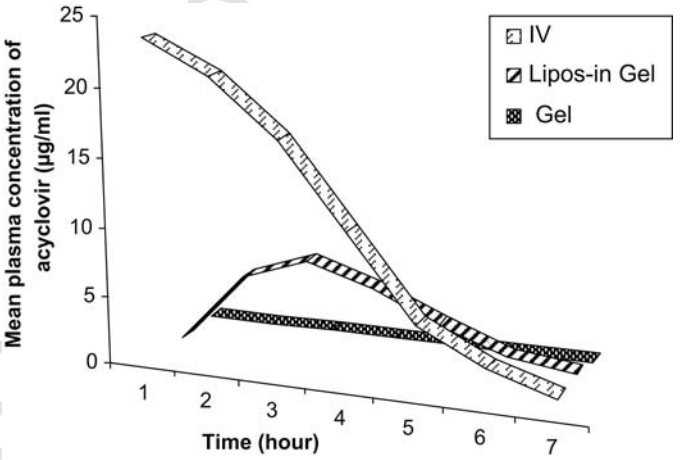

Fig. 8.4. Mean acyclovir plasma concentrations versus time profile for intravenous and nasal administration (mean ± SD, $n = 5$). Acyclovir was administered by intravenous, acyclovir suspended in gel or acyclovir mucoadhesive liposomal gel administered by nasal route.

Ding et al. have studied nasal administration of liposomes formulation of levonorgestrel and evaluated their pharmacokinetic properties; they found that administration of levonorgestrel liposomes via nasal route increases its bioavailability when compared with levonorgestrel suspension by oral route. It was concluded that the liposomes greatly facilitated levonorgestrel nasal absorption and may provide a rapid onset of action of levonorgestrel for emergency contraception (86).

Nasal administration of a liposomal leuprorelin acetate formulation with chitosan produced contraception in rats when compared to subcutaneous (87). Liposomes also provided protection

of entrapped drugs from enzymatic degradation (80) and disrupted the mucosal membrane to increase absorption (88). Taking into considerations that leuprorelin acetate was administered by nasal route at low dose, it would be expected that the nasal route can increase the therapeutic index and reduce the adverse effects of the drug (87).

Zhang and coworkers concluded that the encapsulation of recombinant hirudin-2 in liposomes provided high entrapment capacity, greater stability, and enhancement of nasal absorption of recombinant hirudin-2 (89).

In a comparative study, nicotine base-proliposomes and nicotine hydrogen tartarate-proliposomes and a mixture of powdered nicotine hydrogen tartarate salt and sorbitol were administered intranasally to rats at dose of 1 mg/kg. Nasal absorption of nicotine from these formulations was very rapid (less than 10 min) and showed substantially sustained plasma nicotine levels compared to saline solutions of nicotine base and salt. Nicotine base-proliposomes demonstrated the best characteristics in terms of the area under the plasma concentration, mean residence. It was found that nasal application of proliposomes containing nicotine base could provide a very rapid absorption with prolonged delivery to the systemic circulation (90).

Proliposomes containing propranolol hydrochloride were also evaluated for their potential as a nasal drug delivery system to sustain the plasma concentration of the drug. The proliposomes were administered intranasally to the rats and plasma concentrations of the propranolol hydrochloride obtained after nasal administration of proliposomes were compared with those after nasal, oral, and intravenous administrations of aqueous solution. Nasal administration of the proliposomes resulted in low propranolol concentration at the initial phase and sustained at the terminal phase. Plasma concentrations of oral propranolol solution were much lower than those after the intravenous and nasal administrations. The absolute bioavailability of the orally administered propranolol was only 14.2%, the bioavailability obtained from nasal proliposomes reaching 97.5% (91).

5.2. Gene Therapy

Gene delivery is a challenging task in the treatment of genetic disorders. In this case, the plasmid DNA has to be introduced into the target cells, which should get transcribed and the genetic information should ultimately be translated into the corresponding protein. To accomplish this objective, a number of hurdles are to be overcome by the gene delivery system. Transfection is affected by (1) targeting the delivery system to the target cell, (2) transport through the cell membrane, (3) uptake and degradation in the endolysosomes, and (4) intracellular trafficking of plasmid DNA to the nucleus (92).

