CHAPTER 2 LITERATURE REVIEW

2.1Cerebral ischemia

Stroke is the second most common cause of death and a major cause of disability worldwide. The burden of this disease will only increase in the coming decades as the population ages. Currently, treatments with proven benefits for acute ischemic stroke (AIS) patients include management in a stroke care unit, intravenous administration of tPA i.e tissue plasminogen activator within 3 h, or administer aspirin within 48 h of stroke onset (1) .

However, only a small number of AIS patients (3%) receive specific therapy, and stroke prediction is poor, with approximately a quarter of patients dying in 1 month and half by 1 year (2). It is obvious that in spite of improvement in stroke care and therapy, there is still much room for improvement. Challenge in increasing the percentage of treated patients include extension of the 3-h window for i.v. thrombolytic by improving patient selection using imaging techniques such as diffusion and perfusion magnetic resonance imaging (DWI/PWI MRI) that identify 'ischemic' brain tissue, the so-called ischemic penumbra (3). Other approaches focus on developing alternate thrombolytic agents, such as desmoteplase, having fewer side effects and wider time window; or use of intra-arterial approach to increase the treatment window (4). In addition to these approaches, variety of neuroprotective agents have been evaluated; but till date no successful candidates have been identified, though several promising candidates are currently under investigation in Phase II or III clinical trials. Stroke triggers an array of mechanisms that lead to infarction. When a blood vessel is occluded, oxygen and glucose supply are immediately disrupted which activates excitotoxic mechanisms by increasing extracellular glutamate concentrations. Successively, intracellular calcium rises, leading to activation of enzymes that damage or destroy neurons. The ischemic cascade also induces and worsens mechanisms such as inflammation and apoptosis in a vicious cycle of positive reinforcement, leading to further tissue destruction(5). It becomes apparent that blocking just a single mechanism in the complex cascade using a pharmaceutical agent is unlikely to improve stroke patients' clinical outcome. Conversely, a combination of differently-acting drugs, or using a drug that has multiple mechanisms of action, is promising strategy for AIS treatment. Developing such pharmacologic agents is a worthwhile endeavor that has the potential of impacting upon millions of lives.

2.1.1 Current Research Goals and Scientific Rationale for Treatment

An alternate approach for treating AIS is neuroprotection. The basic principal behind targeting neuroprotective agent at one or more points in the ischemic cascade and deliver them to penumbral ischemic tissue as blood flow persists in this region, allowing the drug to prevent irreversible injury (6). The resulted reduction of infarct size is expected to improve clinical outcome. This hypothesis has been validated in animal models of stroke, with many neuroprotective agents reducing infarct size and some improving behavioral/functional outcomes (7). But till date none of these agents demonstrated efficacy in stroke patients in clinical trials. The current aim for treatment should include development of a drug targeting, multiple points in the ischemic cascade and/or the use of multiple acting drugs that can be safely co-administered. It is anticipated that a neuroprotective agent with even modest benefits would be extensively used as a stand-alone treatment, since there are many instances where i.v. or Intra Arterialthrombolysis is difficult to administer or contraindicated (8). Hence, neuroprotective agents could also be used to extend the survival of the ischemic penumbra and therefore extend the time window for thrombolytic or mechanical reperfusion. Another potential use would be use as an adjunct to thrombolysis or mechanical reperfusion, to reduce hemorrhagic risk or tPA toxicity (9). In that case, the agent must have safety in a clinical trial when used in conjunction with these therapies.

2.1.2 Current Therapies in Ischemic Stroke

Once the patient is diagnosed for ischemic stroke treatment should be followed. The first aim of therapy is to prevent or reverse brain injury. After initial stabilization, an emergency non-contrast head CT scan should be performed to differentiate ischemia from hemorrhagic stroke. Treatments designed to reverse or lessen the amount of tissue infarction fall within following categories:

- Medical support
- Thrombolytic therapy
- Anticoagulation
- Antiplatelet agents
- Neuroprotection

2.1.2.1 Medical Support

The first and foremost goal is to regularize cerebral perfusion in the tissue surrounding ischemic pneumbra. Second keep an eye towards common complications like infections (pneumonia, urinary tract, and skin) and deep venous thrombosis (DVT) with pulmonary embolism. As collateral blood flow within the ischemic brain depends upon blood pressure, there is argument about whether blood pressure should be lowered acutely. Blood pressure should only be lowered if there is malignant hypertension or affiliated myocardial ischemia or if blood pressure is >185/110 mmHg and thrombolytic therapy is anticipated.

When faced with the competing demands of myocardium and brain, lowering the heart rate with a β1-adrenergic blocker (such as esmolol or labetolol) can be a first step to decrease cardiac work and maintain blood pressure. In case of cerebral ischemia, fever is harmful and should be treated with antipyretics. As consequences of cerebral stroke, edema develops causing brain herniation in around 5 to 10% of patients. The larger the infarct, the greater the likelihood that clinically significant edema will develop.

Special attention is given to cerebellar infarction patient as cerebral edema can acutely increase intracranial pressure (ICP) in the posterior fossa or directly compress the brainstem resulting in coma and respiratory arrest and require emergency surgical decompression. Water restriction and intravenous mannitol may be used to raise the serum osmolality, but hypovolemia due to water restriction should be avoided as this may lead to hypotension and worsening infarction.

2.1.2.2 Thrombolytic Therapy

Thrombolytic therapy is one of the most promising therapies for acute ischemic stroke. Thrombolytic therapy restores cerebral blood flow in some patients with acute ischemic stroke and may lead to improvement or resolution of neurologic deficits. The majority of strokes are due to blockage of an artery in the brain by a blood clot. Thrombolytic drugs are mainly responsible for severe bleeding in the brain, which may be fatal. Thrombolytic therapy has now been evaluated in several randomized trials in acute ischemic stroke.

rt-PA (alteplase) is the only approved drug for clinical use in acute ischemic stroke. The molecule was approved on the basis of results of two Phase III trials, where its efficacy was proved when given within 3 h of onset of the symptom. The evidence base for

thrombolysis in stroke includes 21 completed randomized controlled clinical trials enrolling 7152 patients, using various drugs, dose, time frame and intravenous or intra-arterial route of administration(10). To increase the drug concentration at clot and to minimize systemic bleeding complications, there is growing interest in using thrombolytics via an intraarterial route. Although, USFDA approval is not obtained, the Prolyse in Acute Cerebral Thromboembolism (PROACT) II trial found benefit for intraarterial pro-urokinase for acute middle cerebral artery (MCA) occlusions up to the 6 hrs following onset of stroke.

2.1.2.3Anticoagulation

Thrombolytics accelerates the natural degradation of fibrin, anticoagulant reduces the formation of fibrin. As such, the role of anticoagulants lies in the secondary prevention of thromboembolic events, rather than as an agent that can improve the outcome of an established vessel occlusion. Theoretically, since a thrombus will be in a state of dynamic equilibrium with its soluble precursors and degradation products, any drug that reduces fibrin formation should also promote its net resolution. However, these processes seem to occur too slowly to have a practical effect on an occlusive thrombus in the context of stroke.

The prototypical anticoagulants are heparin and warfarin. Heparin is a liver-derived glycosaminoglycan that enhances the action of circulating antithrombin-III, itself an endogenous inhibitor of factors IIa (thrombin), Xa, and IXa. As a safer alternative to unfractionated heparin, Low-molecular-weight (LMW) heparins have been introduced and they achieve more stable bioavailability, results in less vascular permeability and hemorrhage, and complication of thrombocytopenia and paradoxical thrombosis are less with respect to that parent compound.

Warfarin, by contrast, acts at a level far removed from the dynamic chemical inter-conversions of the growing fibrin– platelet complex. By inhibiting the synthesis of vitamin-K dependent coagulation factors, its effects can only be appreciated after several days, during which existing coagulation factors are gradually catabolized.

