CHAPTER II
LITERATURE REVIEWS

1. Alzheimer's Disease

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder that occurs gradually and results in memory loss, unusual behavior, personality changes, and a decline in thinking abilities (Parihar, 2004). Neurons death begins insidiously, many years before the onset of memory loss or other symptoms of the disease. During this symptomatic period, there are no obvious symptoms because the remaining neurons compensate for the loss. When neurons begin to die in alarming rate, the symptoms become pronounced and the process is irreversible. AD affects the cognition (learning, abstraction, judgment, etc.) and the memory with behavioral consequences such as aggression, depression, hallucination, delusion, anger and agitation (Olson et al., 1969). Pathologically, ventricular enlargement and atrophy of hippocampus (the limbic structure responsible for the memory) and the cerebral cortex (gray matter) of the temporal lobe (controlling memory, hearing, and language), parietal lobe (language and senses of touch, pain, space, and temperature) can also be observed (Brumback, Leech, 1994).

1.1 Alzheimer's disease and its pathology

AD is pathologically characterized by the presence of extracellular senile plaques, which consist of a core of Aβ, and intracellular neurofibrillary tangles (NFTs), and loss of synaptic connections within entorhinal cortex and progressing into the hippocampus and cortex (Smith, 1998). The granulovascular degeneration in the hippocampus and amyloid deposition in blood vessels are not required for the diagnosis. To date, the diagnosis of definite AD continues to require postmortem histological analysis of the brain to document the presence of the characteristic senile plaques and tangles that define this disease.

1.1.1 Amyloid plaques

Beta amyloid plaques or senile plaques are primarily composed of amyloid beta peptide (Aβ), a 40- to 42-amino-acid peptide, which is derived by proteolytic cleavage of an integral membrane protein known as amyloid precursor protein (APP) by the action of β- and γ-secretases (Swartz et al., 1999; Selkoe, 2001). Accumulation of Aβ peptides may be the key event in pathogenesis of AD. The exact mechanism by which Aβ peptide deposition induces neurotoxicity is unclear, but it appears the oxidative stress plays an important role. Oxidative stress is extensive in AD (Butterfield DA et al., 2002) and Aβ peptides stimulate oxidative stress by both direct and indirect mechanisms. Aβ peptides by themselves may act as enzymes (Opazo et al., 2002) as they are capable of directly producing hydrogen peroxide and generating free radicals through metal ion reduction (Huang et al., 1999). In addition, Aβ peptides can bind to mitochondrial proteins resulting in the generation of free radicals leading to neuroinflammation and finally resulted in the neuronal cell death (Cummings et al., 1998). However, the mechanism by which Aβ causes neuronal injury and cognitive impairment is not yet clearly understood.

1.1.2 **Neurofibrillary tangles** (NFTs)

NFTs are found inside of the brain's cell. They consist of paired helical filaments protein call tau. Tau is a neuronal microtubule-associated protein and is regulated via its phosphorylation by various protein kinases. The microtubule play important role in the transport of nutrients and other important substances. Under hyperphosphorylation state, tau protein formed tangles that were deposited within neurons located in the hippocampus and medial temporal lobe, the parieto-temporal region, and the frontal association cortex resulting in neuronal cell death (Grundke-Iqbal et al; 1986) (Figure 1).

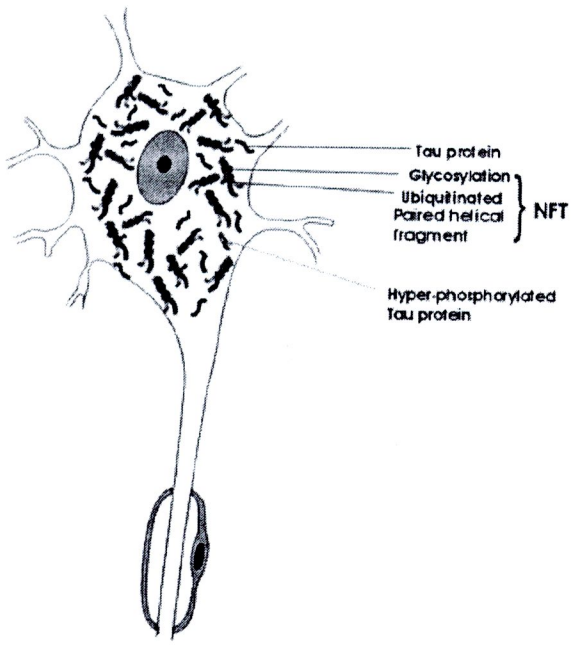


Figure 1 Structural of neurofibrillary tangles (NFT) (Parihar and Hemnani, 2004).

1.1.3 Neuron and synaptic loss

Areas of neuronal cell death and synaptic loss are found throughout a similar distribution pattern as the neurofibrillary tangles, but greatly affect neurotransmitter pathways. The death of cholinergic neurons in the basalis nucleus of Meynert leads to a deficit in acetylcholine (ACh) in the target areas (cerebral cortex), a major transmitter thought to be involved with memory. In addition, serotonergic neurons in the median raphe and adrenergic neurons in the locus coerulus lead to deficits in serotonin and norepinephine (hypothalamus, caudate, limbic and spinal cord) (Tavee and Sweeney, 2002).

1.1.4 Chromosomal mutations

Three genes are associated with early onset familial AD including APP, presenilin-1 (PS-1), and presenilin-2 (PS-2) (Hardy, 1997; Tilley et al., 1998). Mutations in presenilin-1 (PS-1) on chromosome 14, presenilin-2 (PS-2) on chromosome 1 cause AD. In addition, Mutations in the APP gene on chromosome 21 resulted in the increasing of Aβ levels, which are associated with early onset AD (Jankowsky et al., 2004).

1.2 Hypothesis of Alzheimer's disease

1.2.1 Cholinergic hypothesis

Numerous studies reported about the crucial role of cholinergic system could produce memory deficit in Alzheimer's disease (Bartus et al., 1999). It was found that the brains from AD patients showed the degeneration of cholinergic neurons in the basal forebrain (Auld et al., 2002) and the reduction of cholinergic markers, including cholineacetyltransferase (ChAT) and acetyl cholinesterase (AChE) in cerebral cortex (Bowen al., 1988) (Figure 2).

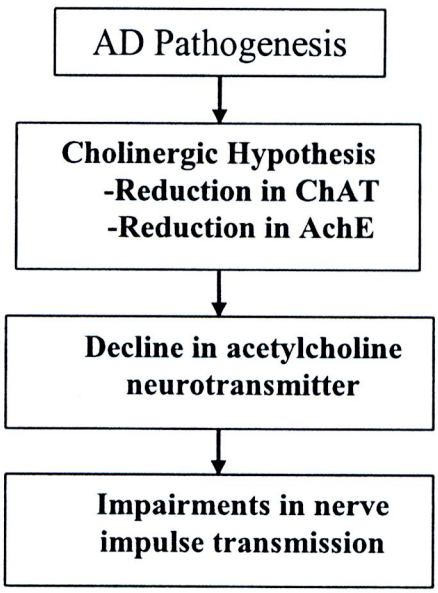


Figure 2 Schematic diagram of the cholinergic hypothesis of Alzheimer's disease (Parihar and Hemnani, 2004).

1.2.2 Amyloid cascade hypothesis

Various document supported that the deposition of Aβ play a crucial role in the pathogenesis of AD. By triggered via various mutations in the amyloid precursor protein gene (APP or AβPP) that causes production, aggregation, deposition and toxicity of Aβ derivative and finally leading to detrimental cellular changes and neuronal cell death (Checler et al., 2002) (Figure 3).