Macrophages play an important role in host immune functions; therefore, several strategies have been developed to transfer genes directly into macrophages but most of them use viral vectors (93). Despite the high transfection efficiency of viral vectors, questions remain regarding to their potential toxicity (94).

The use of nonviral vectors is attractive for in vivo gene delivery because it is safer than using simple viral systems. Cationic liposomes were considered to be one of the most promising nonviral gene delivery systems. Various kinds of cationic lipids have been synthesized and shown to be able to deliver genes into cells both in vitro and in vivo (95). Introduction of ligands for cell-surface receptors into liposomes has been attempted for achieving optimum transfection efficiency in vivo (96).

Dioleoylphosphatidyl-ethanolamine (DOPE) is one of liposomes constituent; this type phospholipids is known to accelerate the endosomal escape of plasmid DNA due to its pH-sensitivity and high transfection efficiency (97). Plasmid DNA complex with mannosylated liposomes is recognized and taken up by mannose receptors, mannosylated exhibited a higher transfection in macrophages based on a receptor-mediated mechanism. A mannosylated cholesterol derivative itself has a positive charge, and a high density of mannose residues that can be deposited on the liposome surface without affecting the binding ability of cationic liposomes to DNA. These properties reflected in their superior in vivo gene transfection and it can be effectively introduced in cell-specific ligand structures to liposomes.

Goncharova et al. demonstrated the importance of nasal mucosa for the immunization against Tick-Borne encephalitis. To study intranasal immunization against TBE virus, they chose biodegradable micelles, cationic liposomes, and live attenuated bacterial/viral vectors. Their results showed the expression of the gene in transfected cells, thereby indicating that the liposomal formulations are suitable for mucosal immunization (98). Complexes of cationic lipid and plasmid DNA (lipoplexes) are the most widely used nonviral vectors for gene delivery. To improve the efficiency of nonviral gene delivery, pharmacological agents such as sex hormones and glucocorticoids have been shown to enhance liposome-mediated gene uptake (99). Estradiol is a female sex hormone steroid that can enhance liposome-mediated gene delivery by increasing gene uptake in vitro and promote nuclear accumulation of the transgene (100). Incorporating β-estradiol into lipoplexes consistently increased gene expression in the lungs and nasal epithelia (sub-confluent and confluent) to human airway epithelial cells, in particular the bronchial epithelial cell line. The greatest enhancement was found using polarized cells (101). These data indicated that β-estradiol and methyl-prednisolone are promising adjuvents for improving gene delivery. Methyl-prednisolone has also been incorporated into lipoplexes and assessed

on confluent and polarized cells where it increased delivery 70-fold and 48-fold, respectively, compared with lipoplex alone. This represents a considerably larger enhance calcium phosphate based delivery to human myoblasts (102).

Tanaka et al. reported that nasal administration of insulin gene packed in cationic liposome produced a sufficiently high expression, processing, and secretion of insulin in mice with streptozocin-induced type 1 diabetes. Within 8 days of treatment, plasma insulin levels in streptozocin-injected mice given the luciferase gene decreased to <100 pg/ml. In comparison, streptozocin-injected mice given the insulin gene exhibited plasma insulin levels at least ten times higher and is actually greater than the mean level in normal nondiabetic mice. These high levels of total insulin corrected hyperglycemia without producing hypoglycemia even after a 16-h fast and devoid of adverse effect of insulin gene expression in treated mice. Using fluorescence in situ hybridization (FISH) analysis, intracellular plasmids observed in alveolar epithelial cells of the lung, where extracellular plasmids were observed in muscle, kidney, gastrointestinal system, and immune cells, as well as the respiratory system, suggesting plasmid dissemination, possibly due to hematogeneous spread (103).