Due to risk of hemorrage and recurrence of stroke events, heparin, or its derivatives are not effective or even more dentrimetal (11). International Stroke Trial, used subcutaneous heparin at low and medium doses, and in TOAST trial, intravenous danaparoid - a heparin derivative was used that avoids the side-effect of thrombocytopenia (12). Treatments were started within 48 hr and continued for 1–2 weeks. In both trials, heparin or danaparoid

reduced the risk of recurrent ischemic stroke, but increased the risk of major hemorrhage, rendering the overall functional long term outcome no different relative to placebo. In the TOAST trial, this occurred despite close adjustment of drug dose in line with anticoagulant effect (monitored as factor Xa activity).

Anticoagulants are also frequently used in any type of ischemic stroke when it progresses or when recurrent events occur in spite of optimal antiplatelet therapy, that is, clopidogrel or aspirin–dipyridamole combination. Anticoagulation may also have a specific benefit, over antiplatelet therapy, in the treatment of vertebrobasilar artery stenosis or dissection (13, 14), aortic-arch atheroma (15), and antiphospholipid syndrome (16) or other prothrombotic states.

2.1.2.4 Other Anticoagulants

A different form of anticoagulant, the snake venom-derived, thrombin analogue, ancrod has shown its effectiveness in the treatment of ischemic stroke(17). This agent cleaves fibrinopeptide from fibrinogen to produce non-cross-linked soluble fibrin, thereby depleting fibrinogen levels. The soluble fibrin complexes formed are also stimulators of endogenous tPA, thus conferring ancrod with a fibrinolytic action.

Recently an endogenous anticoagulant, activated protein C,has shown to have anti-inflammatory and neuroprotective properties and could potentially be administered intravenously in the acute phase of ischemic stroke (18).

2.1.2.5 Antiplatelets Agents

Platelet activation, which results in exposure of glycoprotein IIb– IIIa, the main ligand involved in platelet-fibrin adherence, as well as degranulation of a range of prothrombotic mediators (e.g., von Willebrand factor, calcium)is a critical event in ischemic stroke pathogenesis. Several chemical messenger pathways are involved in this dramatic switch of platelet configuration. Two of these pathways involving the secondary messenger thromboxane and cAMP serve as targets for current antiplatelet therapies.

Aspirin is the only anti platelet agent that has been prospectively studied for the treatment of acute ischemic stroke. The recent large trials, the International Stroke Trial (IST)

and the Chinese Acute Stroke Trial (CAST), found that the use of aspirin within 48 h of stroke onset reduced both stroke recurrence risk and mortality minimally.

Based on the two trials (19); it was clear that acute treatment of stroke with aspirin reduces death and dependency, but the size of this effect is small. Study of IST trial showed that aspirin therapy reduced the risk of recurrence of stroke within 14 days with no significant excess of hemorrhagic strokes. In CAST trial, study showed that there was no significant improvement in outcomes with combination treatment of aspirin and low– molecular-weight heparin(19).

The benefit of aspirin in reducing further ischemic events does not appear to depend on dosage, although maximal thromboxane inhibition during the first few days of stroke may only be achievable with high doses (20).Aspirin in addition with dipyridamole or substituting clopidogrel for aspirin provide significantly better protection against future strokes than aspirin alone(21).A potential role for GP-IIb–IIIa receptor antagonists in either acute treatment or secondary prevention of ischemic stroke has been the subject of recent trials, given the success of these agents in acute coronary syndromes. Abciximab administration showed improved outcomes at three months, which is given within 6 hours(22). However, due to lack of efficacy and risk of higher rate intracranial hemorrhage in patients receiving abciximab, termination of the phase 3 Abciximab in Emergency Treatment of Stroke Trial (AbESTT-II) was done (23). Long term use of an oral GP-IIb–IIIa receptor antagonist, lotrafiban (plus aspirin), in patients with either ischemic stroke or heart disease resulted in a 3 times higher rate of severe hemorrhage and a 33% increase in mortality, although this was related to aspirin dose (24, 25).

2.1.2.6 Neuroprotection and Current Status

Neuroprotective drugs interfere with ischemic cascade events and block one or more damage mechanisms and pathological processes and thereby prevent death of vulnerable nerve cells in the ischemic penumbra(26). This will in turn inhibit pathological molecular events like calcium influx, free radical activation and neuronal death.

Even after multiple clinical trials, beneficial role of excitatory amino acid inhibitory drugs have not yet been proven in humans. . Still interest in neuroprotection continues because of the limited risk potential for such agents, even when administered in the pre-hospital setting or in conjunction with thrombolytic agents.

With improved understanding of the pathophysiologic complexity of ischemic brain injury and the ischemic cascade, the categories of neuroprotective agents have also grown to include promoters of membrane repair, suppressors of neuronal metabolism, apoptosis inhibitors, free radical scavengers, calcium entry blockers, excitotoxic neurotransmission blockers, nitric oxide–related interventions, hyperpolarization agents inhibitingperi-infarct depolarization, anti-inflammatory and anticytokine agents, and neurotrophic agents.

Nearly 200 neuroprotection clinical trials are ongoing or have been completed, with none achieving successful translation to clinical practice so far. Huge number of neuroprotective drugs has been tested in phase 2 and phase 3 trials so far. These agents include, among others, GABA antagonists, inflammation blockers, calcium channel blockers, calcium chelators, AMPA antagonists, nitric oxide inhibitors, competitive and non-competitive NMDA antagonists, serotonin antagonists, free radicals scavengers and antioxidants, Glycine site antagonists, polyamine site antagonists, growth factors, adhesion inhibitors, opioid antagonists, sodium channel blockers and potassium channel blockers. Some of the drugs evaluated like piracetam, were of uncertain mechanism.

Various in vitro and in vivo animal models for ischemic injury have been developed and validated. The cytopathology, biochemical and molecular events, intracellular mediators and several important modulator influences were studied. In unison, these advances provided the productive background for logical approach of ischemic neuroprotection. Neuroprotectives provoke, disrupt, or slow down the series of injurious biochemical and molecular events and would help in irreversible ischemic injury. From the recent research on neuroprotection a principle was formed stating that ‘‘everything works in animals but nothing works in human.’’ Thus it was assumed that ischemic neuroprotection may not be an attainable clinical goal. Thus it is necessary to call attention to numerous studies in experimental animals which provide considerable proofs that protection of the ischemic brain is indeed achievable.

2.2 Nasal Delivery system

Nasal route is used from ancient time for therapeutic and recreational purposes. Psychotropic drugs and hallucinogenic substances such as cocaine and heroin were snuffed for these purposes by the India and South America(27). Over the recent years, importance of nasal delivery for systemic of drugs have been expanded. This route offers an alternative to those

drugs which find difficulty in parenteral administration or oral administration. Due to highly permeable epithelium and richly vascularised nasal tract, bypassing of hepatic first-pass metabolism, large surface area, high blood flow, ease of administration, this route is getting attention of various scientists(28, 29). At present, sumatriptanis given for migraine, desmopressin is given for diabetes insipidus and oxytocin for stimulation of breast milk ejection by nasal route(30). The drugs in pipeline for nasal administration include Vitamin B12, Olanzepin, Calcitonin and apomorphine.

2.2.1 Nasal Anatomy and Physiology

The nose actively contributes to two major functions, the first function is the sense of smell (olfaction) and the second is respiration or breathing The nasal septum divides the nose into two nasal cavities(31). Each cavity contains three distinct functional regions(32). The vestibular region, containing long hairs which serves as a filter for incoming particles(33). Between the vestibular and olfactory regions, the respiratory region is located which contains largest surface area.