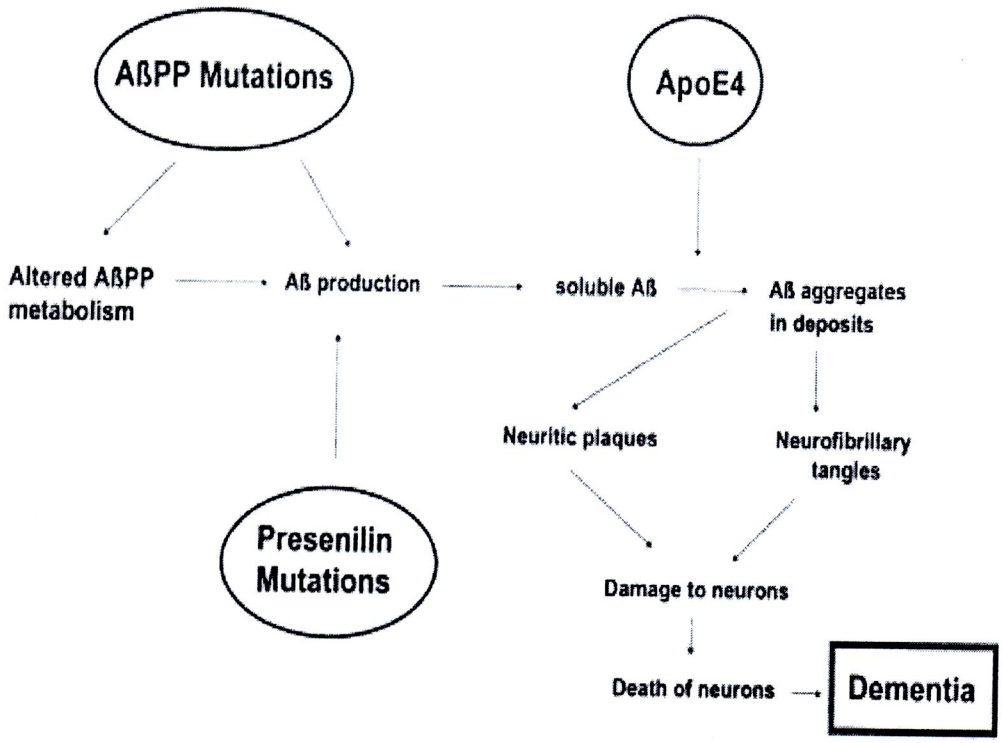


Figure 3 Schematic diagram of amyloid cascade hypothesis of Alzheimer's disease. (Hardy, 1997).

1.2.3 Oxidative stress hypothesis

To date, oxidative stress is considered to play a significant role in the onset and progression of AD (Maiese and Chong et al., 2004). Numerous studies reported that overproduction of Aβ by genetic [i.e. mutations in the amyloid precursor protein (APP) or the presenilins or the presence of apolipoprotein E4 allele (ApoE4) or other mechanisms (i.e. oxidative stress) leads to Aβ-associated free-radical oxidative stress. This oxidative stress is manifested by reactive oxygen species (ROS) formation, lipid peroxidation and subsequent modification of proteins by the reactive lipid peroxidation products 4-hydroxy-2-trans-nonenal (HNE). Other consequences of Aβ-associated oxidative stress are free fatty-acid (FFA) release (which can cause tau polymerization), protein oxidation, Ca^{2+} dyshomeostasis, mitochondrial impairment, peroxynitrite formation, inflammatory response, apoptosis and other cellular responses leading to the neurotoxicity and resulted in the neuron died. This is consistent with the concept of Aβ-associated oxidative stress and neurodegeneration in AD brain (Varadarajan et al., 1999) (Figure 4).

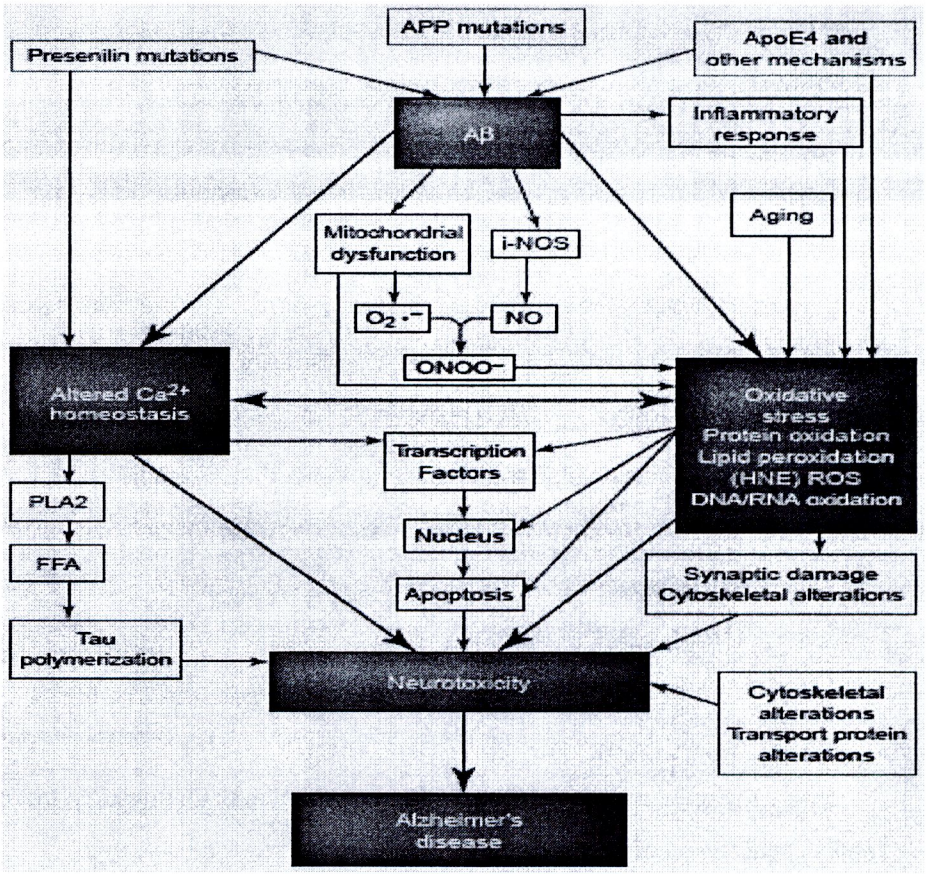


Figure 4 Schematic diagram of the oxidative stress hypothesis of Alzheimer's disease (Butterfield, 2001).

2. Oxidative Stress and Alzheimer's Disease

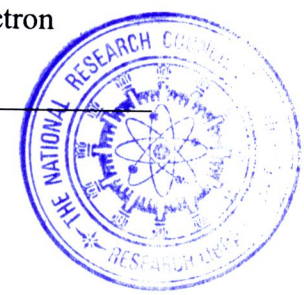
2.1 Biosynthesis of oxidative stress

Oxidative stress occurs when oxygen free radicals are generated in excess through the reduction of oxygen. Reactive oxygen species (ROS) consist of oxygen free radicals and associated entities that include superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide (NO), and peroxynitrite. Several of these species are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems that include superoxide dismutase (SOD), glutathione peroxidase, catalase, and small molecule substances such as Vitamins C and E.

However, problems occur when production of ROS or the free radicals exceed their elimination by the natural antioxidant defense system, or when the latter is damaged. This imbalance between cellular production of ROS and the ability of cells to against them is called oxidative stress (OS) (Ebadi et al., 1996; Jenner and Olnaw, 1996; Simonian and Coyle, 1996). The free radicals produced in the body are toxic, and if not removed or neutralized, they react with lipids, proteins, and nucleic acids and damage cellular functions. Generally, oxidative damage to the cellular components results in alteration of the membrane properties such as fluidity, on transport, enzyme activities, and protein cross-linking. Excessive oxidative damage eventually results in cell death (Simonian and Coyle, 1996; Gorman, 1996).

Generation of ROS is shown in table 1. Oxygen (O_2) reacts with the electrons (e) released from respiratory chain reactions, resulting in the formation of oxygen-based free radical (O_{2-}, superoxide), which is converted to hydrogen peroxide (H_2O_2) by the action of superoxide dismutase (SOD). H_2O_2 is a powerful oxidizing agent that reacts with metal cations such as ferrous ions (Fe^{2+}), and produces highly reactive hydroxyl radicals (OH^-).

Table 1 Formation of ROS by reduction of molecular oxygen in the electron transport chain.