Inclusion of free liposomes into DNA/lipid complexes may be important to accomplish optimal transfection activity in vivo (104). Liposome/DNA complexes can be delivered into organs with high cationic lipid to DNA ratios when administered intravenously. However, through nasal inoculation of liposome/DNA complexes, liposomes are trapped within the lung epithelium. Thus, the cationic lipid to DNA ratio is changed during transit through the lung epithelial cells. In addition, there are inhibitors which inhibit the transfection activity of DNA/lipid complexes in serum. Therefore, in other organs, transfection efficiency was very low, and plasmid DNA was detected only in the extracellular spaces. Liposome-mediated in vivo gene transfer via nasal administration may provide an efficacious route for delivery of hormonal and other gene products into the blood stream.

The major cause of mortality in patients with cystic fibrosis is a lung malfunction. Therefore, gene transfer to correct the underlying genetic defect is a potential treatment for cystic fibrosis. A DNA-liposome formulation was delivered to the patients with no immune tolerance, in repeated doses (three doses). It was concluded that the DNA containing liposomes can be successfully re-administered without apparent loss of efficacy for cystic fibrosis treatment (105).

5.3. Vaccine Delivery

Many of the available vaccines including protein antigens and DNA vaccines are very unstable and need to be protected from degradation in the biologic environment. In addition, their efficacy is limited by their poor capacity to cross biologic barriers and

reach the target sites. Usually parenteral vaccination involves intramuscular administration of antigens. This route of immunization stimulates the immune system to produce IgG antibody in the serum but fails to generate a mucosal antibody response. In contrast, intranasally administered vaccines stimulate IgA antibody response along the mucosal surfaces. IgG facilitates the phagocytosis of bacteria and activates the complement, whereas IgA principally acts by preventing attachment and colonization of bacteria on mucosal surfaces (106).

Mucosal delivery is a highly effective route for the stimulation of local and systemic immunity. However, soluble drugs usually provide poor immunization when administered by mucosal routes and require the adjunct of a mucosal adjuvant or a drug delivery system (107). Mucosal immunization required five times less DNA than epidermal inoculation to induce the same level of protection against rotavirus challenge. This result indicated that mucosal immunization was superior to epidermal inoculation using the same vaccine dose (108). Many diseases such as measles, pertussis, tuberculosis, meningitis, and influenza are associated with the entry of pathogenic microorganisms across the respiratory mucosal surfaces. Therefore nasal vaccines delivery system is a good candidate for induction of both mucosal and systemic immune responses.

A range of different vaccine systems have been described in literature using either whole cells, spilt cells surface antigens, or DNA vaccine (genetic immunization) with or without adjuvant (109). Genetic immunization works by using host cells as protein factories to produce the plasmid encoded antigen. The translated protein is then processed and presented by the immune system in a mode similar to that, which occurs following a natural infection (110). The DNA was administered either directly in saline solution or in combination with carriers or adjuvants such as saponin, liposomes, and cochleates (108). The efficacy of DNA vaccines was monitored by humoral or cellular immune responses or resistance to virus challenges (111). Additionally, DNA vaccine was combined with liposomes or bioadhesive polymers and delivered by the mucosal route. These polymers can form highly viscous aqueous solutions that are thought to attach to mucosal surfaces (112).

Nasal vaccination has received a lot of attention since the nasal cavity is rich in NALT through which viral infections can be acquired. Intranasal vaccination has proven to be safe, easy, with less antigens being required via this route as compared to that needed for oral immunization. Nasal vaccination is thus a cost-effective means for controlling viral and bacterial diseases. The mucosal surfaces are rich in B-cells, T-cells, and plasma cells and such antigen-reactive cells are essential for the induction and maintenance of specific immune responses. Concerning both nasal

vaccination and delivery of therapeutics, it has been shown that nanoscale drug carriers exhibiting mucoadhesive and permeation-enhancing properties have a great potential for improving the delivery through the nasal route (113).

Liposomes might be taken in by M-cell, and transported across the mucosal boundary, thereby transfect immune cells within NALT (114). Liposomes constitute attractive immunoadjuvants that can provide a vehicle or a carrier system into which antigens and coadjuvants can be incorporated (115). The adjuvant potential of liposomes in enhancing the systemic and mucosal immune responses was compared to immunopotentiating adjuvant which could directly activate the cells of the immune system. Intranasal administration of liposomes encapsulated with tetanus toxoid produced high mucosal IgA responses compared to liquid formulation. While liposomes loaded with tetanus toxoid and CpG-ODN produced higher serum IgG, antitoxin titers, and lower nasal IgA titers, compared with tetanus liposomes without adjuvant (116).