To deliver drug systemically, respiratory region is considered as the most important section. The olfactory region responsible for smell functions, is located in the uppermost portion of each cavity and opposite to the septum(32).The epithelium of the respiratory region consists of four different cell types : basal, mucus-containing goblet, ciliated columnar, and nonciliated columnar(34). The ciliated columnar cell is the most predominant. The cilia beat in a wave-like, coordinated manner to transport mucus and trapped particles to the pharynx area for subsequent ingestion. Microvilli cover the cells in the respiratory region, which greatly increases the surface area of nasal cavity. The respiratory region also contains inferior, middle, and superior turbinates(32). Majority of cholinergic innervation to the nasal cavity is provided by the vidian nerve. When stimulated, acetylcholine is released and vasodilatation occurs(31).

Sympathetic innervation to nose arises from the stellate ganglion. Released norepinephrine acts on both α and β2 receptors and the overall result is vasoconstriction, demonstrating dominance of the α receptor(31).

A mucus layer, is present on the nasal passage epithelium resulting from nasal and lacrimal gland secretions as well as plasma transudate (27).In adults and children, the pH of secretions ranges from 5.5 to 6.5 and from 5.0 to 6.7, respectively(35).The mucus consists of

two layers, one is an outer viscous layer of mucus (gel) and second is a watery (sol) layer located along the mucosal surface(28). Mucin, one of the glycoproteins is responsible for the gel-like appearance of the mucus. Mucus contains lysozymes, enzymes, immunoglobulins and other proteins. Mucus composites include approx. 3% proteins, 90–95% water and 1–2% salt.

An important defense function of the nasal passage is mucosal clearance. Clearance occurs as a combined effort of the mucus layer and cilia action. The cilia project into the sol layer where they move in a sweeping motion back and forth. The gel layer of the mucus, along with entrapped particles, is transported to the nasopharyngeal area for ingestion(34). The cilia beat at a frequency of approximately 10–13 Hz (36).This results in movement of mucus at a rate of approximately 5–6 mm/min and therefore clearance of particles from the nose within 20 min(28). Nasal mucosa contains the xenobiotic-metabolizing enzymes(37). In animals, these enzymes are found in larger quantities in the olfactory epithelium compared to the respiratory epithelium of the nose. In humans, this distinction remains unclear owing to difficulty obtaining olfactory epithelium. Respiratory mucosa contains cytochrome P450 enzymes and its identified isoforms like CYP1A, CYP2A, and CYP2E(38)and other enzymes like carboxylesterases, glutathione S-transferases, and rhodanese(39). There are many environmental and pathological factors including common cold, respiratory viruses(40), allergic (41)and non-allergic rhinitis(42), smoking(43)and process of aging (22), which can alter the normal physiology of nasal passage. Estrogen is believed to induce physiological changes in the nose. presence of a deviated septum, immotile cilia syndrome, or nasal polyps(44)also cause alterations in the nasal cavity. The potential effects of these modifications should be taken into account when considering intranasal administration.

2.2.2.1 Advantages of Intranasal Drug Administration(45)

1. Non-invasive and easily accessible route
2. Patient compliance
3. By pass hepatic first-pass metabolism
4. highly vascularized epithelium
5. Useful for gastric fluid sensitive drugs
6. Large surface area for absorption (150 cm^2)
7. Requires low dose to attain therapeutic blood level, there by fewer side effects.
8. High total blood flow per cm^3, there by quick onset of action

9. Direct delivery of drug to brain via olfactory nerves.

2.2.2.2 Limitations of Nasal Delivery System

1. Toxic nature of penetration enhancers used in delivery system
2. Nasal irritation due to drug molecule
3. Less surface area for drug absorption compared to GIT.
4. Once the drug administered cannot be removed.
5. Drugs having molecular weight more than 1kDa are not suitable.

2.2.3 Mechanism of Nasal Drug Absorption

The first step in drug absorption through nasal cavity is crossing through thick mucus layer. High molecular weight and charged drugs find it more difficult to cross. On the contrary neutral drugs and small molecular weight drugs easily cross this layer. Several mechanisms are involved in transnasal absorption of the drugs into the brain.

1. Paracellular transport i.e transport of drug molecule between cell
In this mechanism water soluble and low molecular weight compounds pass through the environment between the cells.

2. Transcellular or simple diffusion i.e across the membrane
In this pathway lipophilic drugs pass through the lipid bilayer of cell membrane either by carrier mediated pathway or through opening in tight junction. It is responsible for transport of lipophilic drugs and their rate of transport depends upon lipophilicity of drug and the carrier.

2.2.4 Factors Affecting Nasal Absorption of Drug across Nasal Epithelium

Drug cross the nasal epithelium by following mechanisms: Across the cell membrane passive diffusion, through the gap between two cells paracellular passive diffusion, carrier-mediated absorption and transcytosis. The factors responsible for reduced nasal absorption of drug are nasal physiology, characteristics of drug and the dosage form.

2.2.4.1Physiological Issue

2.2.4.1.1Mucocilliary Clearance

Mucocilliary clearance is a natural defense mechanism against inhaled hazardous particles. It's a combination of mucus layer and the cilia(46).In healthy human speed of mucociliary clearance is about 5 mm/min. Using viscosity increasing agents the rate can be decreased. Cilia helps in movement of superficial viscoelastic mucus layer towards the nasopharynx and less viscous lower layer of the mucus is relatively stationary(47).

2.2.4.1.2 Enzymes

Nasal tract though have the advantage of avoiding the first pass hepatic metabolism, many metabolic enzymes such as cytochrome P-450, proteolytic enzymes such as endopeptidasesand exopeptidases are present in the nasal mucosa. These enzymes may limit the bioavailability of drugs such as peptides or proteins(48). On the other hand, level of metabolism by nasal enzyme is less in comparison to gastrointestinal tract or liver(49).

2.2.4.1.3 Nasal Pathophysiology

Nasal tract is generally affected by common cold**,** seasonal rhinitis, nasal polyps and cancer. These pathological and physiological conditions hamper the absorption from the nasal cavity either by affecting mucocilliary clearance or nasal epithelium.

2.2.4.1.4 Blood Supply

The nasal cavity is highly vascularised for the heating and humidification of the inspired air and nasal defense. Blood supply to nasal cavity is increased on parasympathetic stimulation and decreased on sympathetic stimulation. An electric stimulation of parasympathetic nerves innervating the nasal mucosa in dogs increased drug permeation due to an increase in nasal blood flow and nasal secretion.

2.2.4.2 Physicochemical Character of the Formulation and Drug Substance

It includes molecular weight (MW), solubility in carrier medium, dissolution rate in physiological fluid, charge either positive, negative or neutral, n-Octanol:Water partition coefficient, pKa of drug , particle size and the presence of polymorphism. Drugs having molecular weight up to 1000 kDa in formulations without adjuvant has showed good

bioavailability i.e. lower the molecular weight more will be absorption. On the other hand, opposing to the result no difference in absorption was observed between gastrointestinal and nasal mucosa in rats. Therefore, unknown mechanisms may be involved in transport of drug having molecular weight more than 1kDa.

Absorption increases with increasing lipophilicity of compound in nasal drug delivery, but in case of quaternary ammonium compounds reduction in absorption was found with increased lipophilicity and molecular weight (50).Nasal formulations contain excipients such as solubilizers for solubilizing drug, preservatives to prevent microbial growth, antioxidants for protection from oxygen.

2.2.4.2.1 Solubility Enhancer

Solubility is a major hurdle in formulating a nasal dosage form. As for administration of high dose more solubility is required due to limitation of spray administration in each cavity. Solubility of drug is improved by using co-solvents, surfactants or solubilizers, cyclodextrin complexes keeping in mind their toxicity and biocompatibility. E.g. Glycols, alcohol, Transcutol®, medium chain glycerides and Labrasol®, Hydroxypropyl–beta-cyclodextrin.

2.2.4.2.2 Preservatives

As most nasal formulations manufactured using are water-based systems; use of preservative is must to prevent microbial growth. E.g Parabens, phenyl ethyl alcohol, benzalkonium chloride, benzyl alcohol and EDTA.