(i) $O_2 + e + H^+ \rightarrow HO_2$ (hydroperoxyl radical)
(ii) $HO_2 \rightarrow H^+ + O_{2-}$ (superoxide radical)
(iii) $O_{2-} + 2H^+ + e \rightarrow H_2O_2$ (hydrogen peroxide)
(iv) $H_2O_2 + e \rightarrow OH^- + OH$ (hydroxyl radical)
(v) $OH + e + H^+ \rightarrow H_2O$
(vi) $O_{2-} + H_2O_2 \rightarrow OH^- + OH + O_2$ (Haber–Weiss reaction)
(vii) $Fe^{2+} + H_2O_2 \rightarrow OH^- + OH + Fe^{3+}$ (Fenton reaction)

(Cui et al., 2004).

The superoxide is an anionic radical formed by the reduction of molecular oxygen through the acceptance of a single electron. The hydroperoxyl radical, which is unstable at physiological pH, dissociates to superoxide. In vivo, it is mainly produced by the electron transport chains in the mitochondria and microsomes through electron leakage—a phenomenon that increases with an increase in oxygen

utilization. This is especially important in brain tissue, which uses elevated quantities of oxygen for its metabolism. The superoxide anion does not cross cell membranes and, by itself, is not very reactive towards cell constituents. Hydrogen peroxide is also a comparatively inactive molecule, but unlike superoxide, it can easily cross cell membranes. Hydrogen peroxide and superoxide may undergo further transformations in the presence of transition metals (particularly iron and copper) (Halliwell and Gutteridege, 1990) to give rise to the highly reactive hydroxyl radicals, by the Haber–Weiss or Fenton reactions. This property, combined with the membrane permeability of hydrogen peroxide, gives superoxide and hydrogen peroxide the ability to affect the integrity of distant molecules within the cell.

2.2 Oxidative stress and the brain

There are many studies suggested that the brain may be particularly vulnerable to oxidative stress for several reasons.

1) Brain has a high content of easily peroxidizable unsaturated fatty acids (especially high in 20:4 and 22:6 fatty acids).

2) Brain requires very high amounts of oxygen per unit weight (about 20% of the total amount used in humans).

3) Brain has a high content of both Fe^{2+} and ascorbate (i.e., they are key ingredients in causing membrane lipid peroxidation).

4) Brain is not highly enriched in antioxidant protective defenses and this then adds to it's otherwise readily poised potential for oxidative damage.

2.3 Effect of oxidative stress in Alzheimer's disease

2.3.1 Lipid peroxidation

The free radical reaction of these lipids with molecular oxygen is a process known as lipid peroxidation, a process that involves intermediate oxygen containing free radicals that attack and abstract hydrogen atoms from lipids, particularly polyunsaturated lipids. Excessive lipid peroxidation has been implicated in a number of diseases. In the circulation, the majority of lipids are transported in association with lipoproteins. Oxidation of lipoproteins in particular unsaturated fatty acids carried in lipoproteins is believed to play a key role in the development and progression of AD. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. Extracellular amyloid plaques,

intracellular neurofibrillary tangles and loss of basal forebrain cholinergic neurons in the brains of AD are the result of abnormalities in lipid metabolism and peroxidation (Halliwell et al., 1984).

2.3.2 Protein oxidation

Excess brain protein oxidation, as a marker of oxidative stress, contributes to the pool of damaged enzymes in AD. Level of protein oxidation (hydradize-reactive protein carbonyl moieties) in aging brain was markedly increased particularly in frontal pole and occipital pole (Smith et al., 1991). The content of this substance was much more increase in Alzheimer's disease patients. Previous study had reported that the protein carbonyl content in hippocampus and inferior parietal lobule were increased in Alzheimer's disease (Hensley et al., 1995). Moreover, many researches reported that elevated of low-density lipoprotein (LDL) was correlated with brain Aβ-42 levels. Additionally, several studies have identified within the brains of AD patients, particularly in the neurofibrillary tangles, the end products of peroxidation, malondialdehyde, peroxynitrite, carbonyls, superoxide dismutase and heme oxygenase-1. Heme oxygenase-1 is a cellular enzyme that is up regulated in the brain and in other tissues in response to an oxidative challenge or other noxious stimuli. Protein oxidation has also been observed in elderly individuals with and without AD, but appears to be more marked in AD patients in the regions presenting the most severe histopathologic alteration.

2.3.3 DNA oxidation

Oxidative damage to DNA may play an important role in aging and AD. Attack on DNA by reactive oxygen species, particularly hydroxyl radicals, can lead to strand breaks, DNA-DNA and DNA-protein cross-linking, sister chromatid exchange and translocation, and formation of at least 20 oxidized bases adducts. Modification of DNA bases can lead to mutation and altered protein synthesis. In late stage AD brain, several studies have shown an elevation of the base adducts 8 hydroxyguanine (8-OHG, 8-hydroxyadenine (8-OHA), 5-hydroxycytosine (5-OHC), and 5-hydroxyuracil, a chemical degradation product of cytosine. Most recently, studies have shown elevated 8-OHG, 8-OHA in nuclear and mitochondrial DNA in mild cognitive impairment, the earliest detectable form of AD, suggesting that oxidative damage to DNA is an early event in AD and not a secondary phenomenon.

Mitochondria are a potential major source of oxidative radicals and oxidative in the form of O^{2-} and H_2O_2, respectively, since their production is linked to metabolism. Increases in the oxidation of mitochondrial DNA (mtDNA) in the cell soma of AD susceptible neurons, which in itself might cause increased oxidative potential.

3. Cognition

3.1 Memory and acetylcholine

Acetylcholine (ACh) was the first known neurotransmitter. It was also recognized as an important neurotransmitter in learning and memory process. This neurotransmitter could be found in brain, neurotransmitter junctions, spinal cord and in both the postganglionic terminal buttons of the parasympathetic division of the autonomic nervous system and the ganglia of the autonomic nervous system.

Acetylcholine (ACh) is synthesized from acetyl-CoA and choline using choline acetyltransferase enzyme as shown in the following diagram.

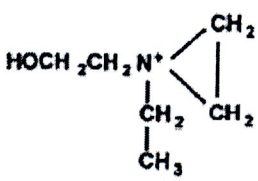
$$\text{Acetyl} + \text{CoA} + \text{Choline} \xrightarrow{\text{ChAT}} \text{Acetylcholine} + \text{Coenzyme A}$$

It was discovered that the nucleus basalis, particularly in the nucleus basalis of Meynert, was a source of acetylcholine. Other central cholinergic projections were also reported including the cholinergic projection from the adjacent medial septum and diagonal band of Broca to hippocampus.The cholinergic system in the neocortex particularly in the basal forebrain was postulated to be the sites for memory storage.

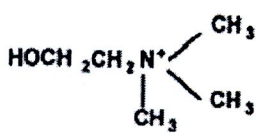
In AD, there are many prominent changes occurred including the degeneration of the cortex, basal forebrain and hippocampus, which led to a profound loss of memory. Although the degeneration of basal forebrain cholinergic neurons was claimed to be an important cause of cognitive impairment in Alzheimer's disease (Coyle et al., 1983), a number of other neurotransmitter systems were also severely affected, including those using the excitatory amino acids aspartate and glutamate as neurotransmitters.

3.2 Effects of ethylcholine aziridinium (AF64A) on cognitive function

Ethylcholine mustard aziridinium (AF64A) was a selective cholinergic neurotoxin that had been used to as chemical toxin to induce selective cholinergic hypofunction in animal model (Hanin 1994; Hanin, 1996) and induced neural degeneration (Hortnagl, 1994). The structure of AF64A was similar to that of choline, except that two of methyl groups were converted to the aziridinium moiety and the third methyl was elongated to an ethyl group (Figure 5). Therefore, the cholinergic nerve terminals via the high affinity choline transport system (Pittel et al., 1987; Rylett, Walters, 1990) into the cholinergic neurons, and caused a specific cholinergic deficit (Baskey et al., 1990; Gomez et al., 1993) could take up this compound selectively.