It is well established that liposomes possess immunoadjuvant activity and have a potential for the intranasal and oral delivery of protein antigen. Immunogenicity of tetanus toxoid has been improved when delivered mucosally (orally and nasally) associated with liposomes producing anti-toxoid IgG antibody titer similar to those obtained via the intramuscular delivery. Nasal delivery of tetanus toxoid entrapped in liposomes improved the immune response compared to delivery of free antigen. Furthermore, if the liposomes were taken up intact, the superficial layer of nose is highly vascularized and therefore a quantity of liposomes will pass directly into systemic circulation resulting in a systemic immune response and some liposomes would probably be taken up and delivered to underlying lymphoid cells of nasal associated lymphoid tissue (117). Antigen uptake by NALT is very important for stimulation immune responses.

Intranasal immunization with liposome-encapsulated influenza hemagglutinin (HA) DNA vaccine-induced T cell proliferation, indicative of $CD4^+$ activity and humoral immune responses, in addition to increasing serum IgG and IgA titres (118). Intranasal immunization with the liposome-supplemented vaccine conferred a better protection against an influenza infection than did intradermal immunization with the antigen alone (119).

Mucosal immunization required five times less DNA than epidermal inoculation to induce the same level of protection against rotavirus challenge. This result indicated that mucosal immunization was superior to epidermal inoculation using the same vaccine dose (108).

Liposome mediated DNA immunization by promoting the disruption of the endosomal membrane after endocytosis/phagocytosis of liposomal-DNA systems and ensuring escape

of the plasmid DNA into the cytoplasm was recorded (120). Entrapment of plasmid DNA into liposomes will protect the nucleic acids against desoxyribonuclease attack which led to enhancing the efficacy the DNA vaccine (121).

Aramaki et al. studied the activation and mucosal response following nasal administration of liposomes in mice and found that IgG level was significantly elevated when bovine serum albumin (BSA)-associated liposomes were administrated intranasally twice with 4-week intervals. They also observed that the contribution of antigen-presenting cells in activation of systemic and mucosal immunity following intranasal administration was different (122).

Intranasal administration of glycol chitosan coated liposomes encapsulating plasmid DNA encoding surface protein of Hepatitis B virus induced significantly higher systemic, humoral, mucosal, and cellular immune responses when compared to naked DNA. This could have resulted from coating liposomes with glycol chitosan which may be able to remain homogeneously dispersed in the mucus, allowing good contact with respiratory mucosa at physiological pH due to electrostatic interaction by chitosan and mucosal surface. In addition, DNA encapsulated into both plain and chitosan-coated liposomes was protected against degradation by DNase, presumably because of the inability of the enzyme to reach its substrate, whereas naked DNA was completely degraded after 15 min (123). It has been reported that intranasal administration of liposomes can provide a promising adjuvant system for stimulation of antibody responses in general and mucosal secretory immunoglobulin (sIgA) responses in particular (124).

The effectiveness of immunizing humans by the intranasal route with *Streptococcus mutans* antigens, either incorporated into liposomes or in free form was investigated, in order to design a more effective approach to prevent oral diseases. Intranasal immunization resulted in primarily a nasal response and the liposomal antigen vaccine induced higher nasal but similar salivary IgA responses, when compared to responses induced with the free antigen vaccine. There may be a mucosal IgA inductive site which preferentially promoted a salivary response resulting in immune responses in nasal secretions, parotid saliva and serum (125).

Effective enhancement of mucosal immune responses was also observed with single intranasal immunization with liposome-formulated *Yersinia pestis* vaccine (formaldehyde-killed whole cell vaccine; KWC). Liposomes significantly enhanced the mucosal and systemic immune responses which were assessed 14 days following a single immunization. Immune responses were characterized by increased levels of specific IgA and IgG in mucosal secretions compared to *Y. pestis* KWC vaccine alone which induced low antibody titers (126). The ability of liposomes to enhance immune responses to vaccine antigens has been

attributed primarily to an increased antigen uptake by antigen presenting cells and consequently increased antigen presentation to T-cells (127). In another study, it was illustrated that single nasal vaccinations with heat-labile toxin (HLT) adjuvant virosomal influenza vaccine can elicit humoral immune response that was comparable to that obtained after a single parenteral vaccination with the same total influenza virus hemagglutinin (HA) content (128).