2.2.4.2.3 Antioxidants

To protect oxidation sensitive drug, antioxidants are required. E.g Sodium metabisulfite, Sodium bisulfite, Butylatedhydroxytoluene (BHT) and Tocopherol (Vitamin E).

2.2.4.2.4 *Humectants*

In many allergic and chronic diseases nasal mucosa become dry leading to tissue irritation. And some excipients especially like preservatives and antioxidants when used in large quantities cause nasal irritation. Hence humectants added into formulation for hydration of nasal tract and prevention of nasal irritation. E.g. Glycerin, Sorbitol and Mannitol.

2.2.4.2.5 Drug Concentration, Dose and Dose Volume

A perfect nasal formulation is one with required dose dissolved in dosing volume. Up to only 150 µl dose volume can be delivered to the nasal cavity and hence to solubilize the required dose in this dose volume use of solubilizers is made. And maximum utilization of this dose is carried out using gelling, or viscosity imparting agents.

2.2.4.2.6 Absorption Enhancers

Drugs having molecular weight more than 1 kDa, low solubility and susceptibility to nasal enzymes requires combination with absorption enhancers to improve bioavailability. In order to increase permeation of the drug through nasal mucosa absorption enhancers are used which improves penetration by either changing physicochemical properties of drug or by altering nasal mucosa structure. The selection of concentration of absorption enhancer is carried out based upon the IIG limit and its toxicity limit.

2.2.4.3Delivery Device Related Factors

Nasal delivery of formulation is carried out using different types of devices. The bioavailability of nasal formulation is dependent upon the size of droplet, site and pattern of deposition in the nasal cavity.

2.2.4.3.1 Droplet Size Distribution (DSD) of Droplet or Powder

DSD and disposition site of the spray depends upon the design of delivery device. Globules or particles less than 10µm will travel through nasal tract and deposit into upper respiratory tract, whereas if the particles or globules size is less than 0.5µm they will be exhaled, while globules more than 200 µm size will slide down the tract. Particles or droplets with size between 10 to 200 µm will be retained in the nasal cavity and subsequently permeated (50).

2.2.4.3.2 Site and Pattern of Deposition

Viscosity of formulation, design of actuators and adapters, handling of spray device and the administration technique influence the site and pattern of drug deposition. And bioavailability of drug depends upon the deposition site and percent area on which drops are spread.

2.2.5 Characteristics Feature of Nasal Dosage Forms(45)

1. Mucoadhesive property
2. Penetrability through the mucus
3. Form a viscous layer on the epithelium
4. Protect drug from the environment in nasal tract
5. Stable in different temperature and RH condition
6. Controlled release of the drug

2.2.6 Formulations for Nasal Drug Delivery

Various dosage forms are formulated for intranasal route for local effect, systemic and brain targeting. The deposition, deposition area and residence time are dependent upon the dosage form and delivery device.

2.2.6.1 Nasal Drops

They are simplest and most convenient dosage form for nasal administration. Nasal Solution is generally given in from of nasal drops. One of most disadvantage of this formulation is that exact dose of formulation cannot be delivered. Nasal drops are not preferred for drugs having low therapeutic window.

2.2.6.2 Nasal Sprays (Solution and Suspension Sprays)

Drug having low therapeutic window are administered using metered dose nasal actuators delivering exact dose. The actuation volumes of 25 to 100 µl can be delivered precisely by these type of systems. Suspensions can also be delivered by this type of delivery systems with consideration of particle size and morphology.

2.2.6.3 Micro-emulsions

Microemulsions are isotropic and thermodynamically stable systems. They are now a days most preferred dosage forms for nasal administration, As they offer advantages like high solubilization capacity, high permeation and absorption rates compared to solvent without surfactant, thermodynamic stability (51).

2.2.6.4 Nasal Gels

Gels prevents post nasal drip back into the throat, reduces anterior leakage out of nasal cavity and helps to localize the formulation on the mucosa for maximum period. Such dosage forms are developed for systemic or local drug delivery.

2.2.6.5 Nasal Powders

Powders can be delivered into nasal cavity using metered doses delivery devices called as insufflators. Powder dosage forms are most preferred for drug which acts locally in the nasal cavity. This dosage from do not require preservative and thereby they are devoid of preservative toxicity and they have better stability compared to liquid dosage from. These systems can cause irritation and gritty feel to nasal cavity.

2.2.6.6 Nanoparticles

Nanoparticles are colloidal system with diameters ranging from 1-1000 nm. They are made up of biocompatible polymers in which active substance is dissolved, encapsulated, entrapped, chemically attached or adsorbed. They have an advantage of conjugating receptor specific targeting moiety to their surface (52).

2.2.6.7 Liposomes

Liposomes are phospholipid vesicles composed of lipid bilayers enclosing one or more aqueous compartments and wherein drugs and other substances can be included. Liposomes can encapsulate both hydrophobic and hydrophilic drug molecules. They offer encapsulation of drug molecules with wide range of hydrophilicity and pKa values. Law SL et.al prepared liposome containing desmopressin for intranasal delivery and study showed that positively charged liposome enhanced contact time with negatively charged nasal mucosa which led to high concentration of drug on penetration site at nasal mucosa. After intranasal delivery, permeability shown to be high in case of positively charged liposome compared to negative charge liposome and free drug solution (53).

2.3 Micelles

Currently, various types of drug delivery carriers and drug targeting carriers like liposomes, nanoparticles, soluble polymers, microcapsules, micelles etc. are being developed or under

development, to minimize drug degradation and loss upon administration, prevent harmful or undesirable side-effects, improve drug bioavailability and the amount of the drug accumulated in the pathological zone.

Among those delivery systems, micelles have been paid more attention in recent years as promising drug carriers. Micelles belong to a group of association or amphiphilic colloids. Such colloids are formed spontaneously under certain concentration and temperature. Although such molecules exist separately at low concentrations in aqueous medium, they show aggregation with increase in their concentration.

One micelle is an aggregate of several dozens of amphiphilic molecules and usually has a shape close to spherical. The concentration of a monomeric amphiphile at which micelles forms is called the critical micelle concentration (CMC), while the temperature below which amphiphilic molecules exist as unimers and above as aggregates is called the critical micellization temperature (CMT). The core of micelle consist of hydrophobic fragments of amphiphilic molecules, which help to solubilize poorly soluble pharmaceuticals, while hydrophilic fragments form the micelle's corona (54). In aqueous systems, the micelle core solubilizes nonpolar molecules while polar molecules are adsorbed on micelle surface and intermediate polarity molecules are distributed in intermediate positions.

It is widely known that surfactants have an ability to enhance the solubility of poorly water-soluble compounds or drugs in an aqueous solution and used in many aspects of drug formulation development (55).In addition, biologically relevant surfactants, bile salts as well as lecithin, can form mixed micelles that are responsible for solubilization and transport of fats and oils during digestion and likely facilitate dissolution and transport of poorly water-soluble drugs in the intestinal fluid (56).

Surfactants typically contain discrete hydrophobic and hydrophilic regions, which allow them to orient at polar–nonpolar interfaces, such as water/air interfaces. Once the interface is saturated, the surfactants self-associate to form micelles and other aggregates, whereby their hydrophobic regions are minimized and shielded from aqueous contact by their hydrophilic regions. This creates a discrete hydrophobic environment suitable for solubilization of many hydrophobic compounds (57).

Kabanov et al. (58) suggest that an 'ideal' self-assembling drug delivery systems should spontaneously form from the mixture of carrier components, drug molecules and

targeting moieties. In order to penetrate various tissues their size should be around 10 nm. They should be stable for a satisfactorily long time in vivo and should not aggravate any biological reactions. They should release free drug upon contact with target tissues or cells; and, finally, the components of the carrier should be easily removed from the body when the therapeutic function is completed.