AF64A



Choline

Figure 5 Structural of AF64A and choline (Fan and Hanin, 1999).

Intraventricular injection of AF64A in rats leading to:

(i) Decreases choline acetyltransferase (ChAT) activity in the hippocampus, without altering the activity of this enzyme in the frontal or parietal cortices, the striatum or the amygdale.

(ii) No lasting effect on the regional concentration of dopamine, serotonin, norepinephrine or their metabolites

(iii) Produces a significant loss of cholinergic neurons in the medial septum (Chrobak et al., 1988).

AF64A produces a variety of effects on cholinergic neurons. At low concentrations, AF64A is taken into the nerve terminal where it binds to choline-specific enzymes and alkylates their catalytic sites. AF64A inhibits the activity of choline aceyltransferase, choline kinase, choline dehydrogenase and acetylcholinesterase (Sandberg et al., 1985).

Following intraventricular injection, AF64A does produce a dose-related decrease in the number of cholinergic neurons in the medial septum (Lorens et al., 1991). Indirect evidence suggests that the final phase of AF64A toxicity (i.e., cell death) might relate to the induction of oxidative stress (Jonhson et al., 1988). For example, pretreatment with Vitamin E, one of most important endogenous lipid-soluble antioxidants prevented the place learning deficit and the cholinergic hypofunction induced by intraventricular injection of AF64A (Wortwein et al., 1994). Although oxidative stress has been implicated in the pathology of several neurodegenerative disorders including AD and in some animal models (Gotz et al., 1994).

3.3 Effect of donepezil (Aricept) on cognitive function

The cholinergic system is one of the most crucial neurotransmitter systems in the brain, and it has very profound links with the manifestations of dementia (Perry et al., 1999). The activities of both choline acetyltransferase (ChAT), the enzyme catalyzing acetylcholine synthesis, and acetylcholinesterase (AChE), the enzyme degrading brain acetylcholine, are reported to be decreased in the neocortex and hippocampus of patients with Alzheimer's disease (Davies and Maloney, 1976; Perry et al., 1985), and this decreased activity correlates with the severity of cognitive impairment (Perry et al., 1978). Significant loss of cholinergic neurons in the nucleus basalis of Meynert has been reported in the brains of patients with this disease (Whitehouse et al., 1982). Based on these pathological findings, the use of reversible AChEs was proposed as a means of potentiating cholinergic neurotransmission, with the aim of improving cognitive function.

Currently, Donepezil hydrochloride is a potent acetylcholinesterase inhibitor (Sugimoto et al., 1995), action with an IC50 value of 6.7-26 mM. In addition, it was previously reported that it could act primarily on the cholinergic system. Moreover, it was found to exert the influence on the other neurotransmitter such as NMDA system by potentiating NMDA-induced currents in some of the cortical neurons tested (Moriguchi et al., 2005). Therefore, it is most widely used for the treatment of mild to moderate Alzheimer's disease (Nordberg and Svensson, 1998; Giacobini, 2000).

4. Blood Brain Barrier

All animals with a complex nervous system require blood–brain barrier (BBB). The BBB is the most important site for regulating from capillaries to neurons (typically<10-15 μm) (Schlageter et al., 1999). In addition, it also supplies key nutrients and protects the brain from neuroactive and potentially toxic compounds circulating in the plasma. The barrier function of the BBB is a combination of physical restriction (tight junctions reduce paracellular permeability for hydrophilic molecules), transport regulation (uptake and efflux carriers and selective trancytosis regulate transcellular molecular flux) and metabolic activity (enzymes metabolize many potentially harmful agents) (Begley et al., 2003). The BBB has played a major role in the evolution of the brain as a complex and integrated network but poses problems for therapeutic approaches that require the delivery of drugs and other molecules to the brain for the treatments of CNS disorders (Begley, 2004).

4.1 Cell types at the blood-brain barrier

The main physical barrier is formed by three cellular elements of the brain microvascular including endothelial cells, astrocyte end-feet, and pericytes (PCs) (Begley et al., 2003). The capillary endothelial cells lining the microvessels, which are coupled by much tighter junctions (zonulae occludentes) than found in peripheral vessels (Wolberg et al., 2002). The endothelial cells secrete and surrounded by a basal lamina (BL), with the end-feet of astrocytic glial cells closely apposed to its opposite side. Moreover, pericytes are embedded in the BL between endothelial cell and astrocyte, making particularly close contacts with the endothelial cells (Figure 6).

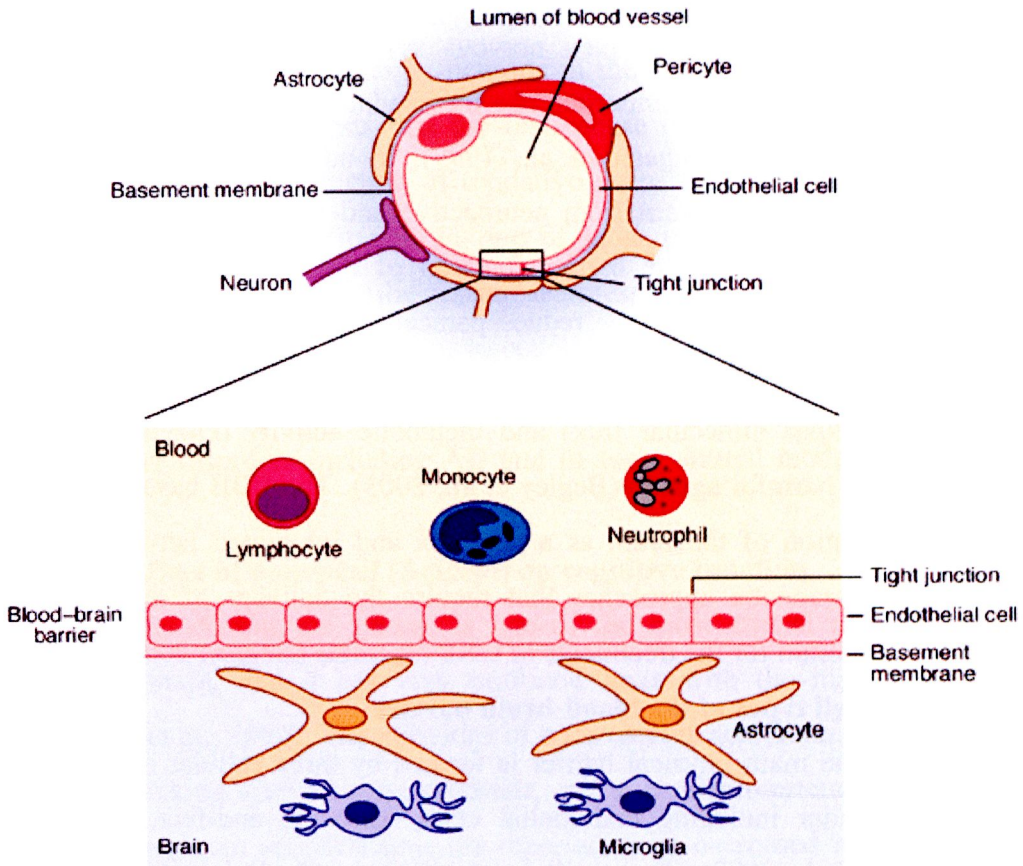


Figure 6 Schematic diagram of cell type in blood-brain-barrier (Begley, 2004).

4.2 Molecular substrusture of the blood-brain barrier

The tightness of the BBB appears not only from the physical complexity of its junctional structure but also from the molecular substructure. The tight junction consists of three integral membrane proteins, namely, claudin, occludin, and junction adhesion molecules, and a number of cytoplasmic accessory proteins including ZO-1, ZO-2, ZO-3, cingulin, and others (Wolberg et al., 2002). The net result is a strong and restricting cell.