Liposomes prepared from conventional ester lipids are usually ineffective as mucosal adjuvants, leading to the use of additional known adjuvants or targeting molecules such cholera toxin B subunit, lipid A, or interleukin-2 to improve the liposomal vaccine effectiveness (126, 129). Therefore, polar archaeal lipids have potential advantages for developing a non-replicating mucosal adjuvant and vaccine delivery system. Intranasal immunization of unilamellar archaeosomes (liposomes made from archaeal polar lipids) with encapsulated ovalbumin (OVA/archaeosomes), induced anti-OVA IgG, IgG1, and IgG2a antibody responses in sera and OVA-specific mucocal IgA in several mucosal sites. Calcium was added in the formulation to interact with the negatively charged archaeal polar lipids and to convert OVA/archaeosomes into an archaeal lipid mucosal vaccine adjuvant and delivery (AMVAD) vaccine (OVA/AMVAD). The ability to induce mucosal immune responses was demonstrated with OVA/AMVAD formulations prepared from a range of different polar lipid compositions (archaeol *H. salinarum*) or caldarchaeol (*T. acidophilum*) or a mixture of these core lipids (*M. smithii*), suggesting a broad applicability of archaeal polar lipids for intranasal immunization (130). AMVAD vaccines consisting of archaeal polar lipids could have potential advantages over the use of other vesicular delivery systems such as those based on ester lipids. Fatty acyl chains of many ester lipids used for preparing liposomes and cochleate formulations were shown to have some degree of unsaturation. Consequently, manufacturing and storage of theses formulations should be conducted under nitrogen to prevent lipid oxidation (131).

5.4. CNS Delivery

Intranasal administration offers a non-invasive alternative route to deliver drugs to the central nervous system, effectively by passing the BBB (**Fig. 8.5**) (132). It was proposed to be an excellent route of administration to target drugs directly to the brain via the olfactory neurons, which provide extracellular and intracellular pathways into CNS (133). It was suggested that substances could be absorbed via the olfactory route by two different mechanisms: the olfactory nerve pathway (axonal transport) and the olfactory epithelial pathway (26). The neural connections between the nasal mucosa and the brain provide a unique pathway for the non-invasive delivery of therapeutic agents to the CNS (134). The

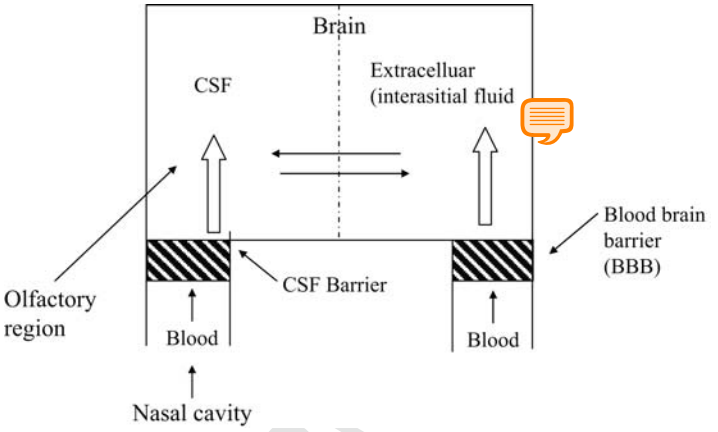

Fig. 8.5. Suggested transport pathways from nose to central nervous system.

olfactory neural pathway provides both an intraneuronal and extraneuronal pathway into the brain (134). The intraneuronal pathway involves axonal transport (olfactory nerve pathway) and it is considered a slow route where substance enters the olfactory neuron via endocytotic or pinocytotic mechanisms and diffuses to the olfactory bulb by utilizing the same mechanisms the cell uses to transport endogenous substances to the rest of the brain (135). The extraneuronal pathway (epithelial pathway) is a faster route for direct nose-to-brain transfer as compounds pass paracellularly across the olfactory epithelium into the perineural space, which is continuous with the subarachnoid space before transport to basolatral side of the olfactory epithelium which delivers drugs directly to the brain parenchymal tissue and/or CSF. Then the molecules can diffuse into the brain tissue or will be cleared by the CSF flow into the lymphatic vessels and subsequently into the systemic circulation (136).