In broad terms, micelles as drug delivery carriers provide a set of advantages including: By use of micelle-forming surfactants, water solubility of a sparingly soluble drug can be improved and so its bio availability is enhanced, reduction of toxicity and other adverse effects, enhanced permeability across the physiological barriers and substantial changes in drug biodistribution. The use of certain special amphiphilic molecules as surfactants can also introduce the property of micelle extended blood half-life upon intravenous administration (59).

Besides, micelles may be targeted by chemical attachment of targeting moiety to their surface. In the latter case, local release of the loaded drug from the micelles in the target organ should lead to increased efficacy of the drug. On the other hand, being in a micellar form en route to the target organ or tissue, the drug is well protected from possible inactivation under the effect of biological surroundings, and itself does not provoke undesirable side effects on non-target organs and tissues.

2.3.1Polymeric Micelles

Polymeric micelles consist of a core and shell structure, in which the inner core is the hydrophobic part, which solubilizes poorly water-soluble drug, whereas outer shell or corona consist of hydrophilic block of the copolymer protects the drug from aqueous environment and stabilizes the PMs against RES uptake. The core can be rendered hydrophobic, by the chemical conjugation of water-insoluble drug (60), which is made up of water soluble polymerby complexation of the two oppositely charged polyions, called polyion complex (PIC) micelles (61).

PIC micelles are formed by block copolymer, in which part is charged segment and other part is neutral polymer chain; the whole molecule is totally water-soluble and narrowly distributed (62). The polymer always contains a nonionic water-soluble segment [e.g. polyethylene glycol (PEG)] and an ionic segment that can be neutralized by oppositely charged surfactant to form a hydrophobic core.

The electrostatic interaction between the ionic segment of the block polymer and the surfactant group changes these segments from water-soluble to water-insoluble, leading to a hydrophobic core in the micelles. The hydrophobic core of micelle is solubilized by the nonionic water-soluble shell (63).

Due to having low CMC (10^{-6} M) values compared with surfactant micelles, Polymeric micelles are very stable (59). All these issues related to PMs make them ideal carriers for anticancer drugs and tumor targeting. These PMs are considered as a suitable carrier for poorly water-soluble drugs, genes and imaging agents (64, 65).

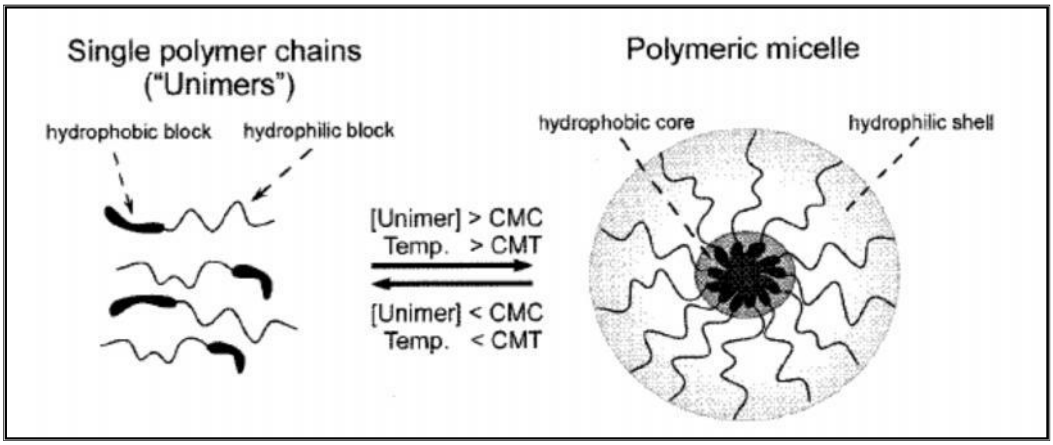


Figure 2.1 Self assemble structure of block copolymer micelles.

For targeting the tumor at inaccessible sites the drug should be administered by the parenteral route, and pharmaceutical drug carriers carrying drug in plasma should possess properties like small particle size, biodegradability, prolonged circulation, high loading capacity, and accumulation in the required pathological site(s) in the body (66). All these properties are satisfactorily executed by PMs.

For active targeting, polymeric micelles can be modified by ligand coupling or addition of pH-sensitive moieties according to the biological properties of the pathological site. Various ligands like epidermal growth factor, transferrin sugars, folate, α2-glycoprotein and peptides have been attached to polymeric micelles. Thus, PMs act as ideal drug carriers for targeting cancerous cells. On reaching the target site, micelles are internalized into the cells via fluid-state endocytosis, even without any surface ligand for targeting (67).

Aqueous solubility of poorly water soluble drugs can be enhanced by polymeric micelles. However, sufficient hydrophobicity to penetrate a cell membrane and the presence

of hydrophobic group(s) for sufficient affinity toward the receptor is required. To encounter these problems, amphiphilic copolymers are used to encapsulate poorly water-soluble anticancer drugs in PMs. These have an inner core made up of hydrophobic block copolymer in which the drug becomes entrapped, and an outer shell of hydrophilic block copolymer that reduces the interactions of drugs with the outer aqueous environment, keeping them stable. Interestingly, the hydrophilic micelle corona keeps the PM stable in plasma for longer duration and also prevents their opsonization and RES uptake.

Micelles have a generally size ranging from 10 nm to 100 nm with a substantial narrow distribution. Size of micelles is important with respect to stability and long circulation in the blood stream. The small size of polymeric micelles is more advantageous in the sterilization processes. Micelles can be easily sterilized by filtration easily and inexpensively using sterile filters, without particles clogging and without need of another separation process.

In case of other pharmaceutical nanosized carriers like liposome's and nanoparticles requires removal process of contaminated micron-sized particles which is contrast to micelle carriers.

2.3.2 Targeted Polymeric Micelles

To increase the efficiency of micelle encapsulated drug or diagnostic agents, they can be targeted to specific site or accumulated in preferential body compartments or pathological zones (i.e. making targeted micelles).

First approach to achieve this is the preferential accumulation of drug-loaded micelles in areas with 'leaky' vasculature (tumors and infarcts) via the EPR effect. Which is based on the spontaneous penetration of long-circulating macromolecules, molecular aggregates and particulate drug carriers into the interstitium through the compromised vasculature in certain pathological sites in the body? It has been shown that the EPR effect is typical for solid tumors and infarcts (68). This approach is called passive targeting. Circulation time of micelles increases with increase in size of PEG block by providing steric protection against opsonin penetration to the hydrophobic micelle core. The increase in size of a PEG block increases micelle circulation time in blood, due to continuous clearance of unimers with a micelle-unimer equilibrium being shifted towards the unimer formation (69).Slow dissociation of micelles under physiological conditions occurs which also plays its role.

By utilizing the EPR effect, smaller size of micellar carriers than other carriers of larger size have an advantage for tumor drug delivery, because they accumulate in tumor interstitium. Diffusion and accumulation parameters were shown to be strongly dependent on the cut off size of tumor blood vessel wall, and the cut off size varies for different tumors (70).

Higher accumulation of different PEG conjugated formulations in tumors are shown in comparison to non-target tissue in experimental Lewis lung carcinoma (LLC; tumor with a relatively small vasculature cut off size in mice (71). It was found that highest tumor uptake is shown in micelles formed by PEG conjugates formed from PEG_{5000}-PE among relatively shorter conjugates. This was explained by the fact that these micelles had the longest circulation time and little extravasation into the normal tissue compared to micelles prepared from the 'shorter' PEG-PE conjugates.

Micelles prepared from PEG-PE conjugates with shorter versions of PEG, be a more efficient carriers for poorly soluble drugs, due to greater hydrophobic-to-hydrophilic phase ratio and more efficient drug loading on a weight-to-weight basis.