4.3 Potential routes for drug delivery to the brain via blood-brain barrier

4.3.1 Passive diffusion

Like all cells, the brain endothelium possesses an outer cell membrane composed of a lipid bilayer with embedded proteins, some of them membrane spanning. Small gaseous molecules such as oxygen and carbon dioxide can

diffuse freely through the membrane, enabling oxidative metabolism of the brain and facilitating its pH regulation. The majority of drugs used to treat the CNS are lipid-soluble (lipophilic) and able to diffuse through the endothelial membrane (Bodor et al., 2003).

4.3.2 Uptake transporters

In peripheral tissues, most of the nutrients and waste products that require molecular exchange with the blood are able to move via the intercellular cleft-the junctions here are sufficiently tight to restrict penetration of large molecules such as plasma proteins but permeable to small hydrophilic solutes. However, the paracellular pathway for small molecule traffic between blood and brain is severely restricted by comparison with the peripheral vasculature; where traffic is needed; it must be handled by specific transport systems in the endothelial membranes. More than 20 such carriers/transporters for uptake into the brain endothelium (uptake transporters) have been identified for glucose, amino acids, nucleosides, nucleobases, and a number of organic anions and cations (Begley et al., 2003).

Many BBB transporters, such as the GLUT-1 glucose carrier, are equilibrate (facilitating transfer but not energy requiring), moving solute down an electrochemical gradient (in this case, net movement from blood to brain), while others require energy to transport against a gradient, either derived directly from ATP (active transport, e.g. Na^+, K^+-ATPase) or from coupling to another molecule enabling use of a favorable concentration gradient (e.g. Na^+-coupled, secondary active transport). Some transporters are located on both the lumen facing (luminal, apical) and the brain facing (abluminal, basal) membranes of the endothelium, while others are localized predominantly to one or other membrane, allowing the possibility of directional or vectorial transport. Thus the Na^+-dependent glutamate transporters (EAAT1-3) appear to be predominant on the abluminal membrane, facilitating glutamate removal from the brain. Although the brain endothelial expression of many transporters is known, the apical/basal distribution is in many cases still unclear, which has complicated modeling of the kinetics of transport. Moreover, the cellular location of other relevant proteins such as enzymes (e.g. hexokinase in the case of glucose transport) may play an important role in vectorial solute flux (McAllister, 2001).

4.3.3 Efflux transporters

Several families of transporters have been identified that are capable of transporting solutes out of the brain endothelial cells, often with consumption of ATP. These include the ABC (ATP binding cassette) family (P-glycoprotein) (Begley et al, 2004). Their roles in normal physiology are unclear, but they are able to restrict the CNS entry of a number of potentially harmful, toxic, or lipophilic agents circulating in the blood, derived from the diet or metabolism, and may have other housekeeping functions. They have a particular significance for drug delivery, since they can effectively block or reduce entry of lipophilic drug molecules.

4.3.4 Transcytosis: receptor-mediated (RMT) and adsorptive-mediated (AM

Tha nguoi dung noi se yeu minh toi mai thoi thi gio day toi se vui hon. Gio nguoi lac loi buoc chan ve noi xa xoi, cay dang chi rieng minh toi... http://nhatquanglan.xlphp.net/ Most standard membrane transporters are able to handle only small molecules (<1000 MW) because of the spatial and energetic requirements of the substrate-to-transporter docking sites and transfer mechanism; however, the broader specificity ABC family (P-glycoprotein) with less rigorous selectivity sites may not be so restrictive (e.g. transferring β-amyloid, MW~4500 (Zlokovic, 2004). Large molecules such as polypeptides and proteins (e.g. transferring, iron binding protein) can be transported across the BBB via vesicular routes. Two mechanisms have been identified (Pardridge, 2005). One requires specific interaction with a membrane receptor followed by internalization (endocytosis) and transfer across the cell (transcytosis) - receptor mediated transcytosis (RMT). The other is less specific, by which cationic molecules can bind to the surface negative charges of the endothelial glycocalyx and be internalized and transferred: adsorptive mediated transcytosis (AMT). This endocytotic traffic is less active in brain endothelium than non-brain endothelium, consistent with the low protein content of ISF and CSF; however, it may be critical for the transfer of small amounts of highly potent regulatory molecules such as TNFα (Pan et al., 2003). These mechanisms offer promise for the delivery of agents too large to couple to membrane transporters.

4.3.5 The enzymatic barrier

The role of the liver in metabolizing circulating compounds has been intensively investigated to elucidate the mechanisms contributing to the plasma pharmacokinetics of compounds. However, for CNS delivery, it is important to take into account further metabolism, particularly in the cells of the barrier layers, chiefly the brain endothelium and the choroid plexus. Both sites express a range of Phase I, II and III enzymes and, in the case of some (e.g. monoamine oxidase), the activity per gram tissue may approach that of the liver. The influence of brain endothelial enzymes has to be considered on a case-by-case basis thus although certain nucleoside analogues of value as neuroprotectants may be substrates for nucleoside transport at the BBB (Chishty, 2004), their brain concentration will be strongly dependent on their rate of metabolism by adenosine kinase and deaminase (Isakovic, 2004).

As mention earlier, it is clear that there are several potential routes for drug delivery to the brain via BBB (Figure 7).

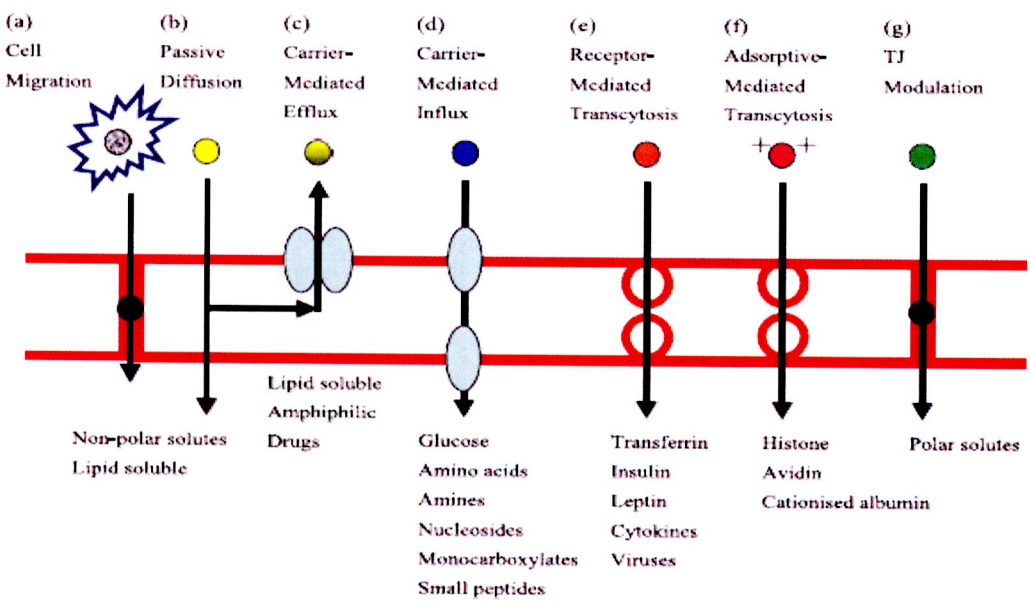


Figure 7 Potential routes for drug delivery to the brain via blood-brain-barrier. (Begley and Brightman, 2003).

5. Nasal Drug Delivery

Nasal drug delivery has generated widespread interest among the scientific community as an alternative route for the administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation and first-pass hepatic metabolism (Krauze et al., 2006).

The nasal cavity is covered with a mucous membrane, which can be divided into non-olfactory and olfactory epithelium areas (Geurkink, 1983). The non-olfactory area includes the nasal vestibule, which is lined with skin-like cells, and the respiratory region, which has a typical airway epithelium.

5.1 The respiratory region

The nasal respiratory epithelium is pseudo-stratified ciliated columnar epithelium. This region is considered to be the major site for drug absorption into the systemic circulation. The four main types of cells seen in the respiratory epithelium are ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells (Figure 8). Although rare, neurosecretory cells may also be seen but, like basal cells, these cells do not protrude into the airway lumen (Petruson et al., 1984).