Depending on the substance administered, axonal transport rates range from 20–400 mm/day to a slower 0.1–4 mm/day (137). Lipophilicity, molecular size, degree of dissociation, and route of administration are very important physicochemical factors must be considered when designing intranasal delivery for brain targeting (31). Formulation factors are also to be considered while designing brain targeted nasal drug delivery systems. The liquid formulations, liquid spray, and drops are the most widely used preparations for intranasal drug delivery. The nasal spray deposits anteriorly in the nasal atrium provide greater residence time, while the drops are dispersed throughout the length of the nasal cavity. Nasal sprays deposit more anteriorly, having more potential for brain delivery. The permeability of the posterior nasal passage is generally higher than the anterior passage (30).

Intranasal delivery is a promising route for delivery of drugs into the CNS and there are 35–40 drugs, which have been reported to reach CNS after nasal administration. Example of theses drugs that rapidly pass into CSF include estradiol and progesterone (138), carbamazepine (139), dopamine (140), risperidone (141), tacrine (142), and neuropeptide (143).

Nasal mucosa in the olfactory region is likely to be a promising target for mucosal immunization to protect the CNS from neurotropic viral infections. Intranasal immunization inducing mucosal and systemic immune responses blocks the propagation of neurotropic virus into the brain via the olfactory pathway and neutralizes the multiplication of virus in visceral organs, allowing more effective protection against neurotropic infections (98).

Rivastigmine is an acetyl cholinesterase which can be rapidly absorbed after oral administration but extensively metabolized by cholinesterase-mediated hydrolysis. Liposomes my provide carrier system for this drug through nasal route to CNS. In a comparative study intranasal liposome was compared with the oral free drug and it was recorded that liposomal formulation can provide ten times higher C_{max}, higher systemic AUC, and higher concentration in the brain compared to oral administration. The liposomal formulation provided better absorption into the brain following intranasal administration compared to the free drug. This might also be due to direct transfer of the drug from nasal mucosa to the brain via the olfactory route (144).

The intranasal administration of quercetin liposome to rats provided opportunity for the drug to enter the central nervous system and act on the central nervous system to promote anxiolytic activity and cognitive enhancing effect with high efficiency (145). The anxiolytic activity of oral quercetin liposomes was compared with intranasal quercetin liposomes, both routes showed anxiolytic and cognitive-enhancing effects. A lower dose and a faster rate were observed with intranasal quercetin liposomes when compared with oral quercetin liposomes. The intranasal quercetin liposome was thus considered as effective in the delivery of quercetin to the central nervous system (146).

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Chapter 8

Query No.	Line No.	Query
AQ1	18	Please check whether the edit made to the sentence "Unfortunately, the mucociliary clearance. . ." is ok.
AQ2	195	In this sentence ("The epithelial cells are. . ."), please confirm if "sit" can be changed to "slit."
AQ3	310	Please check whether the edit made to the senetnec "The physicochemical characteristics of the. . ." is ok.
AQ4	688	In this sentence ("Plasmid DNA complex with mannosylated. . ."), please check if a word or phrase is missing after "receptors, mannosylated."
AQ5	710	"Estradiol is a female sex **hormone steroids can** enhance liposome-mediated gene delivery. . ." has been changed to "Estradiol is a female sex **hormone steroid that can** enhance liposome-mediated gene delivery. . ." Please check if it is OK.
AQ6	792	Please chec whether the edit made to the sentence " A range of different vaccine . . ." is OK.
AQ7	934	In this sentence ("The ability to induce. . ."), please spell out the genus names for "H. salinarum," "T. acidophilum," and "M. smithii."