Adriamycin in polymeric micelles was shown to be much more efficient in experimental treatment of murine solid tumor colon adenocarcionoma than the free drug. Since tumor vasculature permeability depends on the particular type of the tumor, application of micelle as drug carriers could be reasonable for low cut off size tumor vasculature (below 200 nm).

In general, the biodistribution of a microparticulate carrier-associated anticancer drug depends on its circulation time in blood. Thus, it has been shown that long-circulating PEG-grafted liposomes demonstrate increased accumulation in implanted tumor (72). Later, however, it was found that in some cases even the use of long-circulating liposomes could not provide sufficient accumulation in certain tumors. Parr et al. (73) have shown that coating 100-nm liposomes with PEG did not result in increased accumulation of liposome-encapsulated drug in a subcutaneously established murine Lewis lung carcinoma. This phenomenon may be explained by the low vascular permeability (small cutoff size) of this as well as some other tumors. In those cases, drug carriers smaller in size than liposome may provide more efficient drug delivery into tumors. Thus, the micelle-incorporated model protein (soybean trypsin inhibitor or STI, MW 21.5 kDa) accumulates to a higher extent in

subcutaneously established murine Lewis lung carcinoma than the same protein in larger liposome.

It was fact that many pathological processes in various tissues and organs are accompanied with local temperature increase and/or acidosis which is another mechanism for targeting. So, by making micelles capable of disintegration under the increased temperature or decreased pH values in pathological sites, efficiency of the micelle carriers can be further enhanced, i.e. by combining the EPR effect with stimuli responsiveness. For this purpose, micelles carriers are made of thermo- or pH-sensitive components such as poly(N-isopropylacrylamide) and its copolymers with poly(D,L-lactide) and other blocks, and acquire the ability to disintegrate in target areas, releasing the micelle-incorporated drug.

pH-responsive polymeric micelles loaded with phtalocyanine seem to be promising carriers for photodynamic cancer therapy, while acid-cleavable linkage containing micelles loaded with doxorubicin provided an enhanced intracellular drug delivery into tumor cells and thus higher efficiency. Thermo-responsive polymeric micelles were shown to demonstrate increased drug release upon temperature changes (74).

Externally applied ultrasound can enhance the penetration of drug-loaded polymeric micelles into cells (tumor cells) as well as drug release from the micelles (75).Potential of drug delivery of polymeric micelles can further be improved by attaching targeting ligands to the surface of micelles. The attachment of various specific ligands to the water-exposed termini of hydrophilic blocks could be used to improve the targeting of micelles and micelle-incorporated drugs and DNA.

Among those ligands one can name various sugar moieties, folate residues and transferrin as many target cells over express appropriate receptors (such as transferrin and folate receptors) on their surface. Thus, it was shown that galactose and lactose-modified micelles made of PEG-polylactide copolymer interact with lectins, and result in hepatic targeted drug delivery of micelle (76).

Transferrin-modified micelles based on PEG and poly (ethyleneimine) sized between 70 and 100 nm are expected to target tumors with over expressed transferrin receptors. Mixed micelle-like complexes of PEGylated DNA and PEI modified with transferrin were designed for enhanced DNA delivery into cells over expressing the same transferrin receptors. A similar targeting approach was successfully tested with folate modified micelles. Poly(L-

histidine)/PEG and poly (L-lactic acid)/PEG block copolymer micelles carrying folate residues on their surface were shown to be efficient for the delivery of adriamycin to tumor cells in vitro, demonstrating the potential for solid tumor treatment and combined targetability and pH sensitivity (77).

Recent studies demonstrated that transferrin(Tf)-modified poly(ethelene glycol)-phosphatidylethanolamine (mPEG-PE) micelles loaded with the poorly water soluble drug,R547(a potent and selective ATP- competitive cyclin-dependent kinase (CDK) inhibitor, exhibited enhanced targeting efficiency and cytotoxicity in vitro and in vivo to A2780 ovarian carcinoma cells, which overexpress transferrin receptors (TfR) (78).

Other studies showed that stable multifunctional micelle structure formed by spontaneous self –assembly of block copolymers with siRNA containing cRGD peptide resulted in increased gene silencing ability, improved cellular uptake, and broader sub-cellular distribution in vitro and also improved accumulation in both the tumor mass and tumor-associated blood vessels following intravenous injection into mice. In addition, stable and targeted micelles inhibited growth of subcutaneous HeLa tumor models and showed gene silencing in the tumor mass following treatment with antiangiogenic siRNAs (79).

Other research data showed that platinum anticancer drug-incorporating polymeric micelle (PM) with cyclic Arg-Gly-Asp (cRGD) ligand molecules exhibits selective and accelerated accumulation of cRGD/m into tumors via an active internalization pathway, possibly transcytosis, thereby producing significant antitumor effects in an orthotopic mouse model of U87MG human glioblastoma (80).

2.3.3 Advantages of Polymeric Micelle as a Drug Carrier

Polymeric micelles possess certain advantages as a drug carrier including

- ✓ Micelles have capacity to load large amount of drug in the carrier system. Generally, in conventional synthetic polymer-drug conjugate systems and antibody-drug conjugate systems, a loss of the carrier's water solubility resulting from the conjugation of a hydrophobic drug creates a serious problem.
- ✓ Polymeric micelles can incorporate a large number of hydrophobic drug molecules in the micelles' inner core**,** and simultaneously, the micelles can maintain their water solubility

by inhibiting inter micellar aggregation of the hydrophobic cores with a hydrophilic outer shell layer that works as a barrier against inter micellar aggregation. This is a great advantage because many potent drugs that have been developed in recent years are very hydrophobic and are, therefore, water insoluble.

- ✓ Characteristic of low toxicity of micelle carriers systems may be described as an advantage. Generally, polymeric surfactants are known to be less toxic than low-molecular-weight surfactants, like sodium dodecyl sulfate. Additionally, polymeric micelles are considered safe in relation to chronic toxicity. Possessing a much larger size than that for critical filtration in the kidney, polymeric micelles can evade renal filtration, even if the molecular weight of the constituting block copolymer is lower than the critical molecular weight for renal filtration.

- ✓ All polymer chains can be dissociated (as single polymer chains) from the micelles over a long time period. This phenomenon results in the complete excretion of the block copolymers from the renal route if the polymer chains are designed with a lower molecular weight than the critical value for renal filtration. Such a result constitutes an advantage of polymeric micelles over the conventional (non-micelle forming) and non-biodegradable polymeric drug carrier systems.

- ✓ Polymeric micelles are formed from charged polymer chains through ionic interactions. For example, polymeric micelles form from poly (ethylene glycol) (PEG)-b-poly (lysine) block copolymers and poly aspartic acid) (ASP) homo polymers where the poly (lysine) chain is positively charged and the poly(ASP) chain is negatively charged. Negatively charged polypeptides or nucleic acid can replace poly (ASP), which are incorporated into polymeric micelles for protein, gene, and small interfering RNA delivery purposes.

- ✓ Metal ions or chelates can be incorporated into polymeric micelles through coordination bonds or ionic interactions. A platinum chelate cisplatin, which is a widely used anticancer drug, was successfully incorporated into polymeric micelles forming from PEG-b-poly (ASP) through a ligand exchange reaction between a carboxylic acid residue of the poly(ASP) chain and a chloride ion of cisplatin (81).

- ✓ By use of a chelate moiety-conjugated block copolymer, gadolinium (Gd) ions, which can work as a magnetic resonance imaging (MRI) contrast agent, were incorporated into polymeric micelles (82).

As described above, various pharmaceutical agents or drugs, therapeutic genes, and contrast agents can be incorporated into polymeric micelles with appropriate choices of block copolymer structures.

2.3.4 Disadvantages of Polymeric Micelle as a Drug Carrier

It is worthwhile to explain the disadvantages of the polymeric micelle carrier systems. Disadvantages described here are specific to polymeric micelles as well as common for polymeric carriers including non-micelle forming systems.