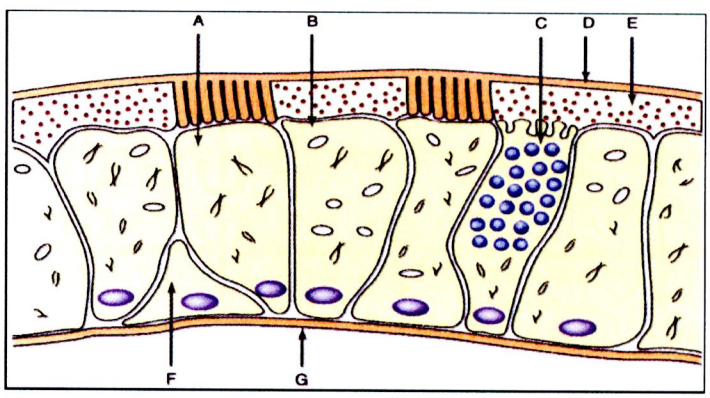


Figure 8 Schematic diagram of cell types in the respiratory epithelium of nasal cavity. Showing ciliated cell (A), non-ciliated cell (B), goblet cells (C), gel mucus layer (D), sol layer (E), basal cell (F) and basement membrane (G) (Petruson et al., 1984).

The proportions of the different cell types vary in different regions of the nasal cavity. In the lower turbinate area, about 15-20% of the total numbers of cells are ciliated and 60-70% is non-ciliated epithelial cells. The numbers of ciliated cells increase towards the nasopharynx with a corresponding decrease in non-ciliated cells (Popp and Martin, 1984). The high number of non-ciliated cells indicates their importance for absorption across the nasal epithelium. Both columnar cell types have numerous (about 300–400 per cell) microvilli (Mygind, 1975). The large number of microvilli increases the surface area and this is one of the main reasons for the relatively high absorptive capacity of the nasal cavity. The role of the ciliated cells is to transport mucus towards the pharynx.

Basal cells, which vary greatly in both number and shape, never reach the airway lumen. These cells are poorly differentiated and act as stem cells to replace other epithelial cells. About 5-15% of the mucosal cells in the turbinates are goblet cells, which contain numerous secretory granules filled with mucin. In conjunction with the nasal glands, the goblet cells produce a secretion, which forms the mucus layer.

5.2 The olfactory region

Humans have relatively simple noses, since the primary function is breathing, while other mammals have more complex noses better adapted for the primary function of olfaction. In a morphometric analysis of mice and rats cavaties, respectively, about 47% and 50% of the total nasal epithelium consists of olfactory epithelium (Gross et al., 1982). In humans, however, the neuroepithelium covers an area of 2-10 cm^2, i.e. around 3% (Morrison and Costanzo, 1990). These size differences in the olfactory area reflect the importance of the sense of smell for the different species. Many common animal models are classified as macrosmatic (i.e. the olfactory epithelium occupies a large area of the total nasal epithelium) while humans are classified as microsmatic (Reznik, 1990).

The olfactory epithelium rests upon a thick connective tissue, lamina propria, which contains blood vessels, olfactory axon bundles and Bowman's glands. Like the epithelium of the respiratory region, the olfactory epithelium comprises pseudo-stratified columnar cells of three principal types: olfactory receptor cells, supporting cells and basal cells (Figure 9). The basal cells are flattened to an elongated ovoid shape and are located close to the epithelial side of the basal lamina. The olfactory neurons are interspersed between the supporting cells that form a distinct layer in the upper third of the olfactory epithelium (Uraih and Maronpot, 1990).

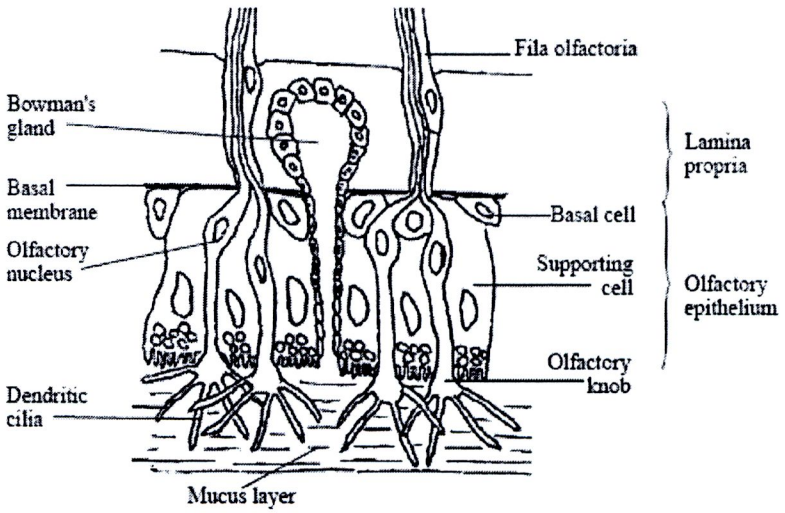


Figure 9 Cell types in the olfactory epithelium of nasal cavity(Mathison et al., 1998).

5.3 Absorption across the nasal epithelium

The pathways for absorption across the nasal respiratory epithelium are no different from those across other epithelia in the body. The four main absorption routes are transcellular and paracellular passive absorption, carrier-mediated transport and absorption through transcytosis. Transcellular passive diffusion is the main mode of absorption for most drugs but, for large or ionised molecules, the paracellular route can provide an opportunity for absorption.

Although nasal delivery avoids hepatic first-pass metabolism, the nasal mucosa provides a pseudo-firstpass effect. However, because of the lower activity per mg respiratory mucosa compared to the gastrointestinal tract and the liver (Longo et al., 1988) and the higher drug to enzyme ratio, it is easier to overcome the degradation problem when using the nasal route. Advantages and limitations of nasal delivery were concluded in Table 2.

Table 2 Advantages and limitations of nasal delivery

Advantages	Limitations
Avoids degradation of drug in gastrointestinal tract resulting from acidic or enzymatic degradation	Volume that can be delivered into nasal cavity is restricted to 25–200 µl
Avoids degradation of drug resulting from hepatic first pass metabolism	High molecular weight compounds cannot be delivered through this route (mass cut off ~1 kDa)
Results in rapid absorption and onset of effect	Adversely affected by pathological conditions
Results in higher bioavailability thus uses lower doses of drug	Large interspecies variability is observed in this route
Easily accessible, non-invasive route	Normal defence mechanisms like mucocillary clearance and ciliary beating affects the permeability of drug
Self-medication is possible through this route	Enzymatic barrier to permeability of drugs
Direct transport into systemic circulation and CNS is possible	Irritation of nasal mucosa by drugs
Offers lower risk of overdose	Limited understanding of mechanisms and less developed models at this stage
Does not have any complex formulation requirement	

(Longo et al., 1988)

5.4 Transport pathways from nose to brain

The different routes by which a drug delivered nasally can reach the CSF and the brain are shown in schematic diagram (Figure 10). When drugs are administered nasally the drug will normally be rapidly cleared by the mucociliary clearance system (Illum et al., 2000). Some of the drug (for lipophilic drugs up to 100% but normally much less) will be absorbed into the bloodstream reaches to the systemic circulation and eliminated from the bloodstream via normal clearance mechanism (Hussain et al., 2000). The drug can reach the brain from the blood by crossing the blood brain barrier but can also be eliminated from CSF into the blood. However, the drug can also be absorbed from the nose via the olfactory region into CSF and possibly further into the brain. The amount of drug absorbed or lost via the different pathways is depend on the characteristics of the drug, especially lipophilicity, molecular weight and the drug formulation (Sakane et al., 1995).