- ✓ It is a fact that relatively high levels of polymer chemistry are needed in the polymeric micelle studies. An AB type of block copolymer is one of the most favorable structures for polymeric micelle carriers. The architecture of the AB block copolymer is very simple; however, its synthesis is more difficult than that of random polymers, where different units are aligned on a polymer chain in a random manner.

- ✓ Further, synthesis of the block copolymer of a large industrial scale in a highly reproducible manner is the problem. Specifically, the polymeric micelle systems are the immature technology for drug incorporation in a physical manner. Yokoyama et al reported that physical incorporation efficiencies were dependent on various factors in drug-incorporation processes. Presently, there seem to be no universal incorporation method applicable to any polymer. Furthermore, in some methods the drug incorporation may be difficult on a large industrial scale, whereas the drug incorporation is easy and efficient on a small laboratory scale.

- ✓ Another disadvantage is much slower extravasation of polymeric carrier systems than that of low-molecular weight drugs. This results from a difference in extravasation mechanisms between polymeric carrier systems and low molecular weight drugs. The polymeric systems translocate from bloodstream to the interstitial space of organs and tissues through intra-cellular channels and inter-cellular junctions, whereas the drugs permeate directly through lipid bilayer cell membranes. Therefore, a long circulation character of the polymeric systems is an essential requirement for delivery of a therapeutic amount owing to compensation of the slow extravasation with a large Area under the Curve value that results from the long circulation.

- ✓ Drugs conjugated or incorporated in the polymeric carrier systems are metabolized in liver in a slower manner than free drug, since access of metabolic enzymes to drugs is inhibited because of the conjugation and incorporation. Therefore, toxic side effects of the conjugated and incorporated drug may be exhibited for a longer period than in case of free drug whose toxic effects can be lowered through metabolism in a short period.

2.3.5 Methods of Preparation of Polymeric Micelles

Drug loading can be done by either chemical conjugation (e.g., amide bond) (83) or physical entrapment. The typical methods used for encapsulation of poorly water-soluble drugs are the oil-in water emulsion solvent evaporation method, dialysis method, and solid dispersion method. One another innovative, one-step method of preparation of PMs consists of lyophilization of the solution mixture of drug and polymer in a water–tert-butanol system. Reconstitution of this freeze-dried cake of drug-polymer mixture with injectable vehicle catalyzes spontaneous formation of PMs. Other methods used are direct dissolution (84), complexation (85), chemical conjugation(86), and various solvent evaporation procedures (87).

2.3.5.1 Dialysis Method

The dialysis method involves addition of small amounts of water to the solution of polymer and drug in a water-miscible organic solvent like dimethyl formamide with stirring followed by dialysis against an excess of water for several hours using a dialysis bag for the removal of organic solvent (88, 89).

2.3.5.2 Oil-in-Water Emulsion Solvent Evaporation Method

Drug along with the polymer is dissolved in a water-immiscible organic solvent like tetrahydrofuran (90), chloroform (91), acetone (92)or a mixture of solvents like chloroform and ethanol (93) and this solution is slowly added to the distilled water under vigorous stirring to form an emulsion with an internal organic phase and continuous aqueous phase, which rearranges the polymer to form micelles. Sometimes surfactants like polyvinyl alcohol are used in aqueous solution. This emulsion is then kept open in air with stirring so as to evaporate all the organic solvent.

2.3.5.3 Solid Dispersion Method

In this method, drug along with the polymer is dissolved in the organic solvent, and a solid polymer matrix is obtained after the evaporation of solvent under reduced pressure. Drug-loaded PMs are obtained after the addition of water to the preheated polymer matrix (94).

2.3.5.4 Microphase Separation Method

In this method the drug and polymer are dissolved in (organic solvent) tetrahydrofuran, and the solution is added drop wise in water under magnetic stirring. PMs are formed spontaneously, and drug is entrapped in the inner part of the micelles. Organic solvent is removed under reduced pressure, and a blue-colored PM solution is formed.

2.3.6 Targeted Polymeric Micelles

Making micelles capable of preferential accumulation in desired body compartments or pathological zones (i.e. making targeted micelles) can further increase the efficiency of micelle-encapsulated pharmaceuticals(drugs and diagnostic agents). There are several approaches to achieve this. The first is the already mentioned preferential accumulation of drug-loaded micelles in areas with 'leaky' vasculature (tumors and infarcts) via the EPR effect. The EPR effect is based on the spontaneous penetration of long-circulating macromolecules, molecular aggregates and particulate drug carriers into the interstitium through the compromised vasculature in certain pathological sites in the body. It has been shown that the EPR effect is typical for solid tumors and infarcts (68). Currently intensive research is carried out by various researchers on such passive targeting approach. Thus, micelle formulations from PEG_{750}-PE,PEG_{2000}-PE, and PEG_{5000}-PE conjugates demonstrated much higher accumulation in tumors compared to non-target tissue (muscle) in experimental Lewis lung carcinoma(LLC; tumor with a relatively small vasculature cut-off size (95)in mice. The accumulation pattern of PEG-PE micelles prepared from all versions of PEG-PE conjugates is characterized by the peak tumor accumulation times of about 5 h post-injection. The largest total tumor uptake of the injected dose within the observation period(AUC) was found for micelles formed by PEG_{5000}-PE. This was explained by the fact that these micelles had the longest circulation time and little extravasation into the normal tissue compared to micelles prepared from the 'shorter' PEG-PE conjugates. Micelles prepared from PEG-PE conjugates with shorter versions of PEG, however, might be more efficient carriers of poorly soluble drugs because they have a greater hydrophobic-to-hydrophilic phase ratio and can be

loaded with drug more efficiently on a weight-to-weight basis. The ability ofPEG-PE micelles to accumulate selectively in tumors was also confirmed with another murine tumor model, EL4 T cell lymphoma (EL4) (96). Micelles prepared fromPEG_{750}-DSPE or PEG_{2000}-DSPE demonstrated selective accumulation in this tumor as well. Some other recent data also clearly indicate spontaneous targeting of PEG-PE-based micelles into experimental tumors in mice as well as into the damaged heart areas in rabbits with experimental myocardial infarction.

Thus, the transport efficacy and accumulation of micro-particulates, such as liposomes and/or micelles, in tumor interstitium is to a great extent determined by their ability to penetrate the tumor vascular endothelium (69). Diffusion and accumulation parameters were recently shown to be strongly dependent on the cutoff size of tumor blood vessel wall which is found to vary in different tumors (70).

A similar targeting approach was successfully tested with folate modified micelles. Poly(L-histidine)/PEG and poly(L-lactic acid)/PEG block copolymer micelles carrying folate residues on their surface were shown to be efficient for the delivery of adriamycin to tumor cells in vitro, demonstrating the potential for solid tumor treatment and combined targetability and pH sensitivity (77). Static stability is described by a critical micelle concentration (CMC). Generally, polymeric micelles show very low CMC values in a range from 1 mg/mL to 10 mg/mL. These values are much smaller than typical CMC values of micelles formed from low-molecular weight surfactants. Dynamic stability is described by the low dissociation rates of micelles which may be more important than the static one for in vivo drug delivery in physiological environments that are in non-equilibrium conditions. The high structural stability of polymeric micelles stated earlier is an important key to in vivo delivery in micellar forms and simultaneously eliminates the possible contribution of single polymer chains to drug delivery. Therefore, although they share the root word “micelle,” polymeric micelles are very different from low-molecular-weight-surfactant micelles in their physicochemical properties. This difference is critical in the applications for drug carriers.

2.4Drug and Polymer Profile

2.4.1Nicergoline

Category: An ergot derivative that has been used as a cerebral vasodilator and in peripheral vascular disease. It has been suggested to ameliorate cognitive deficits in cerebrovascular disease.