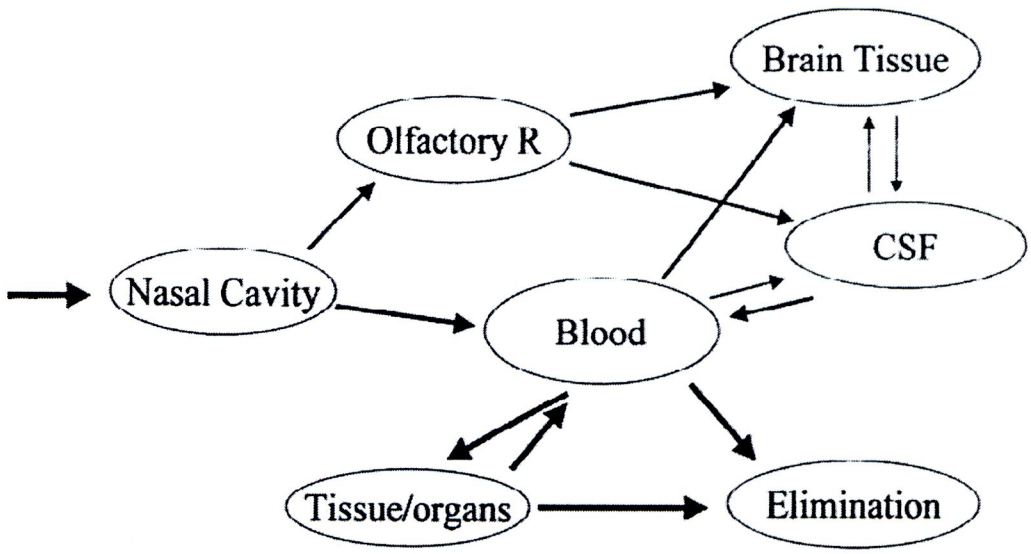


Figure 10 Possible routes of transport between the nasal cavities and the brain

In order to travel from the olfactory region in the nasal cavity to the CSF or the brain parenchyma, drug has to transverse the nasal olfactory epithelium and depends on the pathway followed, including the arachnoid membrane surrounding the subarachnoid space. In principle, three differents pathways across the olfactory epithelium; (i) transcellularly especially across the sustentacular cells, most likely by receptor mediated endocytosis, fluid phase endocytosis or by passive diffusion, the latter pathway most likely for more lipophilic drugs, (ii) paracellularly through tight junctions between sustentacular cells or olfactory neurons, (iii) by the olfactory nerve pathway and the drug is taken up into the neurons by endocytosis or pinocytotic mechanisms and transported via intracellular axonal transport to the olfactory bulb.

5.5 Factors affecting the nasal permeability of drugs

The factors affecting permeability of drug through the nasal mucosa can broadly be classified into three categories as shown in Table 3.

Table 3 Variable factors affecting the permeability of drugs through the nasal mucosa

Biological
Structural features
Biochemical changes
Physiological factors
• Blood supply and neuronal regulation
• Nasal secretions
• Nasal cycle
• pH of the nasal cavity
• Mucociliary clearance and ciliary beat frequency
Pathological conditions
Environmental factors
• Temperature and Humidity
Formulation
Physicochemical properties of drug
• Molecular weight
• Size
• Solubility
• Lipophilicity
• pKa and partition coefficient
Physicochemical properties of formulation
• pH and mucosal irritancy
• Osmolarity
• Viscosity/Density
• Drug distribution
• Area of nasal membrane exposed
• Area of solution applied and Dosage form
Device related
Particle size of the droplet/powder
Site and pattern of disposition

(Sakane et al., 1995).

6. Quercetin

Quercetin (3, 5, 7, 3, 4-pentahydroxyflavone) is one of the most studied flavonoids. It is found in vegetables, fruits such as apples, onions, green tea and red wine (Hertog et al., 1992).

Quercetin, consists of two aromatic rings, A and B rings, and the two rings are linked by an oxygen-containing heterocycle (ring C) (Figure 11).

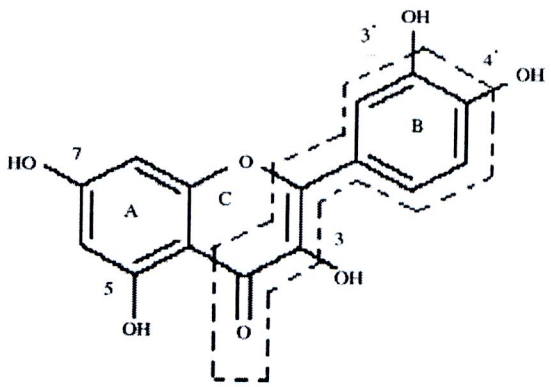


Figure 11 Structure of quercitin (Liu and Guo, 2006).

6.1 Pharmacokinetics

Quercetin was initially suggested to be taken up in the gastro-intestinal tract by passive diffusion (Griffiths, 1982). After absorption, about 20% of an ingested dose of quercetin is absorbed from the small intestine and is transported to the liver via the portal circulation, where it undergoes significant first pass metabolism. Quercetin and its metabolites are distributed from the liver to various tissues in the body. In addition, Quercetin is strongly bound to albumin in the plasma. Peak levels of plasma quercetin occur from 0.7 to 7 hours following ingestion, and the elimination half-life of quercetin is approximately 25 hours (Venkatesh et al., 2002). Regarding pharmacokinetics of the quercetin glycoside conjugates, it appears that the main determinant of absorption of these conjugates is the nature of the sugar moiety. For example, quercetin glucoside is absorbed from the small intestine, whereas quercetin rutinoside is absorbed from the colon after removal of the carbohydrate moiety by bacterial enzymes.

6.2 Safety of quercetin

Toxicity and therapeutic efficacy of different doses of quercetin can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio of LD_{50} /ED_{50}. Doses of quercetin that exhibit high therapeutic indeces are preferred. An effective dose of quercetin can be delivered in a single dose or as multiple doses over a period. After quercetin was administered at dose levels that delivered approximately 40 to 1900 mg/kg/day to male and female rats, there were no treatment related effects on survival and no treatment related clinical signs of toxicity (Davies et al., 2000).

6.3 Antioxidant effects of quercetin

The chemical structure of flavonoids, including quercetin, makes them capable of stabilizing free electrons obtained from free radicals such as ROS in vitro systems (Hanasaki et al., 1994; Heijnen et al., 2001; Pietta, 2002) have shown that particular hydroxyl groups seem to be positively related to abilities of flavonoids to scavenge peroxynitrite. Flavonoids with particular structures may also inhibit ROS production by chelating metal ions that would otherwise contribute to ROS production through Fenton reactions (Pietta, 2002). Flavonoids including quercetin are also known to inhibit superoxide anion production by xanthine oxidase (Hanasaki et al., 1994).

6.4 Adverse reactions

Adverse effects reported with oral quercetin include gastrointestinal effects such as nausea, and rare reports of headache and mild tingling of the extremities. Oral quercetin is generally well tolerated. Intravenous administration of quercetin has been associated with nausea, vomiting, diaphoresis, flushing and dyspnea (Ferry et al., 1996).

6.5 Interactions and usage

6.5.1 Drugs

Quinolone Antibiotics: Quercetin binds, *in vitro*, to the DNA gyrase site in bacteria. Therefore, theoretically, it can serve as a competitive inhibitor to the quinolone antibiotics, which also bind to this site.

Cisplatin: Because of the theoretical risk of genotoxicity in normal tissues in those using cisplatin along with quercetin, those taking cisplatin should avoid quercetin supplements.

6.5.2 Nutritional supplements

Bromelain and papain are reported to increase absorption of quercetin.

7. Liposomes

Liposomes are colloidal particles consisting of an aqueous core enclosed in one or more phospholipid layers, usually 0.05-5.0 μm in diameter that form spontaneously when certain lipids are hydrated in aqueous media (Bangham., 1974). When phospholipids were placed in aqueous medium, the hydrophilic interaction of the lipid head groups with water, results in the formation of multilamellar and unilamellar systems (vesicles). The vesicles consist of simple lipid bilayers that resemble biological membranes, in the form of a spherical shell (Figure 12).

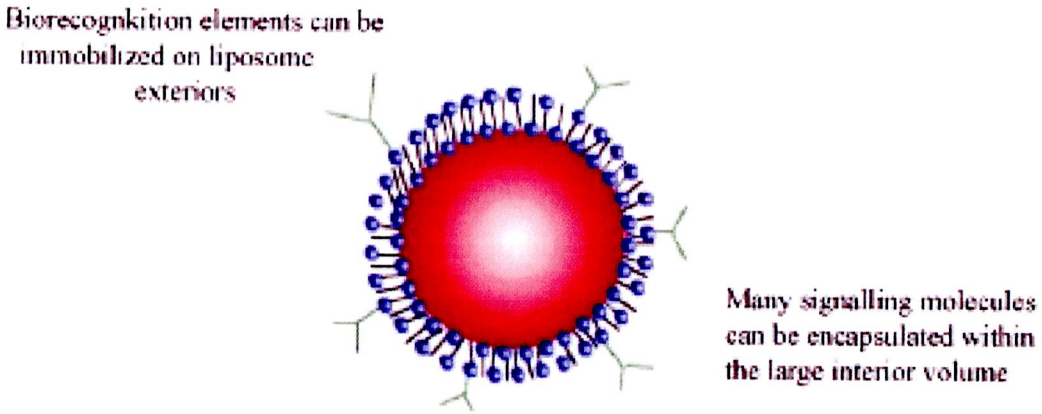


Figure 12 Structure of general liposomes (Edwards and Baeumner, 2006).

Phospholipids with different polar head-groups functionalized for conjugation or to reduce liposome aggregation and hydrophobic regions of different chain length and saturation were used to modify the properties of the resulting liposomes. Cholesterol is often included with membrane phospholipids to reduce the membrane permeability towards encapsulated materials. Their structure is similar to that of cells and thus could be used as a more easily characterized vessel for studying interactions between membrane lipids and biomolecules such as DNA and proteins; permeability of ions and drugs; and elucidating the mechanism of action of pesticides and antibiotics on target organisms (Vemuri and Rhodes, 1994).

Molecules could be associated with liposomes in several ways, including encapsulation within the aqueous inner cavity, partitioning within the lipid tails of the bilayer, and covalent and electrostatic interactions with the polar head-groups of the lipids.

The surface of liposomes could be modified through the choice of lipids to allow conjugation to a variety of biorecognition elements. Typical functionalized lipids include those with an amino group, such as phosphatidylethanolamine (PE); a carboxy-group, such as in *N*-glutaryl-PE; a protected disulfide group, such as pyridyldithio propionate (PDP)-PE; and a hydroxyl group, using cholesterol or polyethylene glycol based entities .

A wide variety of hydrophilic molecules can be encapsulated within the inner cavity, including enzymes, DNA, vaccines, fluorescent dyes, electrochemical and chemiluminescent markers, and some pharmaceutical compounds. The bilayer structure can prolong the longevity of the encapsulated molecules by shielding them from destructive entities within the body (Papahadjopoulos, 1978).

Today, liposomes are potential drug carriers for a variety drugs that includes the traditional small molecular weight drugs, therapeutic proteins, and diagnostic agents. Not only for liposomes biocompatible nature but also because they do not elicit negative biological responses that generally occur when a foreign material is introduced in the system. These lipid vesicles were reported to be non-toxic, non-immunogenic, noncarcinogenic, non-thrombogenic and biodegradable (Ohsawa et al., 1984). In addition, the biophysical properties, such as size, surface charge, lipid composition and amount of cholesterol, was varioused and able to control distribution, tissue uptake and drug delivery (Gregoriadis et al., 1993).

7.1 Classification of liposomes

There are various classes of liposomes. Liposomes are classified either by the method of their preparation or by the number of bilayers present in the vesicle, or by their size. When liposomes were described based on the number of bilayers they are described as unilamellar vesicles (ULV) or multilamellar vesicles (MLV), the reversephase evaporation vesicles (REV), French press vesicle (FPV) and ether injection vesicles (EIV) were the classification based on method of preparation. While large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV) were the classification according to their size (Sharma, 1994).

However, the description of liposomes by the lamellarity and size are more common than by the classification according to method of preparation. The details of the classification of liposomes were shown in table 4 and Figure 13.

Table 4 Classificaion of liposomes by size

Liposome	Classification	Approximate size (μm)
By size	SUV	0.025-0.05
	LUV	0.1
By lamellarity	MLV	0.05-10
	ULV	0.025-0.1
By method	REV	0.5
	FPV	0.05
	EIV	0.02

(Jones, 1995).

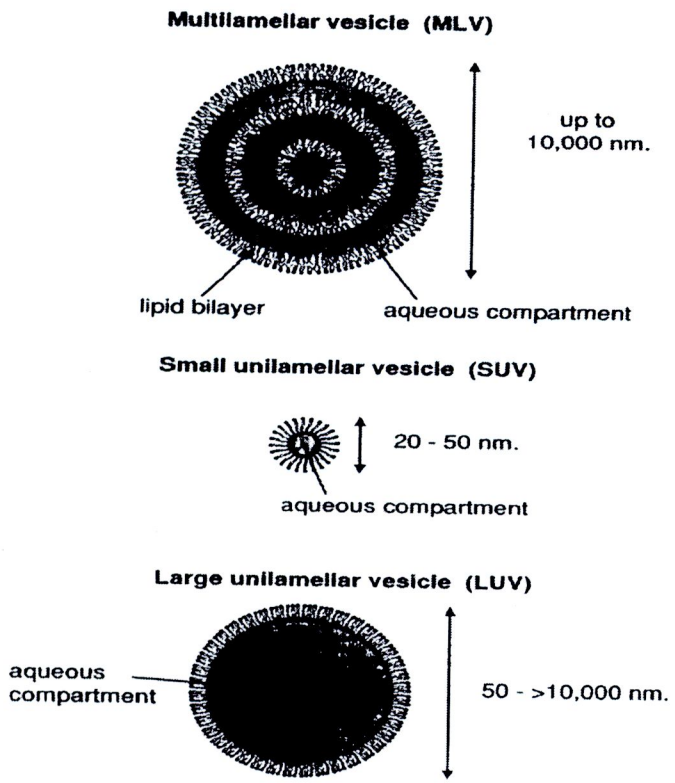


Figure 13 Classification of liposomes (Jones, 1995).

7.2 Limitations of liposome technology

Liposomes have a great potential in the area of drug delivery. However, some of the problems limit the manufacture and development of liposomes including stability, batch-to-batch reproducibility, sterilization method, low drug entrapment, particle size control, and production of large batch sizes and short circulation half-life of vesicles. Some of the remaining problems are reported in detail below.

7.2.1 Stability

One of the major problems limiting the widespread use of liposomes is stability both physical and chemical factors. Depending on their composition, the final liposome formulations may have short shelf lives partly due to chemical and physical instability. Chemical instability might be caused by hydrolysis of ester bond and/or oxidation of unsaturated acyl chains of lipids. Physical instability might be caused by drug leakage from the vesicles and/or aggregation or fusion of vesicles to form larger particles. Both of these processes (drug leakage and change in liposome size) influenced the in vivo performance of the drug formulation, and therefore might affect the therapeutic index of the drug (Sharma et al., 1997; Straubinger et al., 1983).

7.2.2 Sterilization

Identification of a suitable method for sterilization of liposome formulations is a major challenge because phospholipids are thermolabile and sensitive to sterilization procedures involving the use of heat, radiation and/or chemical sterilizing agents. The method available for sterilization of liposome formulations after manufacture was filtration through sterile 0.22 μm membranes. However, filtration was not suitable for large vesicles (>0.2 μm) and is not able to remove viruses. Sterilization by other approaches such as γ-irradiation and exposure to chemical sterilizing agents was not recommended because they could cause degradation of liposome components and might leave toxic contaminants (Zuidam et al., 1995).

7.2.3 Encapsulation efficiency

Liposome formulation of a drug could only be developed if the encapsulation efficiency of therapeutic doses could be delivered in a reasonable amount of lipid, because lipids in high doses might be toxic and also caused non-linear (saturable) pharmacokinetics of liposomal drug formulation. Some new approaches that provide high encapsulation efficiencies for hydrophilic drugs had also developed such as active loading of amphipathic weak acidic or basic drugs in empty liposomes can be use to increase the encapsulation efficiency (Mayer et al., 1986; Clerc and Barenholz, 1995).