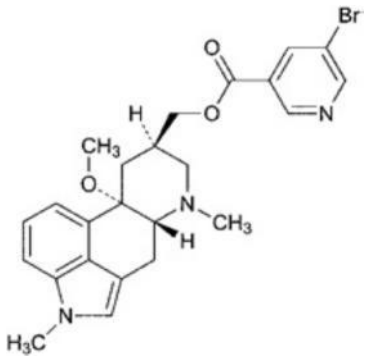
Generic Name: Nicergoline.

Marketed preparations available: Cholergol, Nicerbium, Sermion, Ergotop, Dospan in the form of tablets.

Empirical formula: $C_{24}H_{26}BrN_3O_3$.

Molecular weight: 484.39

Structure:



Appearance: Nicergoline occurs as white to light yellow, crystals or crystalline powder.

Physical properties

Solubility: It is soluble in acetonitrile, in ethanol (99.5%) and in acetic anhydride, and practically insoluble in water.

Melting point: 136°C.

Log P : 3.3

pKa: Strongest Basic at pH 8.13

Toxicity:

- Oral (rat) LD_{50}: 1193 mg/kg Reported, Intra-peritoneal (rat) LD_{50}: 1571 mg/kg
- Subcutaneous (rat) LD_{50}: 776 mg/kg, Intravenous (rat) LD_{50}: 42 mg/kg
- Oral (mouse) LD_{50}: 633 mg/kg, Intra-peritoneal (mouse) LD_{50}: 198 mg/kg\
- Subcutaneous (mouse) LD50: 1025 mg/kg

- **Pharmacodynamics:** Nicergoline is a potent vasodilator (improves brain blood flow). On the cerebral level it prompts a lowering of vascular resistance, an increase in arterial flow and stimulates the use of oxygen and glucose. Nicergoline also improves blood circulation in the lungs and limbs and has been shown to inhibit blood platelet aggregation.
- **Mechanism of action:** Nicergoline acts by inhibiting the postsynaptic alpha(1)-adrenoceptors on vascular smooth muscle. This inhibits the vasoconstrictor effect of circulating and locally released catecholamine (epinephrine and nor epinephrine), resulting in peripheral vasodilation. Therefore the mechanism of Nicergoline is to increase vascular circulation in the brain, thereby enhancing the transmission of nerve signals across the nerve fibres, which secrete acetylcholine as a neural transmitter.
- **Pharmacokinetics:** Nicergoline is rapidly and nearly completely absorbed after oral administration. The half-life of Nicergoline was 13 - 20 hours. Nicergoline is effectively bound to plasma proteins (>90%), with higher affinity for α-acid glycoprotein than for serum albumin. Nicergoline is excreted as its metabolites predominantly in urea (approximately 80% of the total dose) and in feces (10-20%).
- **Metabolism:** By cytochrome P 450 to 10-alpha-methoxy-9,10-dihydrolysergol
- **Adverse effects:** severe hypotension dizziness, dyspepsia, hot flashes, skin rash, sleepiness and insomnia.
- **Indications:** senile dementia, transient ischemia, macular degeneration, migraine.
- **Dosage:** 4-8mg, available as 5mg, 10mg and 30mg tablets.
- **Dosage & When it is to be taken:**
  Adult: PO- The recommended dose is 10 mg 3 times/day. Maintenance: 5-10 mg 3 times/day. Max: 60 mg/day in divided doses.
  Intravenous /Intramascular- The recommended dose is 2 to 8mg/day infusion.
- **Storage Conditions:** Store it at room temperature (25°C).

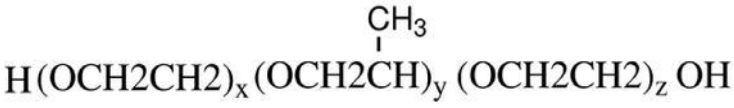
2.4.2 Poloxamer F 127

- Poloxamer F 127is nonionic detergent comprising of both hydrophobic and hydrophilic blocks. It is thermo reversible and achieves high viscosity.

Solubility: Soluble in water and ethanol (95%). Insoluble in ether, paraffin and fatty oils.

Appearance: White granular powder

Structure:



Application:

1. Dissolution enhancer in tablets and capsules
2. Lubricants for drugs incompatible with Mg stearate.
3. Polishing agent for film coated tablet
4. Dispersing and wetting agent

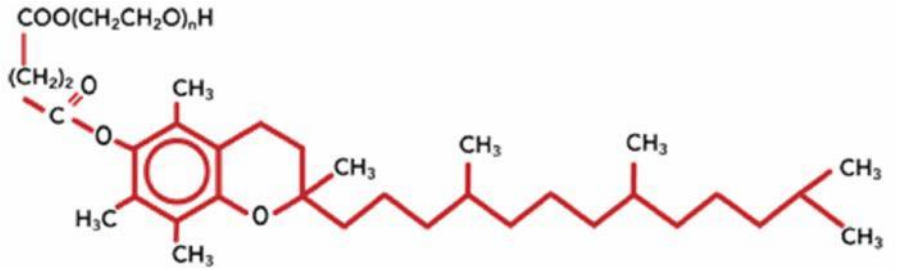
2.4.3Tocopherol Polyethylene Glycol 1000 Succinate (TPGS)

Nonionic surfactant with amphiphilic character, tocopherol succinate part act as lipophilic part and polyethylene glycol part act as hydrophilic part.

Appearance: Waxy solid, off white to light brown

Storage: Preserve in air tight and light resistant container

Chemical name:(d-Alpha Tocopheryl Polyethylene Glycol 1000 Succinate)



Average Molecular Weight: ~ 1513

Waxy solid melting point: 37 -41 °C

Water-miscibility: miscible

Solubility in PEG/PG(1:1): soluble

HLB Value: ~13.2

Critical Micelle concentration: approximately 0.02 wt%

Increases solubility of drug in concentration dependent manner

- **Stability in aqueous media:** Soluble 1g/10 mL, clear to faintly turbid, colorless to faintly yellow, stable at pH 4.5 -7.5 hydrolyzed in the body.
- **Vitamin E Content:**260 mg/g (387 IU/g)
- **Application in Drug Delivery System**

  Drug Solubilization, Prevention of drug crystallization , permeation enhancer, Bioavailability Enhancer for drugs or nutraceuticals, Reduction of drug toxicity, as vehicle for semi-solid dosage form, an emulsifier for injectable formulations, As functional ingredient for inhalation dosage form and self-emulsifying formulations.

2.5 Research Envisaged

The aim of present investigation was to deliver Nicergoline to ischemic zone via nasal route for the effective treatment of cerebral ischemia. It was hypothesized that an intranasal antibody conjugated drug containing nanoconstruct formulation will selectively and effectively deliver drugs to the ischemic zone and will result in reduction of the drug dose and drug associated serious systemic side effects by delivering drug directly to the target organ and minimizing systemic exposure of the drug.

The proposed plan of research includes:

1. Review of literature with reference cerebral ischemia or stroke, intranasal delivery for brain targeting, delivery system based approaches for intranasal delivery of drugs, nanoparticles, microemulsion, mucoadhesive agents, nasal spray, analytical profile and physicochemical properties of the selected therapeutic agents.

2. Preparation of solutions containing selected drug, preparation, optimization and characterization of unconjugated and antibody conjugated nanoconstructs with the help of factorial designing and evaluation of stability of the formulations.

3. Conjugation of the optimized formulation and optimization of conjugated complex for its suitability for *in vivo* studies.

4. Targeting efficiency of conjugated nanoconstruct in animals to ascertain nose to brain transport of drug to ischemic zone.

5. Pharmacodynamic studies of the drug on suitable animal models (middle cerebral artery occlusion model for cerebral ischemia).

6. Preparation of nasal spray of developed formulation. Optimization of nasal spray of formulation with the help of factorial designing and evaluation of stability of the formulations.